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# A case-cohort study of per- and polyfluoroalkyl substance concentrations and incident prostate cancer in the cancer prevention Study-II LifeLink cohort study

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## Abstract

**Introduction:** Per- and polyfluoroalkyl substances (PFAS) are environmentally persistent, potentially carcinogenic chemicals. Previous studies investigating PFAS exposure and prostate cancer yielded mixed findings. We aimed to investigate associations between PFAS exposure and incident prostate cancer in a large cohort of U.S. men, overall and by selected demographic, lifestyle, and medical-related characteristics.

**Methods:** We conducted a case-cohort study among Cancer Prevention Study-II LifeLink Cohort participants who, at baseline (1998–2001), had serum specimens collected and no prior cancer diagnosis. The study included all men diagnosed with prostate cancer (n = 1610) during follow-up (baseline–June 30, 2015) and a random sub-cohort of 500 men. PFAS concentrations [perfluorohexane sulfonic acid (PFHxS), perfluorooctane sulfonate (PFOS), perfluorononanoic



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Ethics approval and consent to participate

At the time of serum sample collection, CPS-II LifeLink Cohort participants provided informed consent for blood sample collection and storage and for future research with the sample. All aspects of the CPS-II cohort study were approved by the Emory University institutional review board.

CRediT authorship contribution statement

**Alyssa N. Troeschel:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Formal analysis, Conceptualization. **Lauren R. Teras:** Writing – review & editing, Resources, Investigation, Conceptualization. **James M. Hodge:** Writing – review & editing, Software, Data curation, Conceptualization. **Juan Rodriguez:** Writing – review & editing, Conceptualization. **Ying Wang:** Writing – review & editing, Conceptualization. **Johnni Daniel:** Writing – review & editing, Conceptualization. **W. Ryan Diver:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Andrea Winquist:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.119560.

acid (PFNA), and perfluorooctanoic acid (PFOA)] were measured in stored serum specimens. We used multivariable Cox proportional hazards models to estimate associations between PFAS concentrations and prostate cancer, overall and by selected characteristics (grade, stage, family history, age, education, smoking status, and alcohol consumption).

**Results:** Prostate cancer hazards were slightly higher among men with concentrations in the highest (Q4) vs lowest quartile (Q1) for PFHxS [hazard ratio (HR) (95% CI): 1.18 (0.88–1.59)] and PFOS [HR (95% CI): 1.18 (0.89–1.58)], but not for PFNA or PFOA. However, we observed heterogeneous associations by age, family history of prostate cancer (PFHxS), alcohol consumption (PFHxS), and education (PFNA). For example, no meaningful associations were observed among men aged <70 years at serum collection, but among men aged ≥70 years, HRs (95% CIs) comparing Q4 to Q1 were PFHxS 1.54 (1.02–2.31) and PFOS 1.62 (1.08–2.44). No meaningful heterogeneity in associations were observed by tumor grade or stage.

**Conclusions:** Our findings do not clearly support an association between the PFAS considered and prostate cancer. However, positive associations observed in some subgroups, and consistently positive associations observed for PFHxS warrant further investigation.

### Keywords

PFAS; Cancer; Prostate cancer; Case-cohort study

## 1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large family of man-made chemicals, widely used in consumer products (e.g., water- and stain-resistant fabrics) and industrial products (e.g., firefighting foam). Early evidence suggesting that perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), two types of PFAS (Buck et al., 2011), may be linked to several adverse health outcomes, including certain cancers, led major producers to phase certain PFAS out of production (Environmental Protection Agency, 2000). Despite this phase out, human exposure to PFAS remains ongoing, as many PFAS, including PFOA and PFOS, do not break down and have already polluted the environment (Agency for Toxic Substances and Disease Registry (ATSDR), 2021). PFAS are detectable in the blood of most people in the United States (U.S.) (Jain, 2018), and people continue to be exposed to PFAS through contaminated water and food products (e.g., eating fish caught from PFAS-contaminated water) (Agency for Toxic Substances and Disease Registry (ATSDR), 2021). Given widespread human exposure to PFAS, it is important to understand their potential effects on cancer development, especially for common cancers like prostate cancer.

