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Concentrations of tetanus and diphtheria antibodies in vaccinated Greenlandic children aged 7–12 years exposed to marine pollutants, a cross sectional study

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ABSTRACT

Previous studies have shown immunotoxic effects of environmental chemicals, and the European Food Safety Authority (EFSA) recently identified a need for more studies on PFAS immunotoxicity in different populations. In the Arctic, populations are exposed to several environmental chemicals through marine diet, and the objective of this study was therefore to examine the association between Greenlandic children's exposure to major environmental chemicals and their concentrations of diphtheria and tetanus vaccine antibodies after vaccination. The study includes cross-sectional data from Greenlandic children aged 7–12 years examined during 2012–2015. A total of 338 children were eligible for the study, and 175 of these had available vaccination records. A parent or guardian participated in a structured interview, and a blood sample from the child was analyzed for specific antibodies against diphtheria and tetanus as well as perfluoroalkyl substances (PFASs), polychlorinated biphenyls (PCBs) and total mercury. Furthermore, for a subgroup, blood samples from pregnancy were available and analyzed for environmental contaminants. The associations between the environmental exposures and antibody concentrations and odds of having antibody concentrations below the protective level were examined in linear and logistic regression models. In crude analyses, elevated concentrations of some of the contaminants were associated with higher concentrations of diphtheria and tetanus antibodies, but the associations were reversed when adjusting for area of residence, and duration of being breastfed and including children with a known vaccination date only. Each 1 ng/mL increase in serum concentrations of perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS) was associated with decreases of 78 % (95 % CI: 25–94 %) and 9 % (95 % CI: 2–16 %), respectively, in diphtheria antibody concentrations. Exposure to PCBs and all PFASs was associated with markedly increased odds of having diphtheria antibody concentrations below the protective level. For each 1 ng/mL increase in serum concentrations of PFHxS, PFOS, perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA), odds of not having protective levels of diphtheria antibodies were increased 6.44 times (95 % CI: 1.51–27.36), 1.14 times (95 % CI: 1.04–1.26), 1.96 times (95 % CI: 1.07–3.60), and 5.08 times (95 % CI: 1.32–19.51, respectively. No consistent associations were seen between maternal contaminant concentrations and vaccine antibody concentrations. In conclusion, we found that increased exposure to environmental chemicals among children in this Arctic population were associated with a decrease in post-vaccination antibody concentrations and with increased odds of not being protected against diphtheria despite appropriate vaccination. These findings emphasize the risk of environmental chemical exposures also in this Arctic population.

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1. Introduction

Through their diet and lifestyle, Arctic populations are exposed to multiple persistent environmental pollutants, including perfluoroalkyl substances (PFASs) and polychlorinated biphenyls (PCBs) as well as methylmercury (Bjerregaard et al., 2001; Johansen et al., 2000; Weihe et al., 2002) that are associated with adverse health effects (Arctic Monitoring and Ass, 2017).

Information from the health care system suggests elevated rates of infectious disease among children in Greenland (Kløvgaard et al., 2016), and in Arctic Canada, exposure to environmental chemicals have been linked to increased risk of infection (Dallaire et al., 2004, 2006), but the possible link between environmental exposures and immune system deficiency has not previously been examined in Greenland. Immune function tests are not easily applied in epidemiological studies, but responses to vaccinations in terms of concentrations of specific antibodies can be used as clinically relevant markers of immune function (Heilmann et al., 2020). This approach has been used to characterize immunotoxic effects of, e.g., PCBs and dioxins (Weisglas-Kuperus et al., 2000; Jusko et al., 2016; Heilmann et al., 2006, 2010) as well as PFASs (Grandjean et al., 2012, 2017a, 2017b; Timmermann et al., 2020; Granum et al., 2013; Stein et al., 2016; Pilkerton et al., 2018), and several studies have shown associations between increased exposure to environmental chemicals, especially PFAS, and decreased concentrations of vaccine antibodies (Heilmann et al., 2006, 2010; Grandjean et al., 2012, 2017a; Timmermann et al., 2020; Granum et al., 2013; Stein et al., 2016). However, sources of exposure varies across populations, and Greenlandic children are exposed to high concentration of environmental chemicals compared to other populations (Timmermann et al., 2019). The European Food Safety Authority (EFSA) recently reviewed the existing literature about PFAS related health effects and identified a need for more studies on immunotoxicity in different populations (Schrenk et al., 2020). Thus, in the present study, we aimed to explore whether the concentrations of diphtheria and tetanus vaccine antibodies in Greenland children were associated with their exposures to major environmental contaminants known to be immunotoxic.

