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## International Journal of Hygiene and Environmental Health

journal homepage: [www.elsevier.com/locate/ijheh](http://www.elsevier.com/locate/ijheh)

# In utero and early childhood exposure to per- and polyfluoroalkyl substances and use of antibiotics in children from the Odense Child Cohort: A Danish cohort study

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## ARTICLE INFO

## Keywords:

Environmental epidemiology  
Infectious diseases  
Antibiotics  
Perfluoroalkyl acids

## ABSTRACT

**Background:** Per- and polyfluoroalkyl substances (PFAS) have been associated with an increased risk of infectious diseases. We aimed to investigate if in utero and early childhood exposure to PFAS was associated with the number of antibiotic prescriptions up to eight years of age.

**Methods:** Among 2448 singleton mother-child pairs from the Odense Child Cohort, 1425 had sufficient information on key variables and were included in the primary analysis. Information on redeemed antibiotic prescriptions from birth to eight years of age was obtained from the Danish National Prescription Registry. Longitudinal discrete-time Poisson models were used to quantify the relationship between PFAS and the number of antibiotic prescriptions redeemed. Analyses were carried out separately for PFAS measured in the mother during pregnancy and in the child at 18 months of age. Missing information was imputed using Multiple Imputation by Chained Equations.

**Results:** We observed no differences in the number of antibiotic prescriptions in the first eight years of life when comparing median and high PFAS concentrations measured in both pregnancy and at 18 months of age (rate ratio PFOA 1.01, 95 % confidence interval 0.94–1.08; PFOS 1.08, 0.98–1.19; PFNA 1.00, 0.94–1.07; PFDA 0.99, 0.94–1.04; PFHxS 1.02, 0.98–1.07).

**Conclusion:** We found no association between in utero or early childhood PFAS concentrations and number of antibiotic prescriptions up to eight years of age. Antibiotic prescriptions may be an unspecific marker of childhood infections, hampering the possibility to observe an association with PFAS exposure.

**Trial registration:** Real World Evidence Registry: <https://osf.io/dyqxm>, registered March 8, 2023.

## 1. Background

Per- and polyfluoroalkyl substances (PFAS) are chemicals widely used in industrial applications including textiles, cookware, and fire-fighting foam due to the water and grease-resistant properties (Fromme et al., 2009). They are characterized by fluorinated aliphatic carbon chains (Fromme et al., 2009; Sunderland et al., 2019). The long-chain PFAS bioaccumulate, have long elimination half-lives of up to three to five years, and can be measured in all human blood samples (Houde et al., 2006; Olsen et al., 2007). Perfluorooctanoic acid (PFOA) and

perfluorooctane sulfonate (PFOS) have been the most widely used PFASs (EFSA CONTAM Panel et al., 2020). Increasing evidence of their adverse health effects have resulted in restricted use (EFSA CONTAM Panel et al., 2020). Knowledge on exposure to other PFAS, including newer short-chain compounds increasingly used as replacements, remains limited (Hull et al., 2023). Human exposure primarily occurs through diet and contaminated drinking water (Sunderland et al., 2019). PFAS is transferred to the fetus through the placenta and in infancy through breastfeeding, confirmed through detection in umbilical cord blood, amniotic fluid, and breast milk (EFSA CONTAM Panel et al., 2020).

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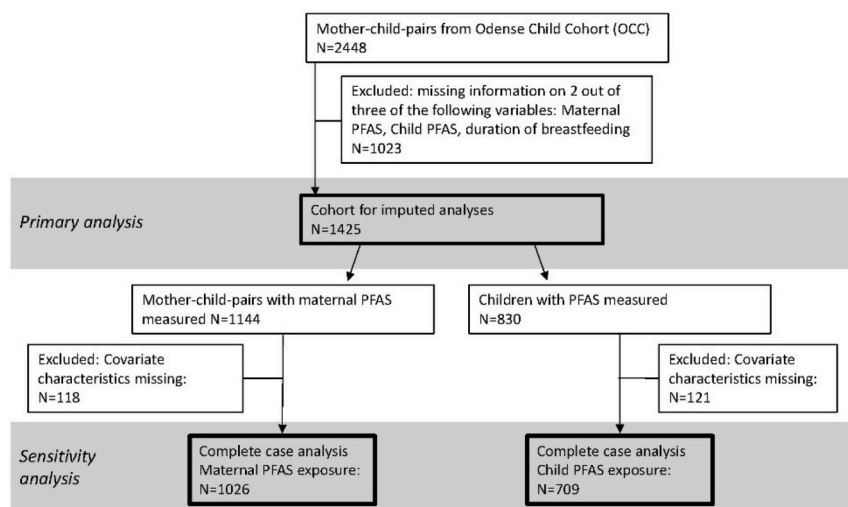
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<https://doi.org/10.1016/j.ijheh.2025.114734>

Received 20 August 2025; Received in revised form 5 December 2025; Accepted 11 December 2025

Available online 24 December 2025

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**Fig. 1.** Participant Flow

Abbreviations: OCC: Odense Child Cohort; PFAS: Per- and polyfluoroalkyl substances.

Infants may be especially sensitive to PFAS exposure, due to the development of the immune system (Kahn et al., 2020).

PFAS have been found to have immunosuppressive properties and exposure has been associated with decreased antibody response to childhood vaccinations and increased risk of infectious disease hospitalizations (EFSA CONTAM Panel et al., 2020). The European Food Safety Authority identified effects on the immune system, such as reduced vaccine antibody levels, as the most critical endpoint assessing human health risks from PFAS exposure (EFSA CONTAM Panel et al., 2020). In the Odense Child Cohort (OCC), both prenatal and postnatal PFAS exposure were associated with a reduction in common childhood vaccination antibodies in 18-month-old children (Sigvaldsen et al., 2024). Furthermore, in the OCC, prenatal PFAS exposure was associated with increased risk of symptoms of infection (especially fever) (Dalsager et al., 2016) and hospitalizations for infections until five years of age (Dalsager et al., 2021a), findings which have been confirmed by others (von Holst et al., 2021). This suggests that PFAS exposure is associated with a more general immune dysfunction. As antibiotic prescriptions may reflect the occurrence of common infections, use may serve as an indirect marker of impaired immune function or increased susceptibility to infection.