Most previous studies that investigated associations of PFAS with prostate cancer incidence and mortality focused on PFOA and PFOS and were conducted among PFAS production plant workers or communities with high exposures due to PFAS-contaminated drinking water. Some studies in these highly exposed populations (Steenland et al., 2015; Vieira et al., 2013; Lundin et al., 2009; Gilliland and Mandel, 1993), but not all (Leonard et al., 2008; Steenland and Woskie, 2012; Consonni et al., 2013; Raleigh et al., 2014), suggested a positive association of PFOA with prostate cancer. Although fewer in number, all studies in populations highly exposed to PFOS suggested a positive association with prostate cancer

(Grice et al., 2007; Olsen et al., 2004). An additional study examined longstanding exposure to drinking water highly contaminated with PFAS (mostly perfluorohexane sulfonic acid [PFHxS] and PFOS, and to a lesser extent, PFOA) in Sweden but found largely inverse associations between higher exposures to the contaminated water (based on a variety of metrics) and prostate cancer (Li et al., 2022). While studies conducted among occupational and highly exposed cohorts can be useful for investigating the health effects of high levels of exposure, most had too few prostate cancer cases or deaths to estimate effects with good precision. Moreover, because PFAS exposure is ubiquitous, it is important to understand whether PFAS exposures at lower exposure levels, like those seen in the general community, can impact prostate cancer risk, but few studies have investigated this link.

To our knowledge, only three studies investigated the association of serum concentrations of specific types of PFAS in general community settings, where PFAS exposure levels were much lower, with the incident prostate cancer risk, yielding mixed findings (Hardell et al., 2014; Eriksen et al., 2009; Rhee et al., 2023). In the first, a prospective case-cohort study among the general Danish population, prostate cancer risk was 30–40% higher, albeit non-statistically significantly, among individuals in the three upper quartiles, relative to the lowest quartile, of serum PFOS (Eriksen et al., 2009). Associations of PFOA with prostate cancer were less clear, although there was a slightly higher risk that was not statistically significant in the highest relative to the lowest PFOA quartile (Eriksen et al., 2009). In the second, a case-control study conducted among the general Swedish population, the authors found no overall association with prostate cancer for a variety of PFAS, but found evidence of an interaction between PFAS and family history of prostate cancer (Hardell et al., 2014). The authors found that the prostate cancer risk was highest among men with a family history of prostate cancer who also had high levels of several individual PFAS, including PFOA, PFOS, and PFHxS, whereas the independent associations of PFAS (among those without a family history) and family history of prostate cancer (among those with low levels of PFAS) with prostate cancer were largely null. In the third, a prospective nested case-control study of U.S. men, the authors found an inverse relationship between PFOA and the risk of aggressive prostate cancer. Associations for other types of PFAS were largely null, except for a possible positive association with PFHxS in overall models and positive associations for PFHxS and PFOS among men with blood draws during later years (1998–2004) (Rhee et al., 2023). The inverse association with PFOA was limited to certain subgroups, such as cases diagnosed ≤3 years of their blood draw, current smokers, and those with nocturia. This highlights the importance of considering heterogeneities in associations across groups defined by various participant characteristics, as doing so may not only help us better understand the complexities of this relationship but also explain discrepancies in findings across studies.

The mixed findings in studies of general population-level PFAS exposures and prostate cancer indicate that our understanding of this potential relationship is incomplete. Therefore, strong, prospective study designs investigating the potential relationship between PFAS and prostate cancer, considering potential heterogeneities by medical and other relevant covariates are still needed. To address these gaps, we investigated whether serum concentrations for 4 PFAS — PFHxS, perfluorononanoic acid (PFNA), PFOA, PFOS — were associated with incident prostate cancer risk in a prospective case-cohort study of men

from the American Cancer Society's (ACS) Cancer Prevention Study-II (CPS-II) LifeLink Cohort study. This study is part of a larger study that investigated PFAS exposure and the incidence of several cancers (e.g., prostate cancer, kidney cancer). In overall models used to examine several cancer types, associations between PFAS serum concentrations and prostate cancer were largely null (Winquist et al., 2023). In the current sub-study, we further investigated associations between PFAS concentrations and prostate cancer, using models that were specifically tailored to prostate cancer and models that investigated potential heterogeneity in PFAS-prostate cancer associations among groups defined by medical (grade, stage, family history of prostate cancer), sociodemographic (age, education), and lifestyle-related (tobacco smoking history, alcohol consumption) factors.