2. Material and methods

The present study relies on clinical examinations of Greenlandic children at age 7–12 years in 2012–2015 (Timmermann et al., 2019). A total of 398 children were invited for clinical examination, and 367 children chose to participate (92 %). Among these, 241 children and their mothers had previously participated in the INUENDO cohort study (Hoyer et al., 2014), 81 of them had previously participated in the IVAAQ cohort study (Bjerregaard et al., 2007), and 45 in both studies.

The INUENDO cohort recruited 598 pregnant women from all regions of Greenland in 2002–2004. On average the women were included in gestational week 24 (25–75 percentiles: week 16.7–32.4) (Toft et al., 2005). The IVAAQ cohort study recruited 450 pregnant women from West Greenland in gestational week 26 during 1999–2005. All children from the two studies presently living in Maniitsoq and Sisimiut, (West coast, north of Nuuk), Ilulissat, Aasiaat, Qeqertarsuaq, Qasigiannguit (in the Disko Bay area), and Tasiilaq (East coast), were invited to participate in the present study, while children living in Nuuk were invited only until the point where the study had reached the required size (Timmermann et al., 2019). A parent or guardian was asked to participate in a structured health interview, which included questions about breastfeeding and socioeconomic status (SES), and the child underwent a physical examination and was asked to provide a blood sample for analyses. From the 367 participating children, blood samples were available from 338 (92 %), and among these, 314 had information about potential confounders (Fig. 1). In Greenland, children are routinely vaccinated against diphtheria and tetanus at ages 3, 5, and 12 months, with a booster at about 5 years. The date of the most recent diphtheria-tetanus vaccination was to the extent possible obtained from the children's vaccination cards or medical records. Vaccination records were, however, not available for 163 children. The overall vaccination rate among Greenlandic children is high. For children born between 2018 and 2019, the coverage of diphtheria and tetanus was 94.4 %, 88.4 %, 83.9 %, and 79.9 % at 3 months, 5 months, 12 months and 5 years, respectively (Albertsen et al., 2020). However, delays frequently occur, and children without a known booster date were assumed to have received their most recent vaccination at age 6 years (average booster age among those with a known date), unless it was known that the booster had not been administered, in which case the most recent vaccination was assumed to have been at 12 months of age. Of note, though C. tetani have been found in Greenlandic soil (Bjerregaard et al., 1986), tetanus have not been registered in the Greenlandic population, and booster vaccinations are therefore not applied at the hospital emergency rooms. Blood samples from pregnancy were available from 91 mothers, and 57 mother-child dyads had complete information on the children as well as blood samples from the mothers (Fig. 1).

2.1. Ethics

The study was performed in accordance with the Helsinki declaration. The Research Ethics Committee for Greenland and the Institutional Review Board of Harvard T.H. Chan School of Public Health (CR- 22769) approved the study protocol, and written informed consent was obtained from a parent/guardian to all children examined.

2.2. Assessment of vaccine antibodies

As previously (Heilmann et al., 2010; Grandjean et al., 2012), serum was analyzed for concentrations of specific antibodies at Statens Serum Institut (Copenhagen, Denmark) which had also produced the vaccines used in Greenland. The samples were analyzed in 2016. Antibodies against tetanus were measured using enzyme linked immunosorbent assay (Hendriksen et al., 1988), and antibodies against diphtheria were measured using a standard Vero cell-based neutralization assay using 2-fold dilutions of serum samples (Miyamura et al., 1974). Calibration was performed using international and local standard antitoxins.

2.3. Mercury, PCB, and PFAS exposure assessment

At University of Southern Denmark, serum samples from the children were analyzed for mercury, PCBs and PFASs. Whole-blood mercury as a marker of methylmercury exposure was measured in child samples with a sufficient volume of 100 μL on a Direct Mercury Analyzer system (DMA-80). The imprecision was less than 5 %. Seven PFASs, i.e. perfluorohexane sulfonic acid (PFHxS), perfluoroheptanesulfonic acid (PFHpS), perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUnDA) were quantified using on-line solid-phase extraction followed by liquid chromatography and triple quadropole mass spectrometry, and serum concentrations of major PCB congeners were determined by use of solid-phase extraction and isotope dilution as previously described (Timmermann et al., 2019). The between batch imprecision for PFASs was <5.2 %, and the limit of detection (LOD) was 0.03 ng/mL. The PCB concentration was expressed in relation to the total lipid concentration determined from the cholesterol and triglyceride contents. The three major PCB congeners CB-138, CB-153, and CB-180 account for approximately half of the total PCB concentration (Grandjean et al., 1995), and a simplified ΣPCB concentration was calculated as the sum of these three congeners multiplied by 2. The limit of detection (LOD) for PCB congeners in these analyses was 0.03 ng/mL, which corresponds to 0.003 μg/g lipid at an average serum-lipid concentration of 10 g/L (Petersen et al., 2006). The between batch imprecision for the PCBs were <10.9 %. All values below the LOD were replaced with the LOD/2. In a subgroup of mothers from the IVAAQ cohort, pregnancy serum was available and was analyzed for five

PFASs, PCBs and mercury using the same methods as described above.