To our knowledge, no previous studies have investigated the impact of in utero or early childhood PFAS exposure on infections treated with antibiotics outside of hospital settings. We therefore aimed to investigate if in utero or early childhood PFAS exposure was associated with higher rates of antibiotic prescriptions until eight years of age in children from the OCC.

## 2. Methods

Using information from the Danish population-based health registries and the OCC, we evaluated the association between 1) in utero and 2) early childhood exposure to PFAS and rates of antibiotic prescribing during childhood.

### 2.1. Odense Child Cohort

The OCC is a prospective population-based birth cohort. From 2010 to 2012, all newly pregnant women living in Odense Municipality ( $n = 6707$ ) were invited; and 43 % ( $n = 2874$ ) participated (Kyhl et al., 2015). From this cohort, 2448 singleton mother-child pairs were eligible for participation in the present investigation. Baseline information on maternal pre-pregnancy BMI, smoking during pregnancy, and maternal

level of education was obtained from self-reported questionnaires. Information on the duration of breastfeeding was obtained from self-reported questionnaires (Bruun et al., 2017; Timmermann et al., 2022).

### 2.2. PFAS exposure

Blood samples were drawn from the pregnant women at the time of inclusion (between gestational week 10 and 16) and from the children at 18 months of age. Both maternal and child blood samples were analyzed for perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA) using online solid phase extraction followed by liquid chromatography and triple quadrupole mass spectrometry (LC-MS/MS) as described previously (Dalsager et al., 2016; Dalsager et al., 2021b). Limit of Quantification (LOQ) was 0.03 ng/mL for all PFAS compounds. PFOA, PFNA, PFDA, and PFOS were detected in all samples. PFHxS was detected in all child samples, and in a subset of maternal samples. Values below LOQ were substituted with LOQ/2 in the statistical analyses. Neither maternal PFAS (mPFAS) nor child PFAS (cPFAS) concentrations followed a normal distribution.

### 2.3. Antibiotic prescriptions

The Danish National Prescription Registry (Pottegård et al., 2017) includes information on all redeemed prescriptions, linked to the individual using a personal identifier given at birth. We identified all antibiotic prescriptions, including antibacterial medication for systemic use (Anatomical Therapeutic Chemical Classification (ATC) J01 and all sublevels), as well as Metronidazole (ATC P01AB01), redeemed for children in our study population from birth to eight years of age. Prescriptions redeemed were used as a proxy for an event of infection requiring treatment with antibiotics. Multiple sequential antibiotic prescriptions within a 21-day period - counting from the first prescription in that period - were interpreted as a lack of compliance or lack of effect of the initial antibiotic and were thus regarded to belong to the same underlying infection and counted as a single event. All children were followed from birth until eight years of age or emigration, whichever came first.

For the analysis of associations with mPFAS concentration, all prescriptions from birth to eight years of age were included, while prescriptions were included only after the age of 18 months (day 550) when examining associations with cPFAS concentrations.

2.4. Covariate selection

Selection of covariates in the analysis model was based on directed acyclic graphs (DAGs) of the *a priori* hypothesized associations between in utero and early childhood exposure to PFAS and offspring antibiotic prescriptions (Figure A.1). Potential confounders included parity, maternal age (18–24, 25–29, 30–34, 35+ years), education level (high school or less, high school plus 1–4 years of education, high school plus more than four years of education), pre-pregnancy body mass index (BMI) (<18, 18–24, 25–29, 30–34, 35+), and self-reported smoking during pregnancy. Offspring sex was included as an independent predictor due to its association with infectious disease (Gehrt et al., 2022) and childhood antibiotic use (Skajaa et al., 2022). Duration of breastfeeding and mPFAS concentrations were excluded from the DAG, as they strongly predict cPFAS concentrations and lie on the causal pathway between mPFAS and offspring antibiotic use (Diop et al., 2021). For analyses on cPFAS concentrations and antibiotic use, gestational age and birthweight were considered potential confounders.

2.5. Missing data

Data on the included variables were missing in a proportion of subjects. For each variable, we evaluated the proportion of missing data. We compared baseline characteristics of complete case observations and individuals with missing data. We imputed missing information using Multiple Imputation by Chained Equation (MICE) (Van Buuren and Groothuis-Oudshoorn, 2011) with predictive mean matching for continuous variables, logistic regression for dichotomous variables, ordinal logistic regression for ordered categorical variables, and multinomial logistic regression for unordered categorical variables. Since a strong relationship exists between mPFAS and cPFAS concentrations - modified through the duration of breastfeeding, we restricted the population to 1425 mother/child pairs with minimum two of these three variables available (Beck et al., 2023) (Fig. 1). We included mPFAS concentration, cPFAS concentration, duration of breastfeeding, birth year, parity, sex, maternal age, the number of antibiotic prescriptions until eight years of age, and all variables that were to be imputed (Table A.1). We imputed 50 datasets; for each dataset, we ran chains with 50 iterations and a burn-in of ten iterations. Convergence of the imputation was assessed by examining trace plots and the distribution of imputed variables stratified by whether the variable was observed or imputed. We assumed variables with missing values to be missing at random. Stata 18 was used for analysis.

2.6. Statistical analyses

Descriptive statistics were used to describe observed and imputed baseline characteristics in the study population. Concentrations of mPFAS and cPFAS were reported stratified by child sex, parity, maternal BMI, maternal smoking during pregnancy, maternal level of education, duration of breastfeeding, and maternal age.

We modeled the relationship between PFAS exposure and the number of antibiotic prescriptions redeemed from birth and up to eight years of age using a longitudinal approach based on generalized linear models in each imputed dataset (Carstensen, 2012). All children’s person time was split into one-month periods, and the number of antibiotic prescriptions was quantified for each month. We then fitted a Poisson model with a log-link, with age at the beginning of each interval as an independent variable, modeled using restricted cubic splines with four knots placed at the 5th, 25th, 75th, and 95th age percentile.

