ANTIBODY RESPONSES TO VACCINES AND PFAS EXPOSURE IN EARLY CHILDHOOD

by

Nancy Bao

Dr. Kate Hoffman,

Advisor

and

Dr. Heather Stapleton,

Advisor

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EXECUTIVE SUMMARY

Per- and polyfluoroalkyl substances (PFAS), are human-made chemicals commonly incorporated into personal care products, cookware, food packaging, and other industrial uses. Previous studies have found that early life exposure to PFAS is associated with health effects in both animal and human studies. There are growing concerns over the potential health consequences such as immunological health associated with prenatal and early childhood PFAS exposure. Studies have found that exposure to environmental stressors during early periods of fetal growth and development may have implications for the development of adverse health effects later in life.

Few studies have assessed the association between PFAS exposure and waning immunity to vaccines during early childhood. Of these studies, PFAS exposure has been inversely associated with antibody responses to vaccines against infectious diseases such as diphtheria, tetanus, and rubella. Antibody responses to vaccines are commonly used as biomarkers to assess immune function and development.

The objective of this study was to evaluate the impacts of PFAS exposures on critical windows of immune function and maturation in early childhood. Early childhood immune function was evaluated using antibody responses to the Diphtheria-Tetanus-acellular Pertussis (DTaP) vaccine. Multiple linear regression analyses (adjusted for child's age, biological sex of child, and maternal age) were conducted to evaluate the associations between maternal and child serum PFAS levels in a North Carolina birth cohort (n=47) and antibody responses to the DTaP vaccine in children (ages 3-6). Maternal serum PFAS were collected during prenatal care visits and used to assess prenatal exposure. Child serum was collected for diphtheria and tetanus antibody titer analyses as well as post-natal PFAS exposure analyses.

Prenatal PFAS exposure measured from maternal serum was not significantly associated with tetanus antibody titers; however, a positive and significant association ($p<0.05$) was observed between prenatal PFNA exposure and diphtheria antibody titers. Postnatal PFAS exposure was not significantly associated with diphtheria antibody titers. Post-natal PFOA exposure was positively associated with tetanus antibody responses ($p<0.05$). The results do not suggest that prenatal and early childhood exposure to PFAS is associated with declines in immune responses to vaccines. Other factors associated with environmental PFAS exposure and vaccine antibody responses should be explored to expand on these findings.

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INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are human-made chemicals used in personal care products, firefighting foams, and many other applications (Sunderland et al., 2019; Rappazzo et al., 2017). With over 4,700 types of PFAS manufactured globally, these compounds became commonplace in various industrial processes and products due to their water and oil repellency, surfactant nature, and chemical stability (Cousins et al., 2020; Buck et al., 2011). However, the chemical stability of these fluorinated hydrocarbon compounds has also contributed to their environmental persistence in drinking water sources and major waterways (Post et al., 2012; Sun et al., 2016; Kaboré et al., 2018).

PFAS exposure in humans primarily occurs through ingestion of drinking water and food as well as inhalation and indirect ingestion of dust and air in indoor environments (Sunderland et al., 2019). PFAS have been detected in human serum, breastmilk, and cord blood (Papadopoulou et al., 2016; De Silva et al., 2020). Persistence of these substances in these biofluids have been used to estimate exposure windows and sources of PFAS in humans (Hu et al., 2018); Many studies have used serum concentrations to provide approximate measures of cumulative exposure to PFAS in humans (De Silva et al., 2020; Grandjean et al., 2012; Xu et al., 2020). Various factors such as diet, age, geographic region, lifestyle, and race/ethnicity can influence serum PFAS duration and levels in humans (Boronow et al., 2019; Hu et al., 2018; Park et al., 2019).

A recent study estimated the elimination half-life of various PFAS in serum of people occupationally exposed in an airport in Sweden (Xu et al., 2020). The authors estimated the following serum half-lives: 2.91 years for linear perfluorooctanesulfonate (n-PFOS), 1.77 years for PFOA (perfluorooctanoic acid), and 2.86 years for perfluorohexane sulfonate (PFHxS) (Xu et. al., 2020). A previous study analyzed serum samples from retired employees occupationally

exposed to PFAS in Alabama (Olsen et al., 2007). From these serum samples, Olsen et al (2007) estimated the arithmetic mean half-lives for PFOS (5.4 years), for PFOA (3.8 years), and for PFHxS (8.5 years).

Zhang et al (2013) assessed the effects of menstruation on serum and urine analyses for an expanded suite of PFAS, which included PFOS, PFOA, PFHxS, PFNA (perfluorononanoate), and PFDeA (perfluorodecanoate). The researchers reported average biological elimination half-lives to be 6.7 years for linear PFOS, 2.3 years for linear PFOA, 7.7 years for PFHxS, 2.5 years for PFNA, and 4.5 years for PFDeA for young females (Zhang et al., 2013). For males and older females, the estimated average biological elimination half-lives were longer than those measured in the young females except for PFDeA—34 years for linear PFOS, 2.8 years for PFOA, 35 years for PFHxS, 12 years for PFNA, and 4.3 years for PFDeA (Zhang et al., 2013). While these biomarkers provide insight to exposure durations, there are still uncertainties and concerns surrounding the ecological and human health consequences of PFAS exposure (Penland et al., 2020; Kotlarz et al., 2020). The recalcitrant nature of these compounds in the human body have prompted further inquiries about the toxicological implications and endocrine disrupting effects of these compounds in humans and other organisms (Beesoon et al., 2012; Pérez et al., 2013; Kang et al., 2016; Spratlen et al., 2020).

Many animal studies have investigated health effects such as endocrine disruption, hepatoxicity, neurotoxicity, and developmental toxicity associated with exposure to the two most common PFAS—perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA) (Shi et al., 2008; Slotkin et al., 2008; Du et al., 2009; Thibodeaux et al., 2003). Gestational exposure to PFOS in mice and rats led to the development of cleft palates and right atrial enlargement in offspring (Thibodeaux et al., 2003). The adverse fetal and maternal health effects in mice and

rats exposed to PFOS revealed potential consequences for exposure during pregnancy and its implications for other species (Thibodeaux et al., 2003).

Human studies have also looked at the health effects associated with prenatal PFAS exposure. A recent study explored the association between prenatal exposure to PFAS and biomarkers of liver injury (e.g. liver enzymes) in six European population-based birth cohorts (Stratakis et al., 2020). The researchers found that children with high levels of prenatal PFAS exposure were associated with higher risk of liver injury characterized by factors such as increased amino acid levels and alanine aminotransferase levels (Stratakis et al., 2020). The results from these *in vivo* and epidemiological studies suggest that early age exposure to PFAS may be detrimental to later life growth and development in humans. Emerging bodies of literatures have expanded on the Developmental Origins of Health and Disease (DOHaD) paradigm, which describes adverse environmental exposures during early development as triggers of later-life disorders and diseases (Haugen et. al., 2015). Fetal and early post-natal stages of growth and development are particularly sensitive windows to external environmental stressors and many studies have explored relationships between maternal pregnancy exposures to environmental contaminants and fetal health effects (Haugen et. al., 2015; Heindel et al., 2017; Curtis et al., 2019). While researchers have found that critical immune cell differentiation and maturation occurs during the prenatal to postnatal periods of human development, there is a limited body of literature assessing the immune consequences of early-life exposures to organic contaminants in the environment (Park et al., 2020; MacGillivray and Kollmann, 2014). Many of these studies have focused on the immune outcomes in response to developmental exposures to chemicals such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs),

pharmaceutical agents, and phthalates (Hertz-Picciotto et al., 2008; Robinson and Miller, 2015; Wang et al., 2014).

Very few studies have explored the potential immunological effects of environmental exposures to PFAS in early life and immune development and function. Grandjean et al conducted a prospective cohort study in the Faroe Islands, which examined the relationship between early-life PFAS exposure and children's antibody levels to childhood tetanus and diphtheria vaccinations (Grandjean et. al., 2012). The authors concluded that increases in prenatal PFOA and PFOS exposure were associated with decreases in anti-diphtheria antibody concentrations in children at five years of age (Grandjean et. al., 2012). Furthermore, they found that childhood exposures to PFOA, PFOS, PFHxS, PFNA, and PFDA at five years of age, were inversely associated with both tetanus and diphtheria antibody concentrations at seven years of age (Grandjean et al., 2012). A follow-up study was conducted in the Faroe Islands cohort in which the researchers found that serum antibody concentrations against diphtheria were negatively associated with PFAS concentrations in serum for adolescents at age 13 (Grandjean et. al., 2017). These studies suggest potential consequences of early life exposure to PFAS chemicals on the immune system (Grandjean et. al., 2012; Grandjean et. al., 2017).