2.4. Statistics

Pairwise correlations between environmental chemicals in child and maternal serum were calculated using Spearman's Rho. The associations between child environmental exposures and antibody concentrations were examined in linear regression models. Distributions of antibodies were skewed to the right and therefore log10-transformed to avoid violation of model assumptions. Estimates from the linear regression models were subsequently back-transformed to express the percent difference in antibody concentrations at each increment increase in contaminant concentrations. Potential confounding variables (area of residence and duration of being breastfed) were identified a priori using a Directed Acyclic Graph (DAG, Supplemental Fig. S1). Vaccine antibody concentration decline over time after vaccination, and in the present study, time since vaccination varied from 312 to 2816 days. Thus, to account for the variance in time interval and improve model efficiency, all linear regression models were adjusted for time since vaccination. The analyses were performed in four different models: 1) crude analyses including children with available information about breastfeeding, 2) analyses adjusted for breastfeeding, 3) analyses adjusted for breastfeeding and area of residence, and 4) analyses adjusted for breastfeeding and area of residence including children with a known vaccination date only. As illustrated in the DAG, maternal environmental exposures could constitute a confounding path if pregnancy exposure also affects the developing immune system and ability to produce antibodies. However, information about maternal exposures were available for only a subset of the children, and we therefore chose to adjust for maternal exposures in sensitivity analyses only. Using logistic regression models, we further examined the associations between the child environmental exposures and not having protective levels ($\geq$0.1 IU/mL) of diphtheria antibody concentrations, while adjusting for area of residence and duration of being breastfed and restricting the analyses to children with a known vaccination date. Among children with available vaccination records, very few children had tetanus antibody concentrations below the protective level, and thus, we did not have sufficient power to perform the logistic regression analysis for lack of protection against tetanus. The associations between maternal environmental exposures and antibody concentrations were examined in adjusted linear regression models identical to those used for the child environmental exposures including children with a known vaccination date.

Assumptions regarding linearity of the environmental exposures were tested by including the chemical concentrations squared along with the chemical concentration in the models (data not shown). Significant ($p < 0.05$) deviations from linearity were found for PFOS and PFDA associations with tetanus and for the PFDA association with diphtheria in analyses including only children with a known vaccination date, and for the maternal PFOS association with diphtheria. However, there is a chance that the significant deviations from linearity are merely chance findings, and with the vast majority of analyses showing linear associations we did not revise our assumption about linearity, but the particular analyses should be interpreted with caution. The assumptions underlying the linear regression models about homoscedasticity and normal distribution of the residuals were inspected visually using plots of residuals against fitted values and quantile-normal plots of the standardized residuals, respectively (data not shown). Generally, the assumptions were met, but in the analyses with less than 60 individuals, slight deviations were found. With the low number of individuals in these analyses, the deviations might be random, but less emphasis should be given to the results from these analyses. All analyses were performed using Stata/IC 16.1 (StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC).