We included the following variables measured at baseline in the model: maternal smoking during pregnancy, pre-pregnancy BMI, maternal level of education, gestational age, maternal age, birthweight, and parity. Dependence of observations for the same individual at different time points was adjusted for using the clustered sandwich estimator of variance, which corresponds to fitting a marginal means/

Table 1

Baseline characteristics of included children with complete and incomplete information on maternal (mPFAS) and child PFAS (cPFAS) concentrations compared with characteristics in the joint Odense Child Cohort.

	All OCC (n = 2448)	Imputed data population (n = 1425)	mPFAS <sup>1</sup> Complete case (n = 1026)	cPFAS <sup>2</sup> Complete case (n = 709)
All				
Sex				
Male	1293 (52.8)	761 (53.4)	546 (53.2)	396 (55.9)
Female	1155 (47.2)	664 (46.6)	480 (46.8)	313 (44.1)
Parity, N (%)				
Primipara	1351 (55.2)	817 (57.3)	589 (57.4)	404 (57.0)
Multipara	1097 (44.8)	608 (42.7)	437 (42.6)	305 (43.0)
Maternal pre-pregnancy BMI				
<18 kg/m <sup>2</sup>	45 (1.8)	27 (1.9)	16 (1.6)	15 (2.1)
18–24 kg/m <sup>2</sup>	1575 (64.3)	890 (62.5)	642 (62.6)	421 (59.4)
25–30 kg/m <sup>2</sup>	556 (22.7)	354 (24.8)	257 (25.0)	192 (27.1)
30–35 kg/m <sup>2</sup>	186 (7.6)	108 (7.6)	77 (7.5)	51 (7.2)
35+ kg/m <sup>2</sup>	85 (3.5)	46 (3.2)	34 (3.3)	30 (4.2)
Missing (n < 5)	–	–	–	–
Smoking during pregnancy				
Yes	132 (5.4)	65 (4.6)	46 (4.5)	32 (4.5)
No	2308 (94.3)	1358 (95.3)	980 (95.5)	677 (95.5)
Missing	8 (0.3 %)	(n < 5)	–	–
Education level				
High school or less	725 (29.6)	391 (27.4)	279 (27.2)	193 (27.2)
High school + 1–4 years	1171 (47.8)	714 (50.1)	521 (50.8)	357 (50.4)
High school + >4 years	492 (20.1)	300 (21.1)	226 (22.0)	159 (22.4)
Missing	60 (2.5)	20 (1.4)	–	–
Breastfeeding duration				
<20 weeks	595 (24.3)	456 (32.0)	340 (33.1)	236 (33.3)
20–40 weeks	576 (23.5)	448 (31.4)	351 (34.2)	244 (34.4)
>40 weeks	539 (22.0)	417 (29.3)	335 (32.7)	229 (32.3)
Missing	738 (30.1)	104 (7.3)	–	–
Maternal age				
<25 yrs	240 (9.8)	134 (9.4)	97 (9.5)	56 (7.9)
25–29 yrs	839 (34.3)	476 (33.4)	343 (33.4)	234 (33.0)
30–34 yrs	934 (38.2)	547 (38.4)	393 (38.3)	273 (38.5)
35+ yrs	435 (17.8)	268 (18.8)	193 (18.8)	146 (20.6)
Gestational age (weeks), median (IQR)	40 (39–41)	40 (39–41)	40 (39–41)	40 (39–41)
Missing	9 (0.4)	6 (0.4)	–	–
Birthweight (grams), median (IQR)	3545 (3194–3870)	3550 (3200–3880)	3542 (3195–3870)	3550 (3220–3875)
Missing	6 (0.2)	(n < 5)	–	–
Maternal PFOA concentration [ng/mL], median (IQR)	1.68 (1.12–2.36)	1.68 (1.12–2.36)	1.68 (1.13–2.35)	1.69 (1.10–2.42)
Missing	1304 (53.3)	281 (19.7)	–	270 (38.1)
Child PFOA concentration [ng/mL], median (IQR)	2.43 (1.54–3.42)	2.43 (1.54–3.42)	2.48 (1.52–3.46)	2.41 (1.50–3.45)
Missing	1618 (66.1)	595 (41.8)	587 (57.2)	–