In looking at other vaccine responses to PFAS exposure, other researchers conducted a cross sectional study of the US National Health and Nutrition Examination Survey (NHANES) data to assess adolescent children's MMR (Measles, Mumps, Rubella) vaccine responses and their current PFAS exposure (Stein et al., 2016). The researchers found increased PFOS serum concentrations in adolescent children were associated with decreased mumps and rubella-specific IgG antibody concentrations, but not measles-specific IgG antibody concentrations (Stein et al., 2016). A birth cohort study in Guangzhou, China recently found increased prenatal PFAS

exposure was associated with decreased antibody responses to Hand Foot Mouth Disease, HFMD in infants, three months postnatal (Zeng et al., 2019). In another birth cohort study, researchers examined the association between children's response to the Rubella vaccine and prenatal PFAS exposure, evaluated by using maternal plasma PFAS (Granum et al., 2013). They found that increased exposure to four PFAS: PFOA, PFOS, PFHxS, and PFNA were associated with decreased anti-Rubella antibody concentrations (Granum et al., 2013). These studies suggest that PFAS exposure may suppress immune responses to childhood vaccinations.

Evaluating antibody responses to early life vaccinations provides a structured approach to elucidating the uncertainties surrounding the functions and maturation of the neonatal immune system (Siegrist, 2001). Vaccinations are designed with a standardized dose and administered based on specified schedules (Robinson et al., 2019). In the United States, children (from birth through adolescence) are advised to undergo routine vaccinations (Robinson et al., 2019). When these routine vaccines are administered, they expose the individual to either components of a pathogen, toxins derived from the pathogen, or deactivated/modified pathogens, triggering the innate immune system to develop antibodies to recognize and protect the body against the designated disease (Vetter et al., 2018). Therefore, antibody titer measurements have been used as conventional surrogate markers for vaccine response (Hogrefe, 2005). Many studies have utilized antibody titers to vaccines as a way to assess immune outcomes and assess whether the immune system is responding adequately to neoantigens (Shen-Orr and Furman, 2013; Klein et al., 2015).

The purpose of this study was to investigate the potential associations between early life exposure to PFAS, as indicated by maternal and children's serum PFAS, and children's vaccination responses. This study focuses on comparing gestational and childhood exposures to

PFAS and early life responses to childhood vaccinations to provide further insights into to critical windows of the immune system that may be impacted by these exposures. This study looks specifically at responses to childhood vaccinations against diphtheria and tetanus

METHODS

Study Design and Participants

Children and mothers (n = 47 pairs) included in this study, are a subset of the children and mothers who participated in both the NEST (Newborn Epigenetics Study) pregnancy cohort study and the TESIE (Toddlers' Exposure to SVOCs in the Indoor Environment) children's follow-up study from central North Carolina (Hoffman et. al., 2018). The NEST study population (n= 2,595 women) consisted of women recruited into the study in their first trimester (approximately 11 weeks) of gestation and their infants recruited from 2005 to 2011 from the Division of Maternal and Fetal Medicine of the Duke University Health System (Hoffman et al., 2018; Fuemmeler et al., 2016; Hoyo et al., 2011). The TESIE study was based on a nested cohort (n=203) of participants from the Newborn Epigenetics Study (NEST) study (Hoffman et al., 2018). The TESIE study subjects were mothers and their children, ages three to six, who were recruited into the study from August 2014 to April 2016 (Hoffman et. al., 2018). The Duke University Medical Center Institutional Review Board approved the NEST and TESIE studies. Mothers provided informed consent before participating in NEST and legal guardians provided consent for children's participation in TESIE. Eight pairs of children and mothers were omitted from further analyses in this study; one pair was omitted as an outlier for both diphtheria and tetanus antibody titers (>99% confidence interval); one pair was removed due to a missing tetanus titer; the remaining four pairs were removed due to missing maternal or child's serum PFAS measurements.

Serum Collection and Analysis

PFAS were measured by maternal serum samples and were used as a proxy for prenatal exposure. Maternal serum samples were collected via venipuncture at prenatal care visits. Children's serum samples were collected via venipuncture or fingerstick procedure by a certified phlebotomist at the children's homes when they were between the ages of three and six (Hoffman et al., 2018). Both maternal and children's serum samples were analyzed for 14 PFAS via isotope-dilution high performance liquid chromatography and tandem mass spectrometry by the Centers for Disease Control and Prevention (Ye et al., 2018). The five most detected PFAS in both children and maternal serum samples were linear forms of PFOA and PFOS, PFHxS, PFNA, and PFDA. Further statistical analyses were restricted to these compounds. Serum PFAS below the LOD of 0.1 ng/mL were replaced with values of the LOD divided by two for the statistical analyses. Children's serum samples were also assessed for antibody production post-vaccination. Serum diphtheria and tetanus specific antibodies were assessed in approximately 50 µL of serum using an enzyme-linked immunosorbent assay (ELISA).

Covariates

Extrinsic and intrinsic variables, considered potential confounders of vaccine response were included for analyses to assess the association between prenatal and post-natal PFAS exposure and vaccine response. The variables intrinsic to the child that were considered were child's age at serum sampling (in months) and biological sex of the child. Among maternal/perinatal variables, parturition age (in years) was included in the analyses. Other potential confounders regarding perinatal health, maternal socioeconomic status and child's behavior and lifestyle were individually explored, but not included in the final analyses because they were not found to be significantly associated with antibody concentrations. These variables

included: race/ethnicity of the mother, gestational period (in weeks), parity, education level, smoking status, source of drinking water, monthly vacuum frequency, residence time in home, daily handwashing frequency of child, and daily time child spent in the home (in hours).

Statistical analyses

Preliminary analyses using the Shapiro-Wilk test and Kruskal-Wallis test, found skewed distributions of antibody concentrations and of PFAS concentrations in serum. The antibody concentrations and PFAS concentrations were natural log-transformed to reduce skewness for further statistical analyses. Pairwise comparisons using Wilcoxon Rank Sum tests were used to assess differences in maternal serum and children's serum PFAS. To evaluate relationships between individual characteristic and antibody concentrations, nonparametric analyses or analyses with transformed data were conducted (i.e. Kruskal-Wallis Test and Chi Square Tests).

To evaluate associations between PFAS and antibody concentrations, unadjusted linear regression analyses were first conducted with individual PFAS: n-PFOA, n-PFOS, PFHxS, PFDeA, and PFNA. These univariate regression analyses were conducted separately for natural log-transformed maternal serum PFAS and natural log-transformed children's serum PFAS. Kruskal-Wallis tests and Pearson's Chi-Squared tests were used to assess the difference in antibody concentrations associated with biological sex and that associated with age. Multivariate regression models were then used to assess anti-tetanus and anti-diphtheria antibody concentrations in association with PFAS while adjusting for the following factors: maternal age, age of child, and sex of the child. From previous literature, maternal age, age of child, and biological sex were considered closely associated with immune function and response and therefore included in the multivariate analyses (Weissenbacher et al., 2012; Simon et al., 2015; Giefing-Kröll et al., 2015; Boef et al., 2018). The parturition age was categorized into maternal

age groups of age <30 and age ≥ 30. Age 30 was chosen as previous literature has found that women ages 30 and older are faced with increased risks during pregnancy (Carolan and Frankowska, 2011). Advanced maternal age for high-risk pregnancy is defined at age 35 and older (Kenny et al., 2013). Due to the limited data size, setting the age at 35 instead of 30 would not have given an even distribution in sample size for further analyses. Sex was another covariate considered as previous studies have observed sex differences in immune responses and innate immune activation in humans and other mammals (Klein and Flanagan, 2016; Rooney et. al., 2003; Furman et al., 2014).

Sensitivity Analyses

According to the United States Centers for Disease Control and Prevention (US CDC), the DTaP (Diphtheria toxoid—Tetanus toxoid—acellular Pertussis) vaccination is administered to children via 5 recommended doses. The first three doses are given at 2, 4, 6 months; the fourth dose is given between 15 and 18 months old, and the fifth dose is given between 4 and 6 years old to protect them against diphtheria, tetanus, and acellular pertussis (Tartof et. al., 2013; Clark and Bobo, 2012). Given the ages of children included in our study, some may have received a booster vaccine just prior to the serum collection. To address booster vaccines, the age of the child was categorized into two groups: ≤ age 4 and > age 4. These designations were based on the DTaP timeline. Since Children are given their fifth DTaP booster dose between the ages of 4 and 6 and setting the cutoff age to 4 provided a more even distribution in sample size for sensitivity analyses (Tartof et. al., 2013; Clark and Bobo, 2012). At this age, it was hypothesized that those four years of age and younger would have lower antibody titers than those older than four years of age who more recently received a booster vaccine.