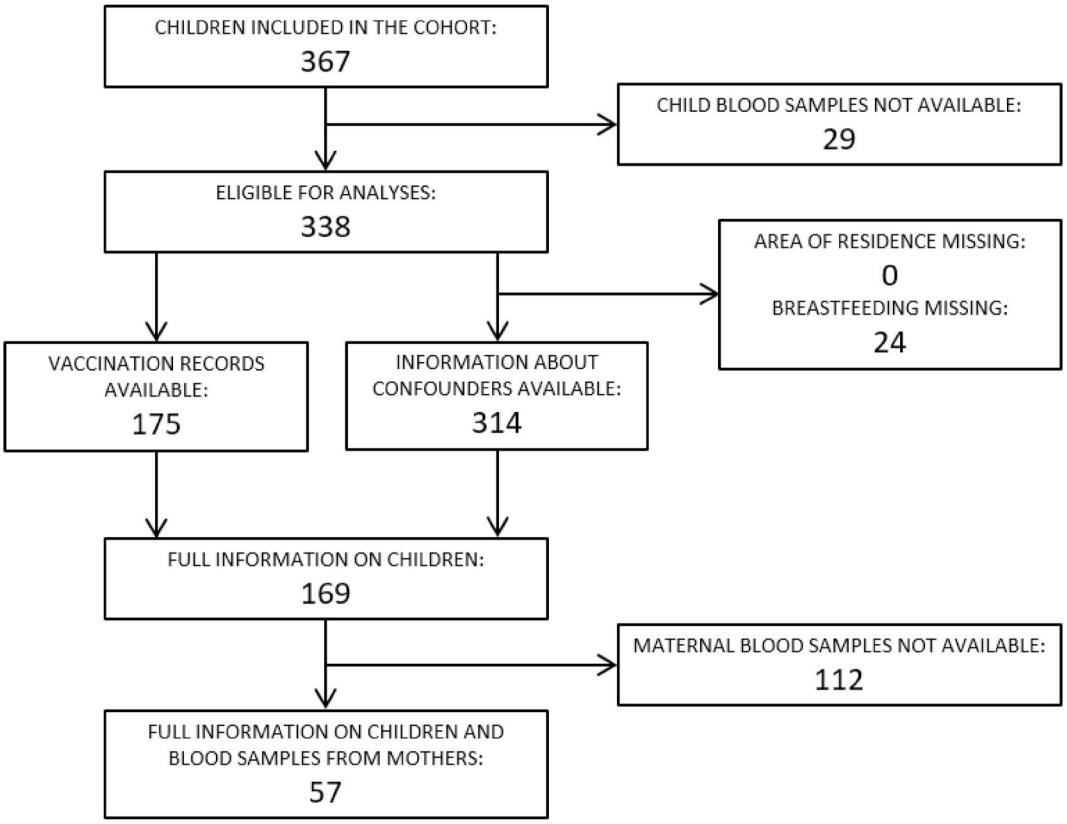

Fig. 1. Flowchart of the data material.

3. Results

The 338 children were between 7.1 and 12.1 years old (median 9.9 years) at the time of examination and approximately half of them were girls. The majority of children were from Nuuk, Sisimiut and Ilulissat, and the median concentrations of tetanus and diphtheria antibodies were 0.92 and 0.07 IU/mL (Table 1). Most (72 %) of children had been breastfed at least 6 months, and only 7 children (2 %) were never breastfed. Forty-two (12 %) had tetanus concentrations below the protective limit and 175 (52 %) had diphtheria concentrations below the limit. Among the 175 children with a known vaccination date, 5 (3 %) and 72 (41 %), respectively, had tetanus and diphtheria concentrations below protective levels. All seven types of PFASs were detected in more than 90 % of the child serum samples. Five children had serum-PFUnDA concentrations below the LOD, CB-138 and CB-153 were below the LOD for 5 children each, and CB-180 were below the LOD for 12 children. One child had concentrations of all three PCBs below the LOD. The children's concentrations of the contaminants were comparable to those found among the mothers during pregnancy with the exception of PFOS that occurred in lower concentrations among the children (Table 2). Child concentrations of PFOS was found to be strongly correlated with PFHxS, PFNA, and PFUnDA, and PFUnDA was also strongly correlated to PFNA. The strongest correlation was found between maternal concentrations of PFNA and PFDA, while exposures in childhood were only weakly to moderately associated with maternal exposures during pregnancy (Table 3).

In crude analyses, elevated concentrations of some of the contaminants were associated with higher concentrations of diphtheria and tetanus antibodies, most pronounced for PFDA, but the association vanished or was reversed after adjustment for potential confounders, especially area of residence, and the reverse associations were strengthened when excluding children with an unknown vaccination date (Table 4). Thus, each 1 ng/mL increase in serum concentrations of PFHxS and PFOS was associated with 78 % (95 % CI: 25–94 %) and 9 % (95 % CI: 2–16 %), respectively, lower diphtheria antibody concentrations. However, confidence intervals were wide, and none of the other associations with immunotoxicants were statistically significant (Table 4). Likewise, exposure to all of the environmental contaminants were associated with markedly reduced odds of having diphtheria antibodies above the protective level (Table 5). For each 1 ng/mL increase in PFHxS, PFOS, PFNA, and PFDA, odds of not having protective levels of diphtheria antibodies were increased by 6.44 times (95 % CI: 1.51–27.36), 1.14 times (95 % CI: 1.04–1.26), 1.96 times (95 % CI: 1.07–3.60), and 5.08 times (95 % CI: 1.32–19.51), respectively (Table 5).

The negative associations between contaminants and concentrations of diphtheria and tetanus antibodies were further strengthened for most of the exposures after adjustment for maternal serum concentrations, but due to the limited sample size, the confidence intervals were wider (Supplementary Table S1). No consistent associations were seen between maternal contaminant concentrations and vaccine antibody concentrations (Table 6).