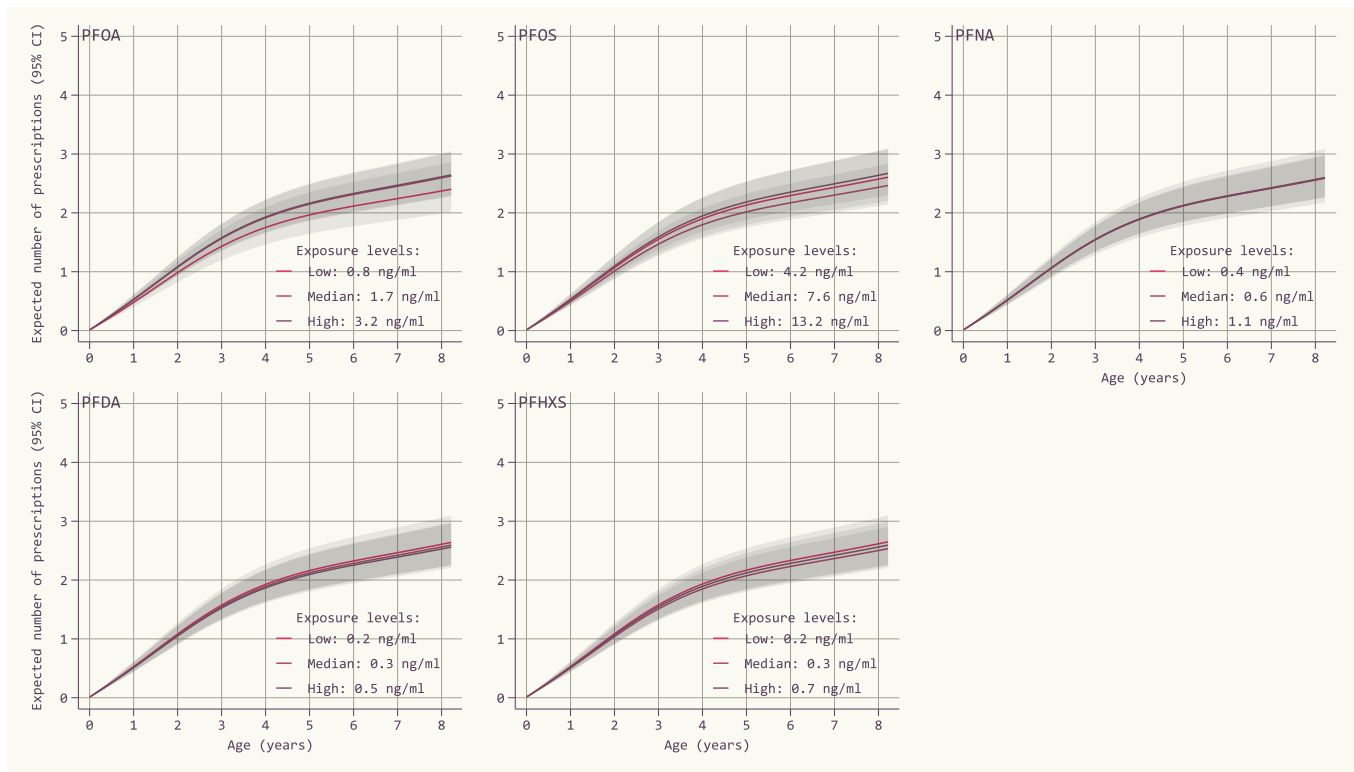
rates model (Amorim and Cai, 2015). The model was fitted to each imputed dataset and regression coefficients were pooled based on Rubin’s rules. From this model, we obtained the expected number of antibiotic prescriptions at the 10th (low concentration), 50th (median concentration), and 90th (high concentration) percentile of the PFAS concentrations with all other covariates set to their mean values. We further estimated rate ratios comparing the expected number of antibiotic prescriptions for median PFAS concentrations compared to high PFAS concentrations (90th percentile). Standard errors and 95 % confidence intervals for cumulative rates of antibiotic prescriptions and rate ratios were calculated using the delta method (Carstensen, 2012). In sensitivity analyses, we performed a complete-case analysis instead of using multiple imputation.

**Table 2**  
Characteristics of maternal and child PFAS concentration.

	Maternal PFAS concentrations [ng/mL] according to covariates. Median (95th percentile)						Child PFAS concentrations [ng/mL] according to covariates. Median (95th percentile)					
	N	PFOS	PFOA	PFHxS	PFNA	PFDA	N	PFOS	PFOA	PFHxS	PFNA	PFDA
All	1144	7.63 (15.19)	1.68 (3.85)	0.35 (0.83)	0.64 (1.43)	0.29 (0.74)	830	4.56 (11.22)	2.43 (6.40)	0.32 (0.81)	0.58 (1.29)	0.17 (0.36)
Offspring Sex												
Male	607	7.73 (15.46)	1.69 (4.19)	0.36 (0.85)	0.66 (1.48)	0.29 (0.79)	458	4.38 (11.62)	2.35 (6.29)	0.31 (0.83)	0.58 (1.25)	0.17 (0.36)
Female	537	7.52 (15.02)	1.67 (3.80)	0.34 (0.80)	0.63 (1.32)	0.28 (0.72)	372	4.81 (11.01)	2.46 (6.74)	0.33 (0.79)	0.57 (1.31)	0.17 (0.35)
Parity, N(%)												
Primipara	651	8.45 (16.25)	2.09 (4.26)	0.40 (0.88)	0.71 (1.52)	0.30 (0.75)	469	4.89 (12.03)	2.74 (7.10)	0.34 (0.92)	0.58 (1.32)	0.18 (0.37)
Multipara	493	6.81 (14.30)	1.20 (2.91)	0.29 (0.71)	0.57 (1.11)	0.27 (0.72)	361	4.18 (9.84)	2.08 (4.17)	0.30 (0.66)	0.57 (1.15)	0.17 (0.35)
Maternal pre-pregnancy BMI												
<18 kg/m <sup>2</sup>	18	6.97 (15.59)	1.27 (4.19)	0.26 (0.78)	0.59 (1.06)	0.29 (2.09)	17	5.82 (18.14)	2.87 (6.82)	0.41 (1.11)	0.69 (1.44)	0.22 (0.44)
18–24 kg/m <sup>2</sup>	725	7.67 (15.14)	1.70 (3.92)	0.36 (0.86)	0.67 (1.43)	0.30 (0.79)	506	4.76 (11.17)	2.53 (6.56)	0.34 (0.83)	0.60 (1.31)	0.18 (0.39)
25–30 kg/m <sup>2</sup>	279	7.73 (15.31)	1.65 (3.66)	0.34 (0.81)	0.62 (1.47)	0.28 (0.67)	214	4.41 (10.88)	2.40 (6.40)	0.31 (0.77)	0.57 (1.21)	0.17 (0.34)
30–35 kg/m <sup>2</sup>	87	7.47 (16.01)	1.65 (3.89)	0.33 (0.67)	0.60 (1.51)	0.24 (0.51)	62	3.49 (11.66)	1.74 (6.29)	0.24 (0.80)	0.44 (0.92)	0.16 (0.28)
35+ kg/m <sup>2</sup>	35	6.95 (17.13)	1.46 (3.76)	0.34 (1.29)	0.58 (1.49)	0.25 (0.42)	31	2.93 (8.44)	1.54 (6.10)	0.20 (0.53)	0.36 (0.99)	0.13 (0.29)
Smoking during pregnancy												
Yes	52	7.64 (12.01)	2.01 (3.31)	0.34 (0.74)	0.60 (1.30)	0.26 (0.55)	37	2.83 (7.31)	1.70 (4.05)	0.21 (0.58)	0.37 (1.04)	0.13 (0.31)
No	1092	7.63 (15.31)	1.67 (3.85)	0.35 (0.83)	0.64 (1.45)	0.29 (0.74)	791	4.65 (11.37)	2.46 (6.62)	0.33 (0.83)	0.58 (1.30)	0.18 (0.36)
Maternal Education level												
High school or less	310	7.79 (16.53)	1.85 (4.19)	0.34 (0.82)	0.65 (1.53)	0.29 (0.74)	226	3.61 (10.95)	2.06 (6.12)	0.25 (0.78)	0.49 (1.07)	0.16 (0.30)
High school + 1–4 years	578	7.73 (14.96)	1.62 (3.69)	0.34 (0.80)	0.63 (1.29)	0.28 (0.73)	414	4.69 (11.54)	2.48 (6.75)	0.33 (0.81)	0.58 (1.32)	0.17 (0.35)
High school + >4 years	243	7.42 (14.91)	1.61 (3.78)	0.38 (0.92)	0.65 (1.40)	0.30 (0.79)	176	5.24 (11.85)	2.69 (6.37)	0.37 (0.98)	0.64 (1.31)	0.20 (0.43)
Breastfeeding duration												
<20 weeks	347	8.14 (16.81)	1.86 (4.15)	0.36 (0.83)	0.67 (1.50)	0.28 (0.76)	243	2.55 (6.48)	1.21 (3.33)	0.17 (0.41)	0.35 (0.77)	0.14 (0.28)
20–40 weeks	356	7.75 (15.59)	1.65 (3.85)	0.35 (0.87)	0.66 (1.47)	0.29 (0.74)	251	5.05 (10.27)	2.71 (7.08)	0.35 (0.80)	0.61 (1.18)	0.18 (0.32)
>40 weeks	337	7.32 (14.30)	1.57 (3.57)	0.34 (0.78)	0.62 (1.39)	0.29 (0.85)	232	6.76 (14.32)	3.15 (7.07)	0.47 (0.99)	0.76 (1.48)	0.22 (0.48)
Maternal age												
<25 yrs	110	7.67 (15.59)	2.09 (4.19)	0.34 (0.81)	0.67 (1.50)	0.28 (0.73)	67	3.49 (11.17)	2.01 (5.72)	0.25 (0.78)	0.44 (1.36)	0.14 (0.36)
25–29 yrs	386	7.96 (16.46)	1.83 (4.14)	0.36 (0.80)	0.69 (1.48)	0.29 (0.73)	280	4.64 (10.99)	2.62 (7.34)	0.33 (0.82)	0.58 (1.20)	0.18 (0.36)
30–34 yrs	434	7.63 (15.17)	1.62 (3.84)	0.34 (0.86)	0.63 (1.35)	0.29 (0.82)	318	4.55 (11.01)	2.27 (6.66)	0.31 (0.82)	0.58 (1.31)	0.17 (0.36)
>34 yrs	214	7.17 (14.68)	1.30 (3.20)	0.34 (0.81)	0.61 (1.16)	0.27 (0.72)	165	4.63 (11.62)	2.24 (6.09)	0.36 (0.80)	0.61 (1.30)	0.18 (0.35)