RESULTS AND DISCUSSION

Table 1 provides information on the demographics of the 47-participant mother-child pairs included in this study. A majority of the children (70.5%) were carried to full term defined at ≥ 39 weeks' gestation (Greene, 2018). The majority of the children were delivered at normal birthweight, defined at ≥ 2500g (Sin et al., 2004). The average age of the children in this study was 4.26 years old and the average age of the mother at delivery (parturition age in years) was 30 years old. More than half of the mothers in this study were non-smokers (90.9%), obtained at least a college degree (53.2%), and were not first-time mothers (61.4%). Mothers were predominantly non-Hispanic Black (46.8%) and non-Hispanic White (38.3%).

Serum PFAS

Five PFAS were included in this study because they were the most commonly detected in this cohort: n-PFOA (linear isomer), n-PFOS (linear isomer), PFHxS, PFNA, and PFDeA. Serum PFOS and PFOA were the highest in both children and mothers (Table 2 and 3; Fig. A1). Pairwise comparisons using Wilcoxon Rank Sum test determined average serum PFOS, PFNA, and PFDeA were significantly different ($p<0.05$) between mothers and children (Fig. A1). Average serum PFOS, PFNA, and PFDeA were respectively 1.89 times, 1.7 times, and 1.56 times higher in mothers than in children. While previous studies have found that children have higher estimated daily intake of PFAS compared to their mothers, the higher maternal PFAS measurements may be characteristic of the subsample of this TESIE cohort or due to other confounding factors associated with maternal age or exposure differences (Winkens et al., 2017). In 2006 and 2015, PFOS and PFOA were respectively eliminated from production and use in the United States (Pontius, 2019). The wane of PFOS and PFOA in industrial uses has led to a decline in serum PFOA and PFOS, which may also explain the higher serum PFOS in mothers

who were exposed to more sources of PFOS for potentially longer durations than their children (Fitz-Simon et al., 2013). Among maternal serum PFAS, PFHxS measurements in mothers of males were 1.76 times higher ($p<0.05$) than those in mothers of females (Table 3). This observation has not been seen in other studies and may be confounded by other variables associated with maternal serum PFAS such as differences in breastfeeding duration (Mogensen et al., 2015). Furthermore, due to the limited statistical power of the small sample size in this study, results should be interpreted with caution. The average children's serum PFAS measurements in this study are comparable to the 2013-2014 National Health and Nutrition Examination Survey (NHANES) serum PFAS measurements of children between the ages of 3 and 5 (Table 2; Ye et al., 2018). The 2013-2014 NHANES measurements provide an approximate measure for PFAS exposure in the general US children's population between the ages of 3 and 5 (Ye et al., 2018). This suggests that our results are relevant for the US general population of children approximately between the ages of 3 and 6.

Serum Antibody Titers

The average serum tetanus antibody titer in children was 1.80 IU/mL and the average serum diphtheria antibody titer in children was 2.83 IU/mL (Table 4). Serum antibody titers were analyzed by their vaccine protection thresholds. Vaccine titers equal to or greater than 0.1 IU/mL are considered to be fully seroprotective against diphtheria while vaccine titers equal to or greater than 0.15 IU/mL are considered to be adequately seroprotective against tetanus (McQuillan et al., 2002; Maple et al., 2000; Schauer et. al., 2003). Antibody concentrations ≥ 1.0 IU/mL have also been found to provide long-term protection against diphtheria and tetanus toxoids (Petráš et al., 2019; Basta et al., 2015). For this study, diphtheria antibody concentrations diphtheria vaccine protection levels were based on the following diphtheria antibody

concentrations: booster recommended (<0.1 IU/mL), full protection (0.1-1.0 IU/mL), and long-term protection (≥ 1.0 IU/mL). Similarly, tetanus vaccine protection levels were based on the following tetanus antibody concentration ranges: full protection (0.15-1.0 IU/mL) and long-term protection (≥ 1.0 IU/mL). Overall, 53.2% of the children had antibody titers that afforded full protection against diphtheria and 29.8% that attained long-term protection (Table 4 and 5). However, 17.0% of the children had antibody titers below the seroprotective level of 0.1 IU/mL and recommended for a booster against diphtheria, corresponding with the recommendations for children between ages four and six (Table 4 and 5). All participants had seroprotective antibody titers against tetanus with 23.4% achieving full protection (0.15 IU/mL to 1.0 IU/mL) and 76.6% achieving long-term protection (Table 5).

Serum antibody titers were also analyzed for potential differences in fetal sex and age of the child. Between males and females, there were no significant differences in serum antibody concentrations for tetanus and diphtheria and no significant sex differences in vaccine protection levels for tetanus and diphtheria (Table 4). Significant differences in serum antibody concentrations and vaccine protection thresholds for tetanus and diphtheria were associated with age of the child categorized as children ≤ age 4 and children > age 4 (Table 5). Antibody titers for tetanus and diphtheria were significantly ($p<0.05$) higher in children older than four years of age. As previously mentioned, age 4 was used as an approximate measure for age of child at their fifth DTaP dose. These findings suggest that the age 5 DTaP booster affords children > age 4 additional protection against diphtheria and tetanus.

Prenatal and Early Childhood PFAS Exposure and Antibody Titers

Univariate correlations between maternal serum PFAS and vaccine titer responses for diphtheria and tetanus in children were non-significant and weak (Fig. A2-A3). Univariate

correlation analyses also found non-significant and weak correlations between serum PFAS in children and their vaccine titer responses (Fig. A4-A5). Multiple regression analyses, adjusted for age of child (grouped by children ≤ age 4 and children > age 4), biological sex of child, and maternal age (maternal age grouped by age <30 and age ≥ 30), were conducted to assess the associations between serum PFAS and vaccine response. From the multiple regression analyses, maternal serum PFAS were not found to be significantly associated with children's antibody titers against tetanus (Table 7). However, maternal serum PFNA, child's age group, child's biological sex, and maternal age group were found to be significantly and positively associated with children's antibody titers against diphtheria (Table 6). A one percent increase in maternal serum PFNA was associated with a 1.4% increase in diphtheria antibody titer (Table 6). All other variables constant in the PFNA regression model, there is an expected 75 (95% CI [21, 93]) percent decrease in children's mean antibody titers against diphtheria from mothers ≥ 30 years old to mothers < 30 years old (Table 6). For the maternal serum PFAS regression models, child's age group was a significant predictor ($p<0.05$) of diphtheria for all five PFAS (Table 6). There was no significant association between children's serum PFAS and children's antibody titers against diphtheria; however, child's age group was a significant predictor for children's antibody titers against diphtheria (Table 8). When adjusted for child's age, child's biological sex, and maternal age, a one percent increase in children's serum PFOA was associated with a 0.51% increase in tetanus antibody titer (Table 9).

These results differ from previous studies evaluating immune response to vaccines in children and PFAS exposure. In particular, Grandjean et al found that diphtheria and tetanus antibody concentrations were negatively associated with serum PFAS in mothers and children (Grandjean et al., 2012). Other studies have found inverse relationships between maternal serum

PFAS and other antibody concentrations against Rubella and Hand Foot Mouth Disease (HFMD) (Granum et al., 2013; Zeng et al., 2019). This study did not find serum PFAS in children and mothers to be negatively associated with children's anti-diphtheria and anti-tetanus toxoid antibody concentrations.

There is not sufficient evidence in this study to suggest that perinatal and early life exposures to PFAS are associated with compromised responses to the DTaP vaccine. Furthermore, this study has multiple limitations, and the results should be interpreted with this in context. This study provided continuous measurements for serum vaccine antibody concentrations and serum PFAS levels from samples taken at one time point. The constraint of a single time point measurement for serum PFAS and serum antibody titers is it hinders the ability to assess exposure duration or immune response over a period of time. In contrast, Grandjean et al assessed associations between serum PFAS and antibody concentrations over multiple time points (Grandjean et al., 2012; Grandjean et al., 2017). Furthermore, in this study, the date of the fifth dose of the DTaP vaccination was not available for analyses; Knowing when each child received their fifth DTaP dose could have provided further insight for stratified analyses of PFAS exposure and vaccine response. The small sample size in this study (n=47 pairs) also reduced the statistical power of the analyses. In comparison to this study, Grandjean et al evaluated associations of more robust sample sizes of 587 children in the Faro Islands birth cohort and 349 children in a follow up study on PFAS exposure and antibody response (Grandjean et al., 2012; Grandjean et. al., 2017).