Table 1
Distribution of characteristics and antibody concentrations among 338 Greenlandic children.

Child characteristics		n/N (%)
Sex		
Girls		162/338 (48)
Boys		176/338 (52)
Residence		
Nuuk		84/338 (25)
Maniitsoq		32/338 (9)
Sisimiut		68/338 (20)
Ilulissat		98/338 (29)
Aasiaat		22/338 (7)
Qeqertarsuaq		10/338((3)
Tasiilaq		24/338 (7)
Breastfed		
<6 months		87/314 (28)
6–12 months		72/314 (23)
>1 year		155/314 (49)
Indoor smoking in house		
Yes		57/300 (19)
No		243/300 (81)
Maternal employment status		
Working		163/215 (76)
Student		23/215 (11)
Other		29/215 (13)
Paternal employment status		
Working		170/188 (90)
Student		8/188 (4)
Other		10/188 (5)
	n	**Median (25;75-percentile)**
Age (years)	338	9.9 (9.1; 10.5)
Time since vaccine booster (days)	175	1201 (890; 1788)
Antibody concentrations	**n**	**Median (25;75-percentile)**
Tetanus (IU/mL)	338	0.92 (0.25; 2.20)
Diphtheria (IU/mL)	338	0.07 (0.02; 0.28)

Table 2
Distribution of contaminant concentrations among 338 Greenlandic children.

Child contaminants	n	n < LOD	Median (25; 75-percentile)
PFHxS (ng/mL serum)	338	0	0.69 (0.54; 0.93)
PFHpS (ng/mL serum)	338	0	0.27 (0.18; 0.45)
PFOS (ng/mL serum)	338	0	8.68 (6.52; 12.23)
PFOA (ng/mL serum)	338	0	2.28 (1.89; 2.88)
PFNA (ng/mL serum)	338	0	1.40 (1.09; 1.95)
PFDA (ng/mL serum)	338	0	0.49 (0.24; 0.80)
PFUnDA (ng/mL serum)	338	5	0.42 (0.26; 0.72)
ΣPCB (μg/g lipid)	338	1	0.26 (0.16; 0.50)
Mercury (μg/L blood)	333	0	3.83 (1.92; 5.62)
Maternal contaminants	**n**		**Median (25;75-percentile)**
PFHxS (ng/mL serum)	91	0	0.70 (0.55; 0.93)
PFOS (ng/mL serum)	91	0	19.16 (15.20; 24.06)
PFOA (ng/mL serum)	91	0	2.13 (1.68; 2.54)
PFNA (ng/mL serum)	91	0	0.90 (0.63; 1.29)
PFDA (ng/mL serum)	91	0	0.46 (0.32; 0.69)
ΣPCB (μg/g lipid)	89	0	0.39 (0.22; 0.52)
Mercury (μg/L blood)	89	0	4.4 (3.1; 8.5)

4. Discussion

As previously described (Timmermann et al., 2019) the children in this study had highly elevated mercury and PCB concentrations compared to American children (Department of Health, 2018) (Nichols et al., 2007), whereas PCB concentrations were lower than among Faroese children (Heilmann et al., 2010). PFOS concentrations were almost twice as high as among American and Faroese children (Grandjean et al., 2017a; Ye et al., 2018), and PFHxS, PFOA, PFNA, and PFDA concentrations were also slightly higher than among the Faroese (Grandjean et al., 2017a).

The results from the linear and logistic regression models were generally internally consistent. Thus, we found decreased diphtheria concentrations after vaccination with increasing PFAS exposure, and higher serum concentrations of PFAS were associated with increased odds of not being protected against diphtheria after vaccination. Similar though slightly weaker associations were seen for tetanus in the linear regression models, but the confidence intervals were wide and in agreement with a null finding. The present study adds to the existing evidence on immunotoxicity of PFAS exposure in humans (DeWitt et al., 2019) and the concerns about PFAS exposures in the Arctic, where some exposures have decreased, while PFNA and PFDA have tended to

Table 3
Pairwise correlations between environmental chemicals in child and maternal serum calculated using Spearman's Rho.