## 2. Materials and methods

### 2.1. Case-cohort study design & population

This case-cohort study was conducted using data from the CPS-II LifeLink Cohort. A case-cohort study design combines the efficiency of case-control studies (e.g., less time consuming and costly than cohort studies) with the benefits of cohort studies (e.g., ability to establish temporality) by sampling all incident cases, and a random sub-cohort (irrespective of disease status) for comparison purposes, from established cohorts (Sharp et al., 2014; PRENTICE, 1986).

**2.1.1. Description of the underlying cohort for the case-cohort study—**The prospective CPS-II LifeLink Cohort study (Calle et al., 2002), is the cohort from which we identified participants for inclusion in the case-cohort study. Briefly, the CPS-II LifeLink Cohort includes a subset of participants from the CPS-II Nutrition Cohort, which includes a subset of participants from the CPS-II mortality cohort (Calle et al., 2002). In 1982, ACS enrolled 1.2 million participants from all 50 U.S. states and the District of Columbia in the CPS-II mortality cohort. During 1992–1993, ACS recruited a subset of CPS-II mortality cohort participants (aged 50–74 years on average, from 21 U.S. states) to participate in the CPS-II Nutrition Cohort. CPS-II Nutrition Cohort participants completed a detailed questionnaire at baseline and received follow-up surveys in 1997 and every two years thereafter to provide updated information regarding lifestyle and newly diagnosed cancers. Later (1998–2001), ACS recruited 39,371 CPS-II Nutrition Cohort participants living in urban and suburban areas of 20 states to participate in the CPS-II LifeLink Cohort, which involved completing a baseline questionnaire and providing a blood sample (1998–2001). All aspects of the CPS-II cohort study were approved by the Emory University Institutional Review Board.

**2.1.2. Case-cohort study selection—**Participants in this case-cohort study were selected using a three-step process. First, among the 17,408 male CPS-II LifeLink Cohort participants, we excluded men who met the following criteria: 1) a previous cancer diagnosis (other than non-melanoma skin cancer) at the time of the blood sample collection (n = 3353); 2) <500 μL of stored serum available (required for PFAS measurements; n = 794); and 3) biologically female (based on genetic testing, n = 3). Second, among the 13,258 men remaining in the eligible cohort, we identified all men diagnosed with incident

prostate cancer (as the first cancer) during follow-up (n = 1610) and considered them case-participants. Third, a random subset of 500 men from the eligible cohort (3.8%) were selected as the comparison sub-cohort, 58 of whom were also considered case-participants. Additional details on the selection process for the case-cohort study were previously published (Winquist et al., 2023).

**2.1.3. Case-participants—**Prostate cancer case-participants in primary analyses included all men with a verified new prostate cancer diagnosis as the first cancer diagnosis (n = 1610), identified during the study follow-up period (from the blood draw through June 30, 2015). Most prostate cancers were self-reported and verified through medical records or linkage with state cancer registries (n = 1587). Additional prostate cancers were identified through linkage with the National Death Index (n = 11) or through the process of verifying another cancer or death (n = 12), with subsequent verification through cancer registry linkage.

To consider potential heterogeneity in PFAS-prostate cancer associations by tumor grade and stage at diagnosis, we conducted additional analyses with a varied case definition to only include cases with a particular tumor grade or stage. For grade, we classified tumors according to Gleason's score as low-grade (Gleason's score <7), intermediate-grade (Gleason's score = 7), and high-grade (Gleason's score >7) and treated these as separate outcomes. For example, for analyses of low-grade prostate cancer, case-participants diagnosed with intermediate- or high-grade prostate cancer were dropped from the analysis unless they were included in the comparison sub-cohort, in which case the participant would be considered only a sub-cohort participant. For stage, we used the American Joint Committee on Cancer (AJCC) staging system to classify tumors as non-advanced stage (AJCC stage 1–2) or advanced stage (AJCC stage 3–4) and conducted analyses similarly to analyses by grade.