It is also possible that the results of this study are confounded by other factors influencing environmental PFAS exposures and antibody responses that were not accounted for here such as geographical location and diet. The primary PFAS exposure routes differed between participants

from this study and those from the Faroe Island birth cohort studies. The Faroe Islands birth cohort is primarily a fishing community in Denmark, where the authors noted seafood diets may serve as a predominant exposure source to PFAS, whereas the TESIE cohort was based in a suburban-urban community in central North Carolina and exposures may be dominated by other sources rather than diet (Grandjean et al., 2012). This is further suggested by the differences in the maternal and children serum PFAS concentrations between the two cohorts. The Faroe Island birth cohort reported average maternal serum PFOS and PFOA measurements that were respectively, 5.4 times and 1.7 times higher than those in this study from the TESIE cohort (Grandjean et al., 2012). The children's average serum PFOS levels and PFOA levels from the Faroe Island birth cohort study were approximately 6.2 times and 2.2 times higher than the average children serum levels measured in this study (Grandjean et al., 2012).

Vaccine-elicited antibody responses can also vary greatly based on behavioral, environmental, and intrinsic factors, among many others (Zimmermann and Curtis, 2019). While this study explored some intrinsic, maternal host, and behavioral/lifestyle factors, there are a myriad of other factors that were collected for consideration that are associated with immune responses to vaccinations (Zimmerman and Curtis, 2019). The significant association observed with maternal serum PFNA and children's diphtheria titers and significant association observed with children's serum PFOA and children's tetanus titers may be attributed to other variables.

Factors involved with vaccine administration, vaccine type, breastfeeding, and other measures of nutrition and diet may influence immune system responses and development as well as exposures to PFAS (Zimmermann and Curtis, 2019; Papadopoulou et al., 2016; Chandra, 2002; Raqib et al., 2007). These other factors may be attributed to the observed positive association between the tetanus antibody titer and children's serum PFOA (Table 6 and Table 9).

For instance, vaccine administration schedules for diphtheria and tetanus are different between the United States and Denmark. The children from the Faroe Islands cohort were given four doses of the diphtheria and tetanus vaccine at 3 months, 5 months, 12 months, and 5 years old, while children from the TESIE cohort were given five doses of the DTaP vaccine, with the initial three doses given at 2 months, 4 months, and 6 months (Grandjean et al., 2012; Tartof et al., 2013). It is important to note that besides the difference in vaccination schedule, the individual doses may also differ between these countries and this may influence the timeline of antibody production. The fifth dose of the

DTaP vaccination for children in the United States may confound associations between PFAS exposure and vaccine response as the dose may produce sufficient antibody responses that cannot be explained by factors such as PFAS exposure (Tartof et al., 2013).

PFAS have also been detected in breastmilk and can act as an elimination pathway for PFAS in breastfeeding mothers (Mogensen et al., 2015). Papadopoulou et al (2016) found that breastfed toddlers had higher serum PFAS concentrations than their mothers. With each month of breastfeeding, the toddler's PFAS concentrations increased between three to six percent (Papadopoulou et al., 2016). Furthermore, growing bodies of literature have found that breastmilk imparts protective antibodies to breastfed children and may confound early life immune responses, though IgG antibodies are not well absorbed through the gut (Hanson and Korotkova, 2002; Hanson, 1998). However, accounting for breastfeeding history may also be important in future studies assessing antibody responses to vaccinations and PFAS exposure during early childhood.

According to the US CDC, the DTaP vaccine is highly efficacious and therefore sensitive immune effects may not be observed in children who have completed the five doses of the DTaP

vaccine (US Centers for Disease Control and Prevention, 2020). It is also important to note that this study did not look at immunity to other infectious diseases such as acellular Pertussis, which is also provided in the DTaP vaccine as well as other vaccine types. Previous studies have found that pertussis immunity decreases following the fifth dose of the DTaP vaccine (Tartof et al., 2013; Klein et al., 2012). Waning immunity against acellular pertussis associated with the DTaP is not well understood and may be associated with environmental exposures to PFAS during early life (Klein et al., 2012). This is an area that requires further investigations into correlations between serum PFAS concentrations and immune responses to other antibody types.

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Table 1. Characteristics of the children from the TESIE study who contributed to antibody serum measurements

	Male (n=28)	Female (n=19)	Total (n=47)	p-value[‡]
Maternal Factors				
Parturition age in years				0.027[*]
Mean (SD)	28.59 (5.28)	32.33 (6.34)	30.09 (5.95)	
Missing	1	1	2	
Maternal age groups				0.002[**]
Maternal Age < 30	19 (70.4%)	4 (22.2%)	23 (51.1%)	
Maternal Age ≥ 30	8 (29.6%)	14 (77.8%)	22 (48.9%)	
Missing	1	1	2	
Race/Ethnicity, n (%)				0.697
Non-Hispanic White	9 (32.1%)	9 (47.4%)	18 (38.3%)	
Non-Hispanic Black	15 (53.6%)	7 (36.8%)	22 (46.8%)	
Hispanic	3 (10.7%)	2 (10.5%)	5 (10.6%)	
Other	1 (3.6%)	1 (5.3%)	2 (4.3%)	

Education, n (%)				0.595
< college graduate	14 (50.0%)	8 (42.1%)	22 (46.8%)	
≥ college graduate	14 (50.0%)	11 (57.9%)	25 (53.2%)	
Smoking status, n (%)				0.557
Non-smoker	24 (88.9%)	16 (94.1%)	40 (90.9%)	
Current smoker	3 (11.1%)	1 (5.9%)	4 (9.1%)	
Missing	1	2	3	
Parity, n (%)				0.977
0	10 (38.5%)	7 (38.9%)	17 (38.6%)	
1	16 (61.5%)	11 (61.1%)	27 (61.4%)	
Missing	2	1	3	
Gestational period in weeks				0.057
Median (Q1, Q3)	39.29 (38.07, 40.00)	40.21 (39.04, 40.68)	39.64 (38.39, 40.29)	
IQR	1.93	1.64	1.89	
Missing	2	1	3	
Child Factors				
Birth status, n (%)				0.292
Pre-term (< 37 weeks)	4 (15.4%)	1 (5.6%)	5 (11.4%)	
Early-term (37-38 weeks)	6 (23.1%)	2 (11.1%)	8 (18.2%)	
Full-term (≥ 39 weeks)	16 (61.5%)	15 (83.3%)	31 (70.5%)	
Missing	2	1	3	
Birth weight in g				0.534
Median (Q1, Q3)	3200 (2948, 3521)	3350.00 (3040, 3462.5)	3245.00 (2954, 3470)	
IQR	573.25	422.50	516.00	
Missing	2	4	6	
Birth weight status, n (%)				0.172
Low birthweight (<2500g)†	3 (11.5%)	0 (0.0%)	3 (7.3%)	
Normal birthweight (≥2500g)	23 (88.5%)	15 (100.0%)	38 (92.7%)	
Missing	2	4	6	
Age in years				0.082
Mean (SD)	4.38 (0.44)	4.10 (0.52)	4.26 (0.49)	
Age groups in years, n (%)				0.027*
≤ age 4	6 (21.4%)	10 (52.6%)	16 (34.0%)	
> age 4	22 (78.6%)	9 (47.4%)	31 (66.0%)	

Note: ‡ p-values assessing for fetal sex differences for continuous variables are from Kruskal-Wallis tests and p-values for categorical variables are based on Pearson's Chi-Squared Test. † Low birth weight status based on the World Health Organization threshold of <2500g (World Health Organization, 2014) * p-value<0.05; ** p-value<0.01

Table 2. Descriptive statistics of PFAS (ng/mL) in children's serum samples categorized by fetal sex