	Child exposures									Maternal exposures						
	PFHxS	PFHpS	PFOS	PFOA	PFNA	PFDA	PFUnDA	ΣPCB	Mercury	PFHxS	PFOS	PFOA	PFNA	PFDA	ΣPCB	Mercury
Child exposures																
PFHxS	1.00															
PFHpS	0.50	1.00														
PFOS	0.82	0.55	1.00													
PFOA	0.38	0.08	0.44	1.00												
PFNA	0.78	0.52	0.86	0.55	1.00											
PFDA	0.65	−0.02	0.70	0.53	0.72	1.00										
PFUnDA	0.70	0.43	0.83	0.34	0.82	0.74	1.00									
ΣPCB	0.56	0.34	0.48	0.26	0.44	0.36	0.44	1.00								
Mercury	0.53	0.23	0.59	0.15	0.57	0.58	0.70	0.34	1.00							
Maternal exposures																
PFHxS	0.30	0.20	0.08	−0.15	0.06	0.00	0.01	0.01	0.08	1.00						
PFOS	0.24	0.15	0.16	−0.12	0.10	0.06	0.08	0.12	0.13	0.62	1.00					
PFOA	0.08	−0.02	0.15	−0.01	0.06	0.10	0.08	−0.02	0.04	0.36	0.57	1.00				
PFNA	0.42	0.22	0.40	0.25	0.43	0.45	0.44	0.48	0.46	0.35	0.55	0.28	1.00			
PFDA	0.47	0.30	0.48	0.28	0.50	0.51	0.51	0.49	0.48	0.31	0.54	0.21	0.92	1.00		
ΣPCB	0.29	0.18	0.15	0.13	0.22	0.23	0.22	0.41	0.22	0.46	0.40	0.05	0.54	0.55	1.00	
Mercury	0.40	0.27	0.38	0.29	0.42	0.43	0.43	0.38	0.47	0.24	0.37	0.00	0.59	0.64	0.51	1.00

increase (Abass et al., 2018).

Postnatal PFAS exposure has previously been associated with reduced vaccine antibody responses in Faroese 5 and 7-year-olds vaccinated against tetanus and diphtheria (Grandjean et al., 2012, 2017a) and in African infants vaccinated against measles (Timmermann et al., 2020). In both studies, antibody titers were assessed in relation to fairly short, known time intervals since last vaccination. However, at longer intervals since booster vaccination, these associations were much weaker (Grandjean et al., 2017b), which is likely due to more factors, such as infectious disease and other vaccinations, affecting the immune system over time, thereby causing random variation. Furthermore, PFAS exposure has been associated with decreased concentrations of rubella but not tetanus antibodies among Norwegian 3-year-olds (Granum et al., 2013). PCB exposure has also previously been associated with reduced antibody response to diphtheria and tetanus among Faroese children (Heilmann et al., 2006, 2010), and in our study we saw a similar tendency for PCB and diphtheria, though not statistically significant. Among Norwegian 3-year olds, PCB exposure was associated with decreased measles antibodies, but no association was seen with tetanus (Stølevik et al., 2013), which is in accordance with our findings. The mercury exposures also did not affect the vaccine antibody concentrations in our study. Likewise, only weak associations between mercury exposure and vaccine responses have been observed in previous studies (Wyatt et al., 2019). Although a direct link to the elevated occurrence of infectious disease (Kløvgaard et al., 2016), could not be explored, the lowered concentrations of vaccine-specific antibodies at elevated exposures suggest an apparent immune function deficit associated with the increased exposure to environmental chemicals, which could possibly also induce impaired protection against infectious disease. A study among Greenlandic children found no association between PCB and otitis media (Jensen et al., 2013), but several studies have linked environmental chemical exposures with child morbidity (Dallaire et al., 2004, 2006; Timmermann et al., 2020; Impinen et al., 2018; Dalsager et al., 2016, 2021).

A number of limitations should be noted when interpreting the results from the present study. First, concentrations of the specific antibodies were fairly low in this study, especially for diphtheria, probably due to the time interval since the most recent vaccination or booster. Tetanus booster vaccinations are not routinely provided in emergency rooms in Greenland. Thus, in the oldest participants, the age-5 booster was probably given seven years prior to study participation, thereby allowing substantial decreases in antibody concentrations over time. The date of the most recent vaccine booster was known only for approximately half of the children in the study, and the analyses in which we used an estimated date of vaccination yielded results that differed from those obtained for the restricted data with exact information on booster time. It seems that using an estimated date of vaccination caused information bias, perhaps in particular due to the long and likely variable time interval since the most recent vaccination. Similarly, in the American National Health and Nutrition Examination Survey (NHANES) 1999–2000 and 2003–2004, one study found PFASs to be associated with reduced concentrations of mumps and rubella but not measles antibodies among 12-19 year-olds of unknown vaccination status (Stein et al., 2016), while another found no association between PFAS and rubella antibodies among 12-18-year-olds (Pilkerton et al., 2018). These findings, like ours, emphasize the need to obtain information on vaccination date in order to use antibody responses as an appropriate marker of immune function.