Abbreviations: PFAS: Per- and polyfluoroalkyl substances; PFOS: perfluorooctane sulfonic acid; PFOA: perfluorooctanoic acid; PFHxS: perfluorohexane sulfonic acid; PFNA: perfluorononanoic acid; PFDA: perfluorodecanoic acid.

Limit of Quantification (LOQ) was 0.03 ng/mL for all PFAS compounds.



**Fig. 2.** Cumulative rate of antibiotic prescriptions from 0 to 8 years of age according to exposure to maternal PFAS concentration in the 10th, 50th, and 90th percentile.

Abbreviations: PFAS: Per- and polyfluoroalkyl substances; PFOS: perfluorooctane sulfonic acid; PFOA: perfluorooctanoic acid; PFHxS: perfluorohexane sulfonic acid; PFNA: perfluorononanoic acid; PFDA: perfluorodecanoic acid.

Estimated among 1425 mother-child pairs using poisson regression with restricted cubic splines with four knots placed at the 5th, 25th, 75th, and 95th age percentile. Multiple redeemed prescriptions within 21 days were counted towards the same event. Missing information on maternal PFAS concentration was imputed using Multiple Imputation by Chained Equation (MICE).

### Ethical approval

The study was approved by the Regional Scientific Ethical Review Committee for Southern Denmark (ProjectID S-20180174), the Danish Data Protection Agency (J.No.19/14001), the Local Data Protection Board (No.11.083), and the Danish Health Data Authority (No. FSEID-00004763), and was performed in accordance with the Helsinki Declaration II. At enrollment, all parents provided written informed consent for participation. Prior to clinical examinations, all parents received written information about the study.

### 3. Results

The characteristics of the imputed cohort ( $n = 1425$  children) resembled the characteristics of the total OCC population (Table 1). For the complete case analyses, 1026 and 709 children were included for analysis with mPFAS and cPFAS concentrations, respectively (Fig. 1). Distribution of baseline characteristics for the complete case analyses was similar to both the imputed cohort and total OCC (Table 1), in terms of maternal age (38.2–38.5 % within the age group 30–34 years), sex of the child (52.8–55.9 % male), parity (55.2–57.4 % primipara), maternal pre-pregnancy BMI (59.4–64.3 % with BMI 18–24 kg/m<sup>2</sup>), and maternal level of education (47.8–50.8 % with high school + 1–4 years of education).