	Male (n=28)	Female (n=19)	Total (n=47)	p-value[1]
Children's serum PFAS concentrations (ng/mL)				
PFOS				0.66
Mean (95% CI)	2.84 (2.06, 3.61)	2.45 (1.68, 3.21)	2.68 (2.14, 3.22)	
Median (Q1, Q3)	2.55 (1.25, 3.90)	2.20 (1.45, 2.80)	2.40 (1.35, 3.20)	
IQR	2.65	1.35	1.85	
Min - Max	0.60 - 8.20	1.00 - 7.90	0.60 - 8.20	
PFOA				0.53
Mean (95% CI)	1.78 (1.41, 2.14)	2.00 (1.53, 2.47)	1.87 (1.59, 2.15)	
Median (Q1, Q3)	1.70 (0.85, 2.35)	1.60 (1.20, 2.75)	1.70 (1.20, 2.65)	
IQR	1.50	1.55	1.45	
Min - Max	0.40 - 3.70	0.60 - 3.90	0.400 - 3.90	
PFHxS				0.53
Mean (95% CI)	1.35 (0.55, 2.16)	0.76 (0.56, 0.95)	1.11 (0.63, 1.59)	
Median (Q1, Q3)	0.75 (0.48, 1.05)	0.60 (0.55, 0.90)	0.70 (0.50, 1.00)	
IQR	0.58	0.35	0.50	
Min - Max	0.20 - 10.50	0.30 - 2.00	0.20 - 10.50	
PFNA				0.23
Mean (95% CI)	0.47 (0.37, 0.57)	0.57 (0.43, 0.72)	0.50 (0.429, 0.592)	
Median (Q1, Q3)	0.40 (0.30, 0.50)	0.50 (0.40, 0.70)	0.40 (0.30, 0.60)	
IQR	0.20	0.30	0.30	
Min - Max	0.10 - 1.10	0.20 - 1.40	0.10 - 1.40	
PFDeA				0.33
Mean (95% CI)	0.20 (0.11, 0.28)	0.17 (0.07, 0.26)	0.18 (0.12, 0.24)	
Median (Q1, Q3)	0.15 (0.05, 0.23)	0.05 (0.05, 0.25)	0.10 (0.05, 0.25)	
IQR	0.18	0.20	0.20	
Min - Max	0.05 - 1.10	0.05 - 0.80	0.05 - 1.10	

Note: 1. p-values from Kruskal-Wallis Tests comparing children's average PFAS by fetal sex; linear PFOS (perfluorooctane sulfonate); linear PFOA (perfluorooctanoate); PFHxS (perfluorohexane sulfonate); PFNA (perfluorononanoate); PFDeA (perfluorodecanoate)

Table 3. Descriptive statistics of PFAS (ng/mL) in maternal serum samples categorized by fetal sex

	Male (n=28)	Female (n=19)	Total (n=47)	p-value[1]
Maternal serum PFAS concentrations (ng/mL)				
PFOS				0.07
Mean (95% CI)	5.58 (4.60, 6.56)	4.32 (3.20, 5.44)	5.07 (4.34, 5.81)	
Median (Q1, Q3)	5.85 (3.80, 7.10)	4.00 (2.50, 5.55)	4.90 (2.95, 6.40)	
IQR	3.30	3.05	3.45	
Min - Max	1.00 - 12.90	1.60 - 9.80	1.00 - 12.90	
PFOA				0.36
Mean (95% CI)	1.95 (1.59, 2.32)	1.82 (1.19, 2.45)	1.90 (1.58, 2.22)	
Median (Q1, Q3)	1.75 (1.18, 2.58)	1.70 (1.00, 2.10)	1.70 (1.05, 2.30)	
IQR	1.40	1.10	1.25	
Min - Max	0.80 - 3.80	0.60 - 6.40	0.60 - 6.40	
PFHxS				0.03*
Mean (95% CI)	1.30 (0.93, 1.66)	0.74 (0.59, 0.90)	1.07 (0.84, 1.31)	
Median (Q1, Q3)	1.00 (0.68, 1.48)	0.70 (0.45, 1.00)	0.80 (0.50, 1.20)	
IQR	0.80	0.55	0.70	
Min - Max	0.40 - 3.60	0.20 - 1.30	0.20 - 3.60	
PFNA				0.59
Mean (95% CI)	0.84 (0.73, 0.96)	0.85 (0.66, 1.05)	0.85 (0.75, 0.95)	
Median (Q1, Q3)	0.80 (0.68, 1.00)	0.70 (0.60, 1.00)	0.80 (0.60, 1.00)	
IQR	0.33	0.40	0.40	
Min - Max	0.40 - 1.50	0.40 - 2.10	0.40 - 2.10	
PFDeA				0.61
Mean (95% CI)	0.28 (0.21, 0.35)	0.28 (0.22, 0.34)	0.28 (0.23, 0.33)	
Median (Q1, Q3)	0.20 (0.20, 0.33)	0.30 (0.20, 0.35)	0.30 (0.20, 0.35)	
IQR	0.13	0.15	0.15	
Min - Max	0.05 - 1.00	0.05 - 0.60	0.05 - 1.00	

Note: 1. p-values from Kruskal-Wallis Tests were used to compare average maternal PFAS by fetal sex; * denotes p-value<0.05; n-PFOS (linear perfluorooctane sulfonate); n-PFOA (linear perfluorooctanoate); PFHxS (perfluorohexane sulfonate); PFNA (perfluorononanoate); PFDeA (perfluorodecanoate)

Table 4. Antibody concentration comparisons (IU/mL) between male and female participants

	Male (n=28)	Female (n=19)	Total (n=47)	p-value†
IgG antibody concentration in serum (IU/mL)				
Diphtheria				0.795
Mean (SD)	1.92 (3.19)	1.63 (3.00)	1.80 (3.08)	
Median (Q1, Q3)	0.32 (0.14, 3.03)	0.34 (0.10, 1.04)	0.34 (0.13, 2.18)	
IQR	2.89	0.93	2.04	
Tetanus				0.051
Mean (SD)	3.28 (2.23)	2.17 (2.03)	2.83 (2.20)	
Median (Q1, Q3)	2.90 (1.84, 4.03)	1.55 (0.80, 2.62)	2.31 (1.06, 3.73)	
IQR	2.20	1.82	2.66	

	Male (n=28)	Female (n=19)	Total (n=47)	p-value†
Vaccine protection level, n (%)				
Diphtheria				0.865
booster recommended	5 (17.9%)	3 (15.8%)	8 (17.0%)	
full protection	14 (50.0%)	11 (57.9%)	25 (53.2%)	
long-term protection	9 (32.1%)	5 (26.3%)	14 (29.8%)	
Tetanus				0.276
full protection	5 (17.9%)	6 (31.6%)	11 (23.4%)	
long-term protection	23 (82.1%)	13 (68.4%)	36 (76.6%)	

Note: † p-values are from Kruskal-Wallis Tests comparing diphtheria antibody titer (IU/mL) and tetanus antibody titer (IU/mL) by fetal sex. The p-values are from Pearson's Chi-square Test comparing the diphtheria and tetanus protection levels by fetal sex. *p-value<0.05.

Table 5. Antibody concentration comparisons (IU/mL) between participants ≤ age 4 and > age 4

	≤ age 4 (n=16)	> age 4 (n=31)	Total (n=47)	p-value†
Antibody concentrations in serum (IU/mL)				
Diphtheria				0.016*
Mean (SD)	0.72 (1.91)	2.36 (3.44)	1.80 (3.08)	
Median (Q1, Q3)	0.18 (0.04, 0.43)	0.59 (0.19, 3.18)	0.34 (0.13, 2.18)	
IQR	0.39	2.99	2.04	
Tetanus				0.014*
Mean (SD)	1.85 (1.81)	3.34 (2.24)	2.83 (2.20)	
Median (Q1, Q3)	1.21 (0.73, 2.46)	2.83 (1.69, 4.25)	2.31 (1.06, 3.73)	
IQR	1.74	2.55	2.66	

	≤ age 4 (n=16)	> age 4 (n=31)	Total (n=47)	p-value†
Vaccine protection level, n (%)				
Diphtheria				0.020*
booster recommended (<0.1IU/mL)	5 (31.2%)	3 (9.7%)	8 (17.0%)	
full protection (0.1-1.0 IU/mL)	10 (62.5%)	15 (48.4%)	25 (53.2%)	
long-term protection (≥ 1.0 IU/mL)	1 (6.2%)	13 (41.9%)	14 (29.8%)	
Tetanus				0.018*
full protection	7 (43.8%)	4 (12.9%)	11 (23.4%)	
long-term protection	9 (56.2%)	27 (87.1%)	36 (76.6%)	

Note: † p-values are from Kruskal-Wallis Test comparing diphtheria titer (IU/mL) and tetanus titer (IU/mL) by approximated booster vaccination age (age 4). The p-values are from Pearson's Chi-square Test comparing diphtheria protection level and tetanus protection level by approximated booster vaccination age. *p-value<0.05.