Lifestyle, health, and compliance with vaccine schedule are likely to vary between the different areas in Greenland (Kløvgaard et al., 2016), and area of residence is a predictor of the environmental exposures (Timmermann et al., 2019). The reversed associations after adjustment for area of residence emphasize the importance of adjusting for this confounder. SES is also known to affect child health, but we did not have complete data on factors related to SES. However, in Greenland, we would expect area of residence to be a stronger predictor of environmental exposures compared to SES, and thus, SES is likely not an important confounder in this Artic setting when adjusting for area of residence. Furthermore, many persistent environmental chemicals are transferred through breast milk (Grandjean and Jensen, 2004; Mogensen et al., 2015), while breastfeeding duration beneficially affects child health (Victora et al., 2016), which makes breastfeeding an important confounder. However, as area of residence and breastfeeding are very closely associated with childhood environmental exposures, the adjustments, though necessary, could have led to a slight underestimation of the true associations. (Budtz-Jorgensen et al., 2003). A main strength of this study is that data on maternal exposures during pregnancy were available for about one-fourth of the children in the study, thereby allowing potential consideration of life-time exposure levels.

In this study, exposure to some of the environmental chemicals were strongly correlated, which makes it difficult to completely separate their effects. We examined nine different exposures measured in child serum and two different outcomes with several different adjustment sets. Furthermore, we examined 7 different exposures measured in maternal serum. Thus, there is a high risk of finding significant associations merely by chance. Therefore, focus should be on general trends in the results rather than single significant findings. Overall, we found that higher childhood exposures to environmental chemicals were associated

Table 4
Percent difference in tetanus and diphtheria antibody concentrations for each increment increase in child concentrations of PFASs, PCB and mercury in crude and adjusted analyses.

		Tetanus [a]	Diphtheria [a]
	N	% difference (95 % CI)	% difference (95 % CI)
Crude			
PFHxS (ng/mL serum)	314	28 (−5, 73)	48 (1, 116)
PFHpS (ng/mL serum)	314	−20 (−60, 60)	−46 (−77, 30)
PFOS (ng/mL serum)	314	3 (−0, 6)	3 (−1, 7)
PFOA (ng/mL serum)	314	21 (−5, 54)	19 (−12, 61)
PFNA (ng/mL serum)	314	17 (−5, 44)	21 (−7, 57)
PFDA (ng/mL serum)	314	74 (12, 168)	124 (30, 286)
PFUnDA (ng/mL serum)	314	38 (0, 89)	40 (−6, 108)
ΣPCB (μg/g lipid)	314	36 (3, 78)	40 (−1, 97)
Mercury (μg/L blood)	309	2 (−1, 6)	2 (−1, 6)
Adjusted [b]			
PFHxS (ng/mL serum)	314	28 (−6, 73)	48 (1, 115)
PFHpS (ng/mL serum)	314	−20 (−60, 60)	−44 (−76, 34)
PFOS (ng/mL serum)	314	3 (−0, 6)	3 (−1, 7)
PFOA (ng/mL serum)	314	21 (−5, 54)	21 (−10, 64)
PFNA (ng/mL serum)	314	17 (−5, 44)	22 (−6, 58)
PFDA (ng/mL serum)	314	74 (12, 169)	126 (32, 289)
PFUnDA (ng/mL serum)	314	38 (0, 89)	42 (−5, 110)
ΣPCB (μg/g lipid)	314	36 (3, 79)	39 (−1, 96)
Mercury (μg/L blood)	309	2 (−1, 6)	3 (−1, 7)
Adjusted [c]			
PFHxS (ng/mL serum)	314	−28 (−53, 10)	−40 (−64, 1)
PFHpS (ng/mL serum)	314	−19 (−66, 91)	−38 (−78, 77)
PFOS (ng/mL serum)	314	−2 (−5, 2)	−3 (−7, 1)
PFOA (ng/mL serum)	314	−1 (−24, 29)	−10 (−35, 25)
PFNA (ng/mL serum)	314	−11 (−30, 13)	−19 (−39, 9)
PFDA (ng/mL serum)	314	−29 (−61, 28)	−39 (−70, 27)
PFUnDA (ng/mL serum)	314	−15 (−42, 25)	−27 (−54, 16)
ΣPCB (μg/g lipid)	314	−2 (−32, 40)	−26 (−52, 15)
Mercury (μg/L blood)	309	−0 (−3, 3)	−1 (−5, 3)
With known vaccination record [c,d]			
PFHxS (ng/mL serum)	169	−38 (−74, 48)	−78 (−94, −25)
PFHpS (ng/mL serum)	169	−22 (−81, 217)	−85 (−98, 8)
PFOS (ng/mL serum)	169	−3 (−8, 3)	−9 (−16, −2)
PFOA (ng/mL serum)	169	−8 (−30, 21)	−22 (−48, 16)
PFNA (ng/mL serum)	169	−8 (−37, 34)	−39 (−64, 4)
PFDA (ng/mL serum)	169	−29 (−68, 61)	−59 (−87, 31)
PFUnDA (ng/mL serum)	169	−1 (−45, 81)	−46 (−77, 25)
ΣPCB (μg/g lipid)	169	−9 (−53, 76)	−53 (−82, 19)
Mercury (μg/L blood)	166	−0 (−7, 7)	−3 (−12, 7)