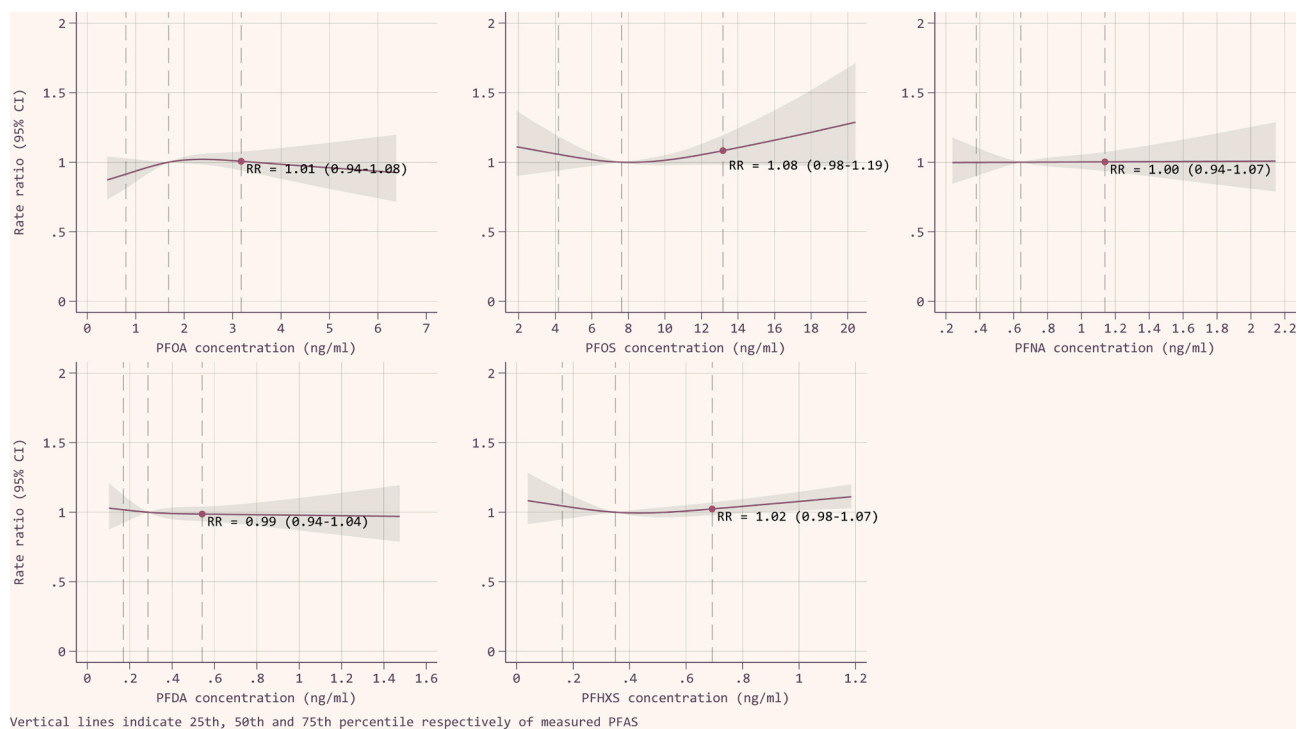
Maternal PFAS concentrations were measured in 1144 mothers and were highest for PFOS (median 7.63 ng/mL, 95th percentile 15.19 ng/mL) followed by PFOA (median 1.68 ng/mL, 95th percentile: 3.85 ng/mL) (Table 2). Observed PFOS and PFOA concentrations were higher in mothers with male offspring, primiparity, lower level of maternal

education, or shorter duration of breastfeeding. cPFAS concentrations were measured in 830 children and were highest for PFOS (median 4.56 ng/mL, 95th percentile: 11.22 ng/mL), followed by PFOA (median 2.43 ng/mL, 95th percentile: 6.40 ng/mL). Observed PFAS concentrations were higher in children who were female, born to primiparous mothers, or whose mothers had lower BMI, did not smoke during pregnancy, had higher level of education, or breastfed for a longer duration (Table 2).

Overall, 84 % of all children redeemed one or more antibiotic prescriptions during the study period. The median number of antibiotic prescriptions was two (10th percentile 0 prescriptions, 90th percentile 7 prescriptions), and the median age at the time of redeeming the first antibiotic prescription was 1.1 years. The expected median cumulative rates of redeemed antibiotic prescriptions at eight years of age in the imputed cohort were 2.43 for mPFOS, 2.59 for mPFOA, 2.56 for mPFNA, 2.50 for mPFHxS, and 2.56 for mPFDA (Table A.2). The 10th and 90th percentile rates showed minimal deviation (Fig. 2). The expected cumulative rates of redeemed antibiotic prescriptions from birth to eight years of age follow a similar pattern for children exposed in utero to low, median, or high concentrations of mPFAS. The cumulative rates of antibiotic prescriptions slightly level off after four years of age. Complete case analyses yielded comparable results to analyses based on the imputed datasets (Figure A.2, Table A.2).

We observed no differences in the number of antibiotic prescriptions at eight years of age when comparing median and high PFAS concentrations measured in both pregnancy and at 18 months of age (rate ratio PFOA 1.01, 95 % confidence interval 0.94–1.08; PFOS 1.08, 0.98–1.19; PFNA 1.00, 0.94–1.07; PFDA 0.99, 0.94–1.04; PFHxS 1.02, 0.98–1.07) (Fig. 3).

The expected cumulative rates of redeemed antibiotic prescriptions



**Fig. 3.** Rate ratio of antibiotic prescriptions according to exposure to maternal PFAS concentration in the 10th, 50th, and 90th percentile. Abbreviations: PFAS: Per- and polyfluoroalkyl substances; PFOS: perfluorooctane sulfonic acid; PFOA: perfluorooctanoic acid; PFHxS: perfluorohexane sulfonic acid; PFNA: perfluorononanoic acid; PFDA: perfluorodecanoic acid. Estimated among 1425 mother-child pairs using poisson regression with restricted cubic splines with four knots placed at the 5th, 25th, 75th, and 95th age percentile. Multiple redeemed prescriptions within 21 days were counted towards the same event. Missing information on maternal PFAS concentration was imputed using Multiple Imputation by Chained Equation (MICE).

from 18 months to eight years of age follow a similar pattern for children with low, median, or high concentrations of all cPFAS at 18 months of age (Fig. 4, Table A.3). However, there was a tendency for children with low cPFAS concentrations to receive slightly more antibiotic prescriptions compared with children with median and high cPFAS concentrations (Fig. 4, Table A.3). Notably, the expected number of antibiotic prescriptions for eight-year-old children with low cPFAS concentrations ranged from 2.12 to 2.18, whereas for those with high cPFAS concentrations, it ranged from 1.56 to 1.75, with the confidence intervals overlapping (Table A.3). The cumulative rates of antibiotic prescriptions slightly level off after four years of age. The results were robust across complete case and imputed model (Figure A.3, Table A.3).