We obtained information on tumor characteristics (Gleason's score, degree of tumor differentiation, and AJCC stage at diagnosis) from abstracted medical records or state registries after a participant reported a cancer diagnosis. When Gleason's score was unavailable, we inferred it based on year of diagnosis and degree of tumor differentiation when possible. Additional details on how Gleason's score was reported and inferred can be found in the Supplemental Methods and Supplemental Table 1. We then used Gleason's score (with missing scores inferred when possible) to categorize tumor grade.

## 2.2. Exposures

The primary exposures of interest include 4 PFAS — PFHxS, PFNA, PFOA, and PFOS — obtained from stored serum samples collected from CPS-II LifeLink participants (1998–2001). The samples were tested for linear isomers of PFOA, PFOS, PFNA, PFHxS, perfluorooctane sulfonamide (FOSA), perfluorobutane sulfonic acid (PFBS), and perfluoroheptanoic acid (PFHpA) using high performance liquid chromatography/tandem mass spectrometry by NMS labs. To assess reliability of the measurements, quality control duplicates were included with the study samples for 5% of the samples. Additional details on laboratory testing methods and quality control analyses can be found elsewhere

(Winquist et al., 2023). Although PFBS, PFHpA, and FOSA concentrations were measured, we excluded them from the present analysis. We excluded PFBS because it had a low frequency of detection in the sub-cohort (6.4%), PFHpA because of low variability in the sub-cohort (values ranged 0.04–1.6 ng/mL), and FOSA because quality control analyses indicated poor reliability (the intraclass correlation coefficient ([ICC] for FOSA was 0.32, whereas the ICC for PFHxS, PFNA, PFOA, and PFOS were >0.95).

We modeled each of the 4 PFAS categorically, based on distribution quartiles or tertiles in the sub-cohort, and continuously. Untransformed PFAS measures were right-skewed (Fig. 1), so we $\log_2$-transformed the data, after which the distributions were approximately normal. We examined the possibility of a non-linear relationship between each of the $\log_2$-transformed PFAS types and prostate cancer non-parametrically with restricted cubic splines (Durrleman and Simon, 1989). Tests for non-linearity were conducted using the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. Likelihood ratio tests indicated that the relationships (if any existed) between the $\log_2$-transformed PFAS types and hazard of prostate cancer were approximately linear (all $P > 0.10$, Supplemental Fig. 1). Therefore, analyses considered the $\log_2$-transformed PFAS concentrations as continuous measures.

### 2.3. Covariates

We selected covariates for models *a priori* based on previous literature (Hardell et al., 2014; Eriksen et al., 2009; Rhee et al., 2023; Barry et al., 2013) and biological plausibility as a confounder: participant age at serum collection, year of serum collection, race and ethnicity, education, smoking status, and alcohol use. We also considered prostate-specific antigen (PSA) screening, occupation, and body mass index (BMI) as covariates but excluded them from models for reasons described in the Supplemental Methods. Participants self-reported all variables. We obtained information on race/ethnicity, education, occupation, and PSA screening from the 1982 CPS-II baseline questionnaire, age at and year of serum collection from the CPS-II LifeLink baseline survey, and smoking, alcohol, and BMI from the 1997 CPS-II Nutrition Cohort follow-up survey (or earlier surveys if the information was missing on the 1997 survey). A family history of prostate cancer (considered in statistical interaction analyses) was defined as a report of a family history of prostate cancer in a first degree relative by a participant on either the 1982 or 1997 survey.

### 2.4. Statistical analyses

Demographic and medical characteristics of study participants were summarized using frequencies and percentages, or medians and inter-quartile ranges, among case-participants and sub-cohort participants. To investigate associations between each PFAS and prostate cancer (regardless of grade or stage), we used multivariable Cox proportional hazards models, weighting study subjects according to the Prentice method (PRENTICE, 1986) to estimate hazard ratios (HR) and 95% confidence intervals (95% CI). Variances were estimated using the robust variance estimator proposed by Barlow (1994). All models adjusted for age at blood sample collection (single year categories), year of blood sample collection (1998–1999, 2000–2001), education (high school graduate or less, some college or trade school, college graduate, graduate school or higher), alcohol use (non-drinker, 1

drink/day or less, 2 drinks/day or more), smoking status (never, former/current smoker), and race and ethnicity (non-Hispanic white, other). For comparison, we also used a minimally adjusted model that only included age and year of blood sample collection as covariates. To consider potential heterogeneity in PFAS-prostate cancer associations by tumor grade and stage at diagnosis, we conducted additional analyses, varying our case definitions to only include cases of a particular grade (low, intermediate, high) or stage (non-advanced, advanced) and treated these as separate outcomes.