Table 6. Maternal PFAS Linear regression models based on the dependent variable: Natural log-transformed serum diphtheria antibody concentrations (IU/mL)

Model	**PFOS**			**PFOA**			**PFHxS**			**PFNA**			**PFDeA**		
Predictors for Diphtheria[§]	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value
Ln(maternal PFAS)	0.63	(-0.31, 1.6)	0.18	0.33	(-0.62, 1.3)	0.49	0.61	(-0.24, 1.5)	0.15	1.4	(0.06, 2.8)	**0.041***	0.47	(-0.37, 1.3)	0.27
Sex															
Female	1.2	(0.00, 2.4)	0.051	1.1	(-0.14, 2.3)	0.081	1.3	(0.07, 2.6)	**0.039***	1.2	(0.02, 2.3)	**0.046***	1.1	(-0.07, 2.3)	0.064
Child's age group															
> age 4	1.3	(0.15, 2.5)	**0.028***	1.4	(0.21, 2.6)	**0.022***	1.6	(0.35, 2.8)	**0.013***	1.3	(0.12, 2.4)	**0.032***	1.5	(0.29, 2.7)	**0.017***
Maternal age group															
Maternal Age ≥ 30	-1.2	(-2.3, 0.03)	0.057	-1.1	(-2.3, 0.13)	0.080	-1.0	(-2.2, 0.17)	0.091	-1.4	(-2.7, -0.24)	**0.020***	-1.3	(-2.5, -0.02)	**0.047***

[1]CI = Confidence Interval; The following models shows multiple linear regression analyses for the following continuous predictors for diphtheria antibody titers: natural log-transformed maternal serum PFAS: PFOS, PFOA, PFHxS, and PFNA, and PFDeA. [§]Each model contains the following categorical independent variables: biological sex of the child, child's age sorted into two age groups ≤ age 4 and > age 4, and maternal age sorted into two groups, mothers ages ≥ 30 and mothers ages < 30. *denotes a p-value <0.05.

Table 7. Maternal PFAS Linear regression models based on the dependent variable: Natural log-transformed serum tetanus antibody concentrations (IU/mL)

Model	**PFOS**			**PFOA**			**PFHxS**			**PFNA**			**PFDeA**		
Predictors for Tetanus[§]	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value
Ln(maternal PFAS)	0.25	(-0.22, 0.72)	0.29	0.16	(-0.31, 0.64)	0.50	0.20	(-0.23, 0.62)	0.36	0.47	(-0.22, 1.2)	0.18	0.24	(-0.18, 0.66)	0.26
Sex															
Female	0.04	(-0.57, 0.64)	0.90	-0.01	(-0.61, 0.59)	0.97	0.06	(-0.57, 0.69)	0.84	0.02	(-0.57, 0.60)	0.96	0.03	(-0.57, 0.62)	0.93
Child's age group															
> age 4	0.57	(-0.03, 1.2)	0.060	0.61	(0.00, 1.2)	**0.049***	0.65	(0.04, 1.3)	**0.038***	0.55	(-0.04, 1.1)	0.067	0.64	(0.04, 1.2)	**0.037***
Maternal age group															
Maternal Age ≥ 30	-0.36	(-1.0, 0.23)	0.23	-0.33	(-0.92, 0.27)	0.27	-0.31	(-0.90, 0.29)	0.30	-0.46	(-1.1, 0.16)	0.14	-0.43	(-1.0, 0.19)	0.17

[1]CI = Confidence Interval; The following models shows multiple linear regression analyses for the following continuous predictors for tetanus antibody titers: natural log-transformed maternal serum PFAS: PFOS, PFOA, PFHxS, and PFNA, and PFDeA. [§]Each model contains the following categorical independent variables: biological sex of the child, child's age sorted into two age groups ≤ age 4 and > age 4, and maternal age sorted into two groups, mothers ages ≥ 30 and mothers ages < 30. *denotes a p-value <0.05.

Table 8. Children's PFAS Linear regression models based on the dependent variable: Natural log-transformed serum diphtheria antibody concentrations (IU/mL)

Model	**PFOS**			**PFOA**			**PFHxS**			**PFNA**			**PFDeA**		
Predictors for Diphtheria[§]	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value
Ln(PFAS)	0.30	(-0.50, 1.1)	0.45	0.51	(-0.44, 1.5)	0.28	-0.12	(-0.79, 0.56)	0.73	0.46	(-0.52, 1.4)	0.35	-0.23	(-0.81, 0.35)	0.43
Sex															
Female	1.0	(-0.16, 2.2)	0.086	1.0	(-0.18, 2.2)	0.095	1.0	(-0.22, 2.2)	0.11	0.90	(-0.30, 2.1)	0.14	1.0	(-0.20, 2.2)	0.10
Child's age group															
> age 4	1.5	(0.24, 2.7)	**0.020***	1.3	(0.08, 2.5)	**0.037***	1.4	(0.18, 2.6)	**0.025***	1.3	(0.03, 2.5)	**0.045***	1.5	(0.26, 2.7)	**0.019***
Maternal age group															
Maternal Age ≥ 30	-1.1	(-2.3, 0.13)	0.080	-1.3	(-2.6, -0.02)	**0.046***	-1.0	(-2.2, 0.17)	0.089	-1.2	(-2.4, 0.05)	0.061	-1.1	(-2.3, 0.12)	0.078

[1]CI = Confidence Interval; The following models shows multiple linear regression analyses for the following continuous predictors for diphtheria antibody titers: natural log-transformed children's serum PFAS: PFOS, PFOA, PFHxS, and PFNA, and PFDeA. [§]Each model contains the following categorical independent variables: biological sex of the child, child's age sorted into two age groups ≤ age 4 and > age 4, and maternal age sorted into two groups, mothers ages ≥ 30 and mothers ages < 30. *denotes a p-value <0.05.

Table 9. Children's PFAS Linear regression models based on the dependent variable: Natural log-transformed serum tetanus antibody concentrations (IU/mL)

Model	**PFOS**			**PFOA**			**PFHxS**			**PFNA**			**PFDeA**		
Predictors for Tetanus[§]	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value	Beta	95% CI[1]	p-value
Ln(PFAS)	0.21	(-0.18, 0.61)	0.28	0.51	(0.06, 1.0)	**0.028***	0.18	(-0.15, 0.51)	0.29	0.32	(-0.16, 0.80)	0.19	-0.06	(-0.35, 0.23)	0.68
Sex															
Female	-0.02	(-0.60, 0.57)	0.95	-0.04	(-0.60, 0.52)	0.87	0.00	(-0.59, 0.59)	>0.99	-0.11	(-0.70, 0.48)	0.71	-0.04	(-0.64, 0.55)	0.88
Child's age group															
> age 4	0.64	(0.03, 1.2)	**0.039***	0.49	(-0.09, 1.1)	0.093	0.60	(0.00, 1.2)	0.051	0.50	(-0.10, 1.1)	0.10	0.62	(0.00, 1.2)	**0.049***
Maternal age group															
Maternal Age ≥ 30	-0.33	(-0.92, 0.26)	0.26	-0.56	(-1.2, 0.04)	0.068	-0.34	(-0.93, 0.25)	0.25	-0.40	(-1.0, 0.20)	0.19	-0.33	(-0.93, 0.27)	0.28

[1]CI = Confidence Interval; The following models shows multiple linear regression analyses for the following continuous predictors for tetanus antibody titers: natural log-transformed children's serum PFAS: PFOS, PFOA, PFHxS, and PFNA, and PFDeA. [§]Each model contains the following categorical independent variables: biological sex of the child, child's age sorted into two age groups ≤ age 4 and > age 4, and maternal age sorted into two groups, mothers ages ≥ 30 and mothers ages < 30. *denotes a p-value <0.05.

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APPENDIX:

Supplementary Figures

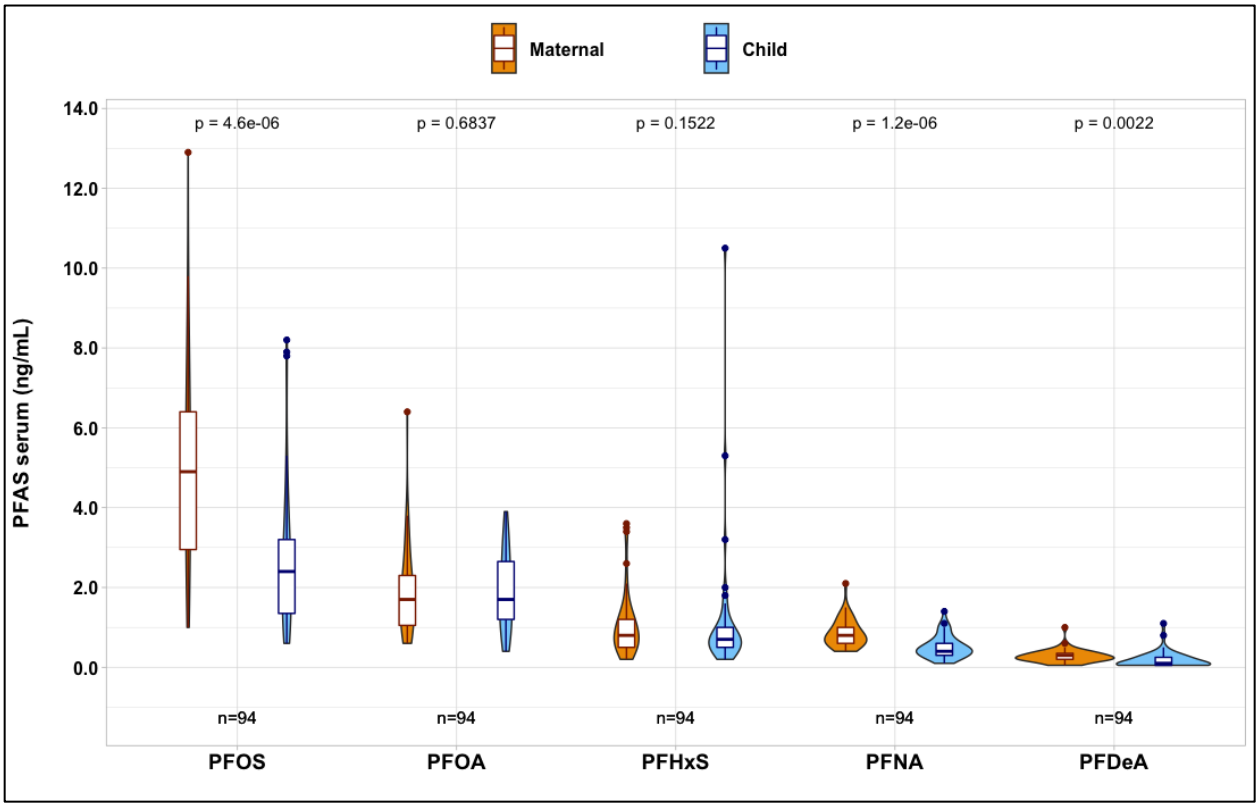

Figure A1. Maternal and children's serum PFAS concentration (ng/mL) distributions. *Note: The p-values are based on pairwise comparisons between mean maternal and children's serum PFAS concentrations using Wilcoxon rank sum test. A total of 94 serum samples from mothers (n=47) and children (n=47) were analyzed per PFAS.*

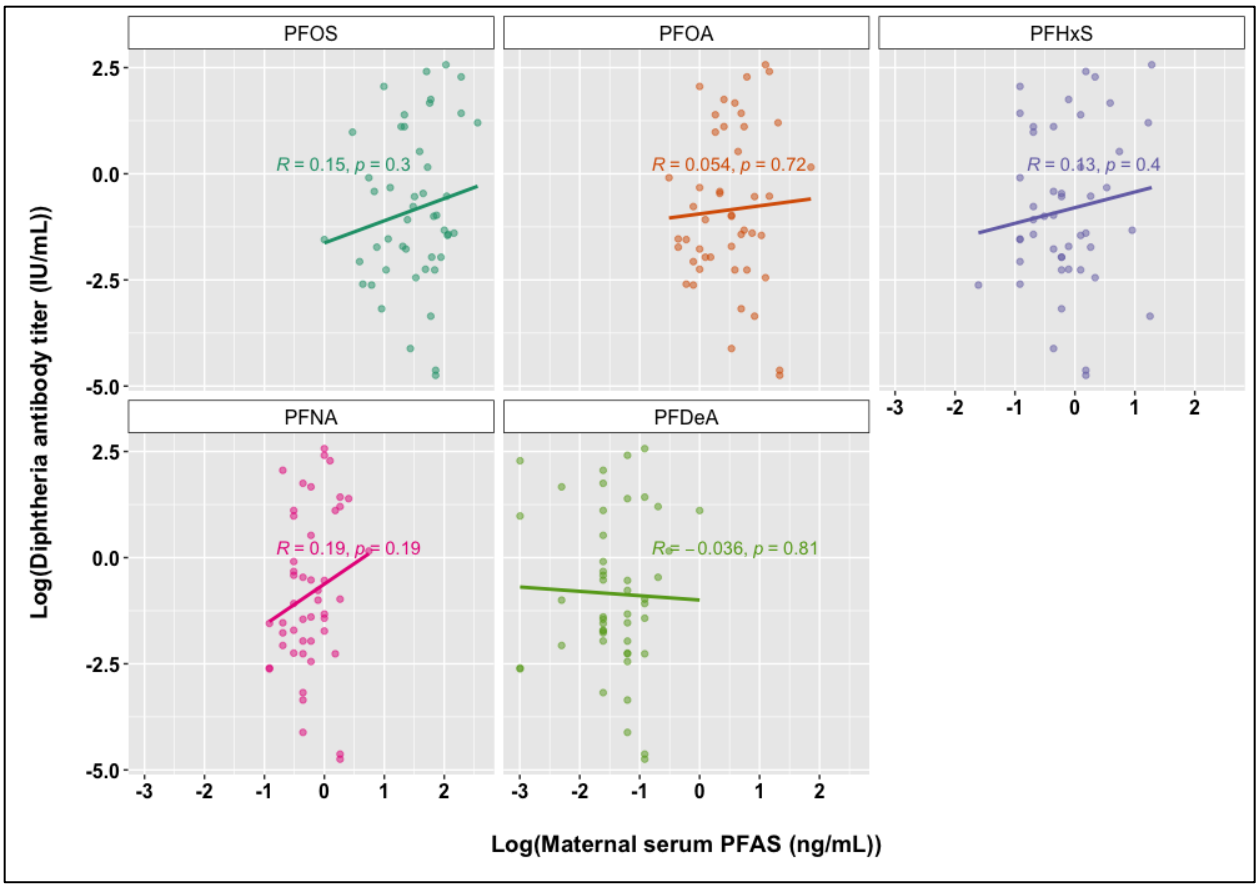

Figure A2. Correlation plots of natural log-transformed diphtheria antibody titer and natural log-transformed maternal serum PFAS concentrations. Pearson coefficient correlations, R, are reported.

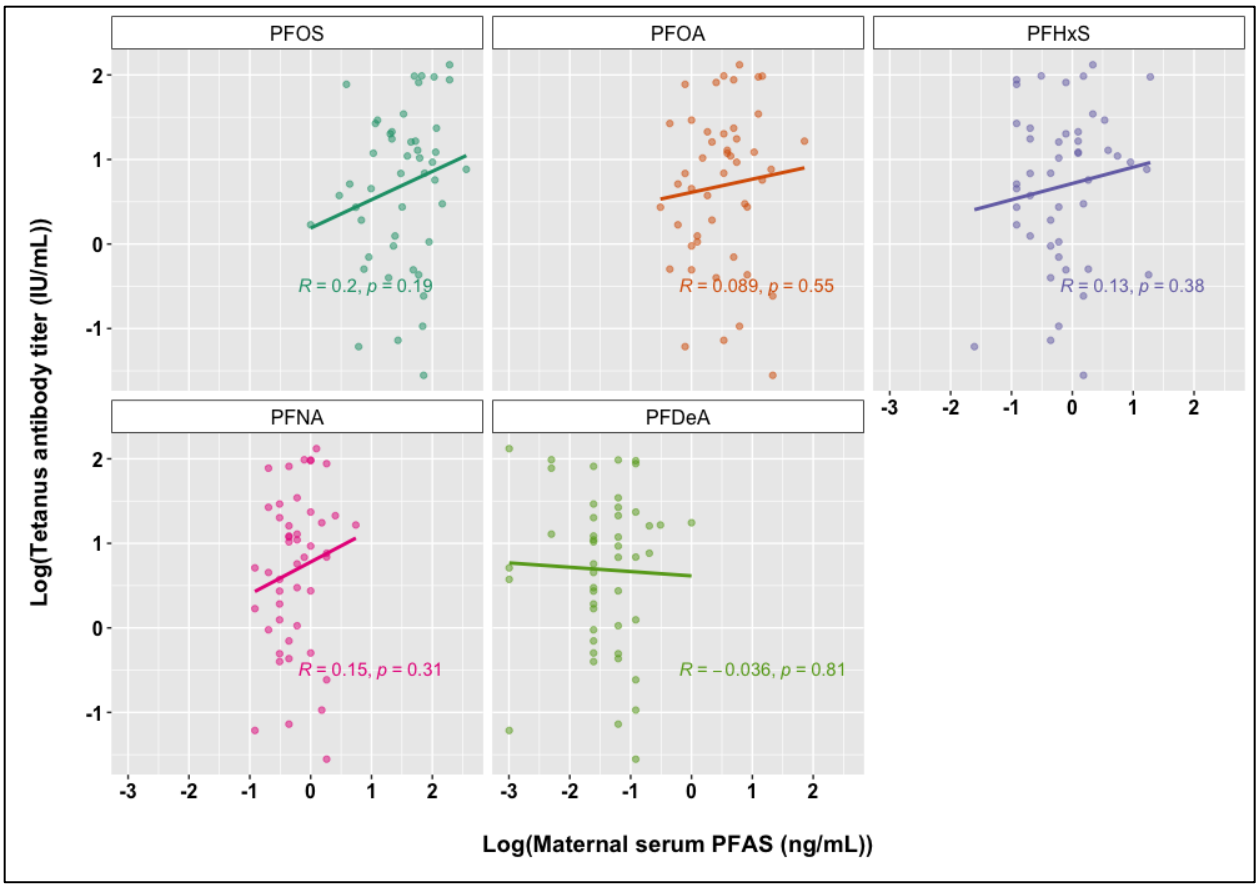

Figure A3. Correlation plots of natural log-transformed tetanus antibody titer and natural log-transformed maternal serum PFAS concentrations. Pearson coefficient correlations, R, are reported.