[a] Adjusted for time since vaccine booster/estimated time since vaccine booster.
[b] Adjusted for duration of being breastfed (<6 months, 6–12 months, >1 year).
[c] Adjusted for duration of being breastfed (<6 months, 6–12 months, >1 year) and area of residence (Nuuk, Maniitsoq, Sisimiut, Ilulissat, Aasiaat, Qeqertarsuaq, Tasiilaq).
[d] Including children with known tetanus-diphtheria booster date only.

with lower antibody concentrations after vaccination and with higher odds of not having a sufficient antibody concentration to be protected against diphtheria.

5. Conclusions

This study emphasizes the potential risk of environmental chemical exposures in this Arctic population, where the exposures are mainly due to biomagnification in the marine food chain that leads to elevated concentrations of contaminants from far-away sources (Abass et al., 2018). The large number of vaccinated children with diphtheria concentrations below the protective level, should raise public health concern beyond the theoretical risk of diphtheria (Vogt, 1999). Prevention of contaminant exposures in the Arctic must rely on dietary advisories (Arctic Monitoring and Ass, 2017), but international efforts to reduce uses and dissemination of immunotoxicants will provide an advantage over time (Grandjean, 2018).

Table 5
Odds ratio of not being protected against diphtheria (antibody concentrations <0.1 IU/mL) for each increment increase in child concentrations of PFASs, PCB and mercury. Only children with known vaccination records were included.

	Diphtheria [a]	
	n < 0.1 IU/mL/N	OR (95 % CI)
PFHxS (ng/mL serum)	68/169	6.44 (1.51, 27.36)
PFHpS (ng/mL serum)	68/169	4.01 (0.43, 37.43)
PFOS (ng/mL serum)	68/169	1.14 (1.04, 1.26)
PFOA (ng/mL serum)	68/169	1.41 (0.91, 2.19)
PFNA (ng/mL serum)	68/169	1.96 (1.07, 3.60)
PFDA (ng/mL serum)	68/169	5.08 (1.32, 19.51)
PFUnDA (ng/mL serum)	68/169	2.61 (0.99, 6.90)
ΣPCB (μg/g lipid)	68/169	1.63 (0.58, 4.56)
Mercury (μg/L blood)	67/166	1.08 (0.97, 1.21)

[a] Adjusted for area of residence (Nuuk, Maniitsoq, Sisimiut, Ilulissat, Aasiaat, Qeqertarsuaq, Tasiilaq), and duration of being breastfed (<6 months, 6–12 months, >1 year).

Table 6
Percent difference in tetanus and diphtheria antibody concentrations for each increment increase in maternal concentrations of PFASs, PCB and mercury.

		Tetanus [a]	Diphtheria [a]
	N	% difference (95 % CI)	% difference (95 % CI)
PFHxS (ng/mL serum)	57	−1 (−72, 245)	−53 (−87, 73)
PFOS (ng/mL serum)	57	2 (−3, 6)	1 (−4, 6)
PFOA (ng/mL serum)	57	−7 (−44, 56)	44 (−15, 145)
PFNA (ng/mL serum)	57	64 (−18, 228)	−11 (−58, 88)
PFDA (ng/mL serum)	57	95 (−45, 591)	−39 (−84, 133)
ΣPCB (μg/g lipid)	55	113 (−27, 521)	−41 (−81, 80)
Mercury (μg/L blood)	56	−2 (−10, 7)	−7 (−15, 2)

[a] Adjusted for time since vaccine booster (only children with known vaccination date were included), area of residence (Nuuk, Maniitsoq, Sisimiut, Ilulissat, Aasiaat, Qeqertarsuaq, Tasiilaq), and duration of being breastfed (<6 months, 6–12 months, >1 year).

CRediT author statement

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Philippe Grandjean has provided paid expert assistance in legal cases involving PFAS exposed populations. Otherwise, the authors have no actual or potential competing financial interests.

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Appendix A. Supplementary data

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