#### 4. Discussion

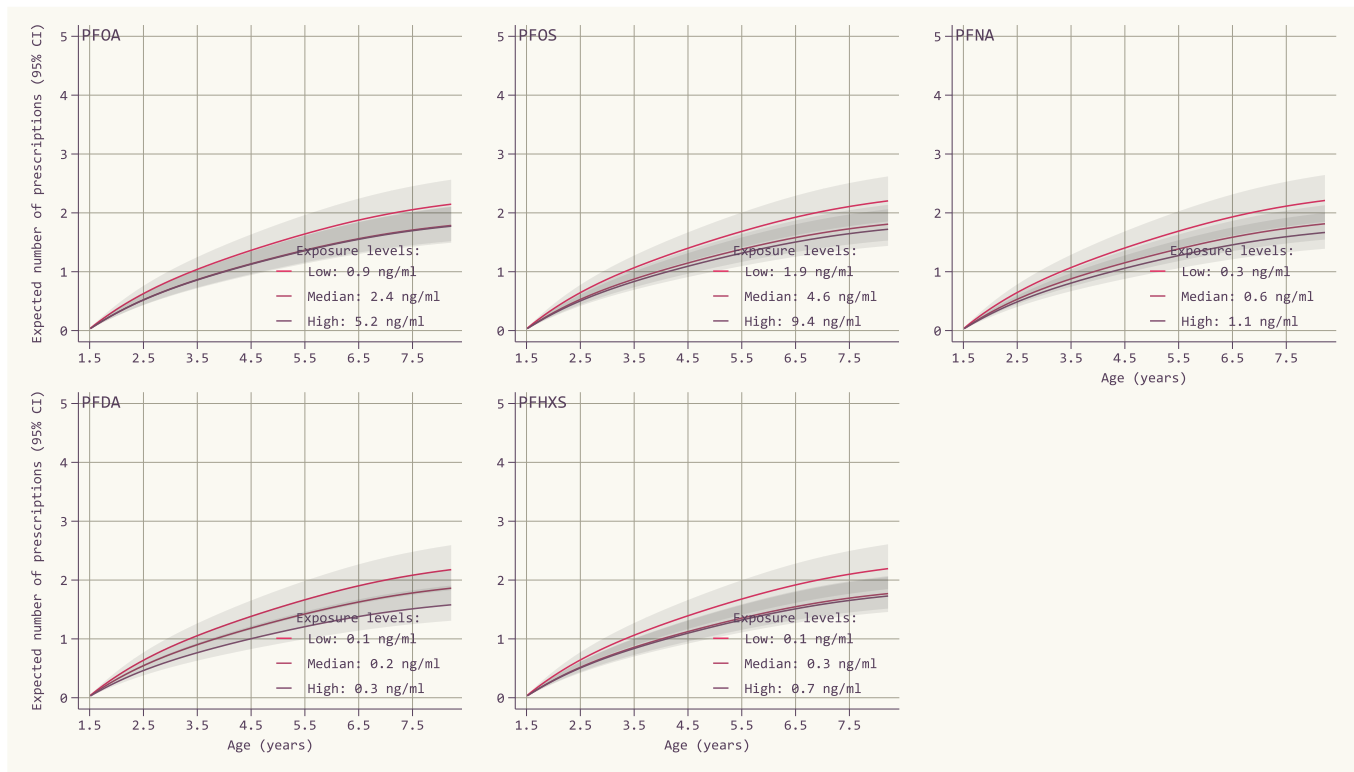
We found no associations between in utero or early childhood PFAS exposure and the expected number of antibiotic prescriptions, as the cumulative rates of prescriptions were similar across children with low, median, and high concentrations of both maternal and child PFAS. However, children with low concentrations of PFAS at 18 months had a slight tendency for more prescriptions at eight years of age.

The present investigation benefited from the prospective follow-up including measures of PFAS exposure in both the mother and child from the OCC. Selection bias is an inevitable challenge in cohort studies with active participation, but the OCC benefitted from a high participation rate (43 %), which helped minimize this potential selection bias. The present investigation was limited to mother-child pairs with information on two out of three of the following variables: mPFAS concentration, cPFAS concentration, and/or breastfeeding duration. Mothers with complete information on breastfeeding duration may be more health-conscious and thus, restricting the cohort to only children with available breastfeeding duration data could introduce selection bias.

Reassuringly, the covariate characteristics of the included mother-child pairs resemble the characteristics of the entire OCC. We adjusted the analyses for measures of socio-economic status likely associated with health consciousness including maternal education and smoking status to minimize the potential selection bias. We undertook the main analyses in a cohort eligible for imputation to maintain statistical power and minimize potential selection bias. The imputation model assumes that the variables were missing at random. We validated the imputation using trace plots and through comparison of imputed and observed values, and we found no major outliers.

We included antibiotic prescriptions as a proxy for the less severe infections that can be handled outside hospital settings. The data from the Danish prescription registry does not allow for distinguishing between different types of infections or the specific indications for use (Diop et al., 2021). Importantly, differences in health care seeking behavior among parents and treatment thresholds among general practitioners may result in misclassification when using antibiotic prescriptions as a marker of infectious diseases (Skajaa et al., 2022). The differential misclassification may both over- and underestimate childhood infection episodes. Some children may be treated with antibiotics for conditions not requiring antibiotics e.g. viral infections and some children may not be treated even if antibiotics are indicated. Redeemed antibiotic prescriptions thus reflect a diverse group of conditions, and the lacking specificity for correctly classifying infections along with lower severity of infections represented entail that antibiotic prescriptions serve as a poor marker of childhood infections. Accordingly, these limitations may explain why we could not confirm previous findings of associations between PFAS exposure and childhood infections (Dalsager et al., 2021a; Goudarzi et al., 2017; Granum et al., 2013; Impinen et al., 2018, 2019).