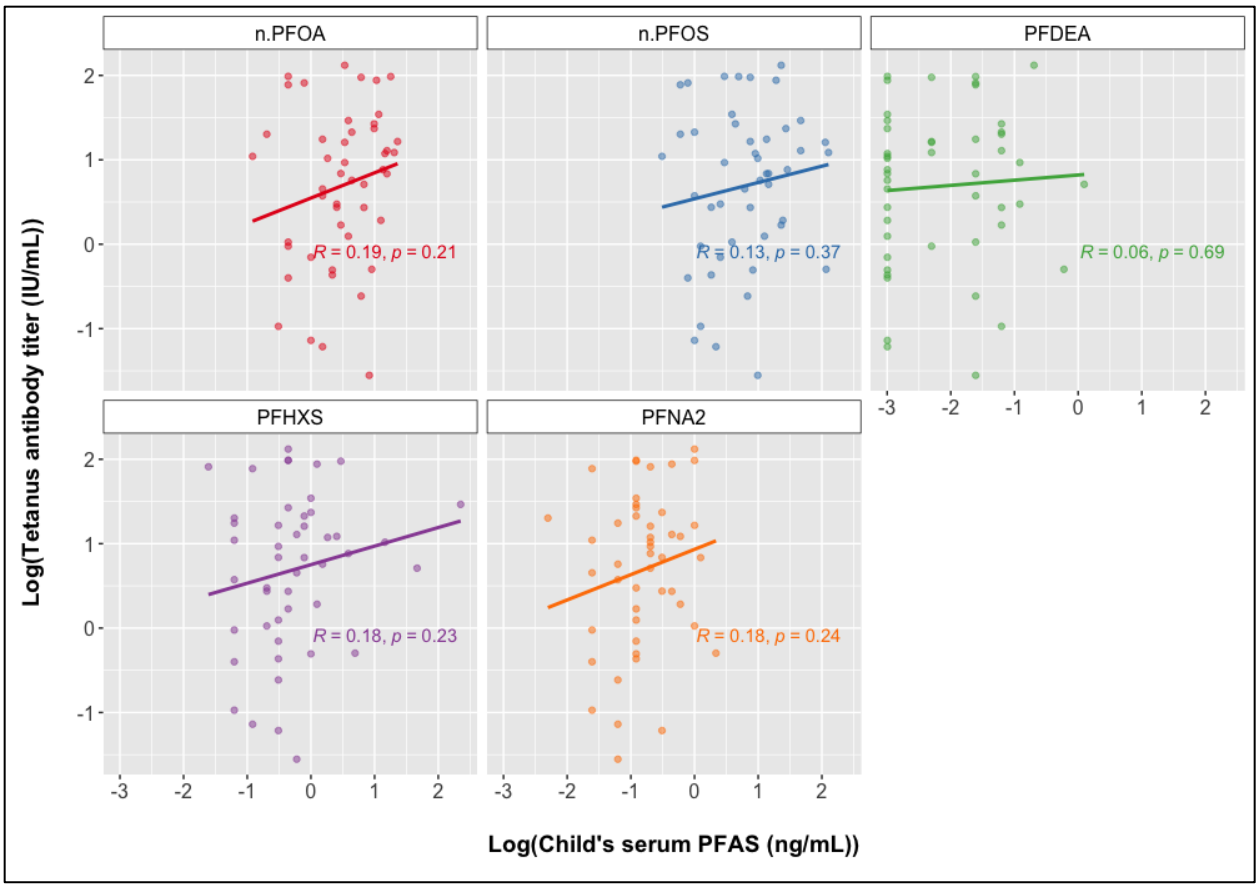

Figure A4. Correlation plots of natural log-transformed tetanus antibody titer and natural log-transformed children's serum PFAS concentrations. Pearson coefficient correlations, R, are reported.

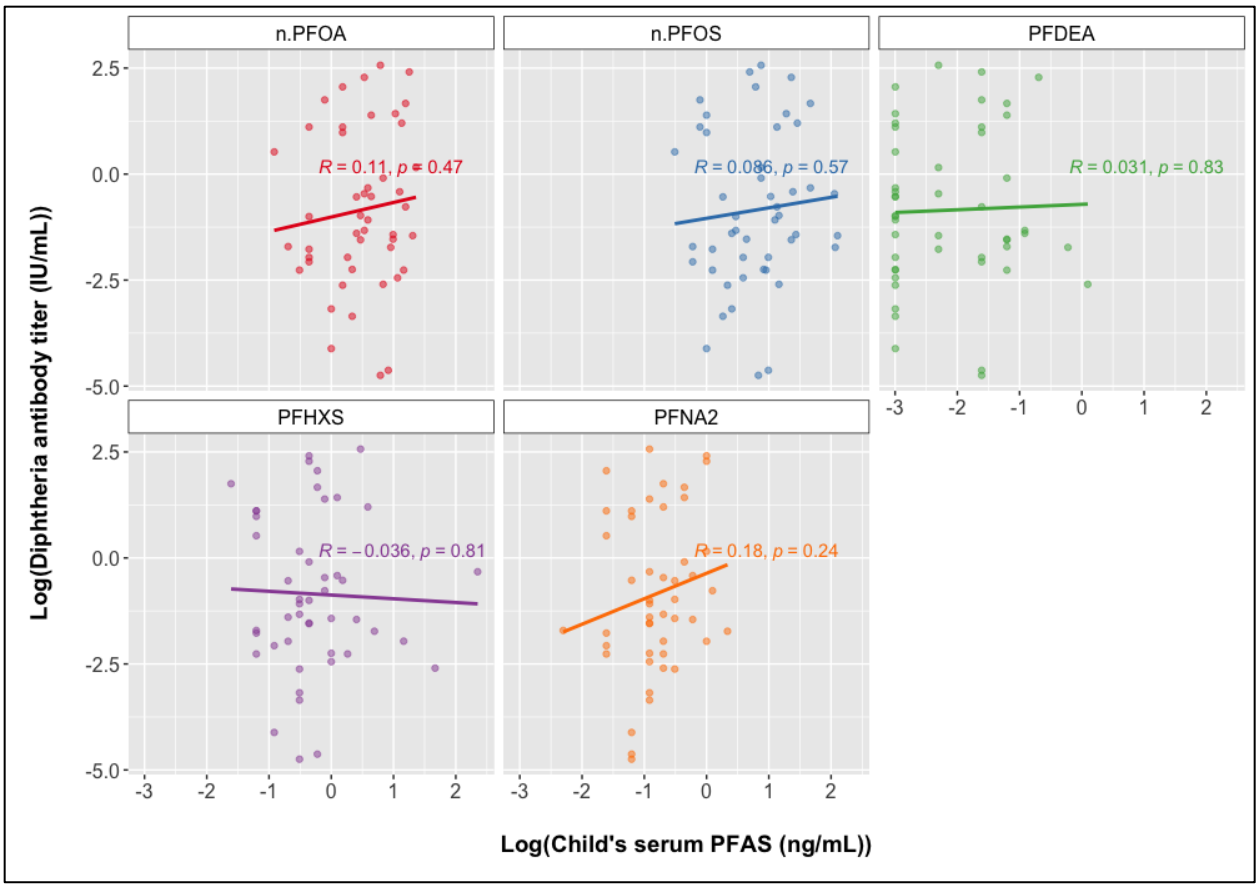

Figure A5. Correlation plots of natural log-transformed diphtheria antibody titer and natural log-transformed children's serum PFAS concentrations. Pearson coefficient correlations, R, are reported.