We found that children with low concentrations of PFAS had a slight tendency for more antibiotic prescriptions at eight years of age, which



**Fig. 4.** Cumulative rate of antibiotic prescriptions from 18 months to 8 years of age according to the child's PFAS concentration in the 10th, 50th and 90th percentile.

Abbreviations: PFAS: Per- and polyfluoroalkyl substances; PFOS: perfluorooctane sulfonic acid; PFOA: perfluorooctanoic acid; PFHxS: perfluorohexane sulfonic acid; PFNA: perfluorononanoic acid; PFDA: perfluorodecanoic acid.

Estimated among 1425 children using poisson regression with restricted cubic splines with four knots placed at the 5th, 25th, 75th, and 95th age percentile. Multiple redeemed prescriptions within 21 days were counted towards the same event. Missing information on PFAS concentration was imputed using Multiple Imputation by Chained Equation (MICE).

should be interpreted with caution and is probably due to residual confounding by breastfeeding. Breastfeeding is associated with lower infection rates in early childhood (Kramer and Kakuma, 2012), but is also a source of PFAS exposure (EFSA CONTAM Panel et al., 2020). Therefore, the higher cumulative number of antibiotic prescriptions observed among children with low cPFAS concentrations could partly be explained by confounding from shorter durations of breastfeeding. However, it was not possible to separate the different effects associated with breastfeeding, including the potential detrimental effects of PFAS exposure and the potential beneficial effects generally attributed to breastfeeding.

Several studies have investigated the association between maternal PFAS concentration and risk of infectious diseases in the offspring (Dalsager et al., 2021a; Dalsager et al., 2016; Fei et al., 2010; Goudarzi et al., 2017; Granum et al., 2013; Impinen et al., 2019; Impinen et al., 2018; Okada et al., 2012; von Holst et al., 2021). However, to our knowledge, no studies have investigated the association between prenatal and early childhood PFAS exposure and infections measured by use of antibiotics. In a previous study undertaken in the OCC, maternal PFAS exposure in the highest tertile was associated with increased odds of having days with fever ( $>38$  C),  $OR_{PFOS} = 2.35$  and  $OR_{PFOA} = 1.97$ , respectively (Dalsager et al., 2016). Similarly, a doubling in maternal PFOS concentration was associated with a 23 % increased rate of hospitalizations for infections; and a doubling in PFOA and PFOS, respectively, were associated with 27 % and 54 % higher rates of lower respiratory tract infections (Dalsager et al., 2021a). Conversely, in a different cohort of Danish mother-child pairs followed from 1996 to 2002, Fei et al. observed an inverse association between higher maternal PFAS concentration and offspring rate of infectious disease

hospitalization (Fei et al., 2010). The inconsistent findings for infectious disease hospitalizations in Denmark may reflect the considerably higher PFAS concentrations in the study by Fei et al., as the samples were collected from 1996 to 2002 (Dalsager et al., 2021a). Three studies from distinct Norwegian cohorts (Granum et al., 2013; Impinen et al., 2018, 2019), and one from Japan (Goudarzi et al., 2017), also reported associations between maternal PFAS and higher rates of parent-reported infections in the offspring. Another study from Japan found no association between maternal PFAS and childhood infections, however, this study only included 343 infants, and only assessed otitis media (Okada et al., 2012). Previous studies have reported associations between child PFAS concentrations and antibody responses to common childhood vaccinations and indicated that PFAS measured in children may better reflect immunological function than maternal PFAS concentrations (Abraham et al., 2020; Grandjean et al., 2012; Granum et al., 2013; Sigvaldsen et al., 2024). PFAS has been found to have immunosuppressing properties in both epidemiological (Grandjean et al., 2012; Granum et al., 2013), toxicological, and animal models (DeWitt et al., 2019). Thus the link between higher PFAS concentrations and risk of infections observed in previous epidemiological studies is both biologically plausible and cooperated by toxicological evidence. Accommodated by insights from in vitro and animal studies, previous investigations furthermore suggest that PFAS may especially increase risk and severity of lower respiratory tract infections (LRTI) (Dalsager et al., 2021a; Kvaalem et al., 2020). However, only one Norwegian cross-sectional study found association between child PFAS measured at ten years of age and higher rates of LRTI (Kvaalem et al., 2020). We were unable to examine the specific indications for antibiotic use in our data, but the total number of antibiotic prescriptions may not be a good

measure of infections.

## 5. Conclusion

Overall, in utero and early childhood exposure to PFAS was not associated with a higher expected number of antibiotic prescriptions from birth to eight years of age. These findings contrast with previous studies suggesting that PFAS exposure is associated with increased risk of infections. The observed difference may be due to the limited precision of antibiotic prescriptions as a marker for infections and relationship with health care-seeking behaviour.

## CRedit authorship contribution statement

**Nete Lundager Klokke Rausgaard:** Writing – review & editing, Writing – original draft, Visualization, Methodology. **Lise Gehrt:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology. **Martin Thomsen Ernst:** Writing – review & editing, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Iben Have Beck:** Writing – review & editing, Methodology. **Flemming Nielsen:** Writing – review & editing, Resources, Methodology, Investigation. **Helene Kildegaard:** Writing – review & editing, Methodology. **Anton Pottegård:** Writing – review & editing, Resources, Methodology, Conceptualization. **Tina Kold Jensen:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Lars Christian Lund:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Project administration, Methodology, Formal analysis, Conceptualization.

## Funding

The work was supported by the European Research Council (101141686); the Independent Research Fund Denmark (2034-00081B); Sygeforsikring Danmark (2021-0173); Odense University Hospital; Region of Southern Denmark; and The Municipality of Odense. The funding sources had no involvement in the study design, collection, analysis, and interpretation, writing of the report, or the decision to submit the article for publication.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

None.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijheh.2025.114734>.

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