We conducted several analyses to assess statistical interaction. First, we investigated associations between each of the PFAS types and prostate cancer within strata of family history of prostate cancer (yes, no) from models that included terms for the PFAS of interest, family history of prostate cancer, and their interaction, while adjusting for the other covariates. We assessed the statistical significance of interactions on the multiplicative scale by comparing a full model that included the interaction term(s) to a reduced model without the interaction term(s) using the likelihood ratio test. Similar to Hardell and colleagues (Hardell et al., 2014), we also investigated the joint associations of PFAS and family history of prostate cancer using the single referent group approach, in which individuals with PFAS concentrations in the lowest tertile without a family history of prostate cancer served as the referent group. We then assessed interactions on the additive scale through the calculation of relative excess risks due to interaction (RERI) and the corresponding confidence intervals (Li and Chambless, 2007). Second, to investigate potential heterogeneity in PFAS-prostate cancer associations by demographic and lifestyle characteristics, we conducted multivariable Cox proportional hazards models with interaction terms to estimate PFAS associations with prostate cancer within strata of age (<70 years, ≥70 years), education (less than college graduate, college graduate or higher), smoking status (never smoker, current/former smoker), and alcohol consumption (yes, no). We described our rationale for selecting variables for interaction analyses in the Supplemental Methods.

For all participants, the underlying time scale was time from serum collection. Follow-up began on the date on which participants completed the blood draw. Follow-up ended on the earliest date of the following events: 1) the participant's first cancer diagnosis (regardless of cancer type); 2) the date of last survey return for a participant who became lost to follow-up; 3) death, or 4) June 30, 2015 (study end date). If a participant died (identified through the National Death Index) before the next survey was sent out (generally ≤2 years after the last survey), that participant was not considered lost to follow-up and instead, was censored upon the date of death.

Statistical analyses were performed using SAS (version 9.4; SAS Institute, Cary, NC) software. PFAS distribution plots were produced using R Statistical Software (version 4.0.3). We considered a two-tailed $P < 0.05$ to be statistically significant. We assessed the proportional hazards assumptions using the ZPH option available in SAS PROC PHREG, which is based on the correlation between the weighted Schoenfeld residuals and failure times. Both age and smoking status appeared to violate the proportional hazards assumption in most models. Therefore, models controlling for age stratified the baseline hazard by age (single year categories), and models controlling for smoking status stratified the baseline hazard by smoking status.

# 3. Results

## 3.1. Study population

The median age at serum collection among sub-cohort participants was 70 years (25th–75th percentiles: 66–74 years; Table 1). Sub-cohort participants were predominantly non-Hispanic white (98%), college graduates or with higher education (53%), and former smokers (58%). The median (25th–75th percentiles) of PFAS serum concentrations in ng/mL among sub-cohort participants were as follows: PFHxS = 3.3 (2.1–5.3); PFNA = 0.7 (0.5–1.0); PFOA = 5.2 (4.0–7.0); and PFOS = 18 (13.5–25.5) [see Fig. 1 for PFAS distributions]. Additional details about PFAS concentrations in the cohort can be found elsewhere (Winquist et al., 2023). Case-participants and sub-cohort participants were largely similar in terms of sociodemographic and lifestyle characteristics (Table 1). Among sub-cohort participants, the total amount of follow-up time was 5458.6 person-years (median 13.1, 25th–75th percentiles: 7.0–15.0 years). Among case-participants, the median time from blood draw to prostate cancer diagnosis was 4.9 years (25th–75th percentiles: 2.1–8.0 years).

## 3.2. PFAS and overall prostate cancer

In multivariable models investigating the overall risk of prostate cancer, men with PFHxS concentrations in quartiles 2–4 (Q2–Q4), relative to men with concentrations in the lowest distribution quartile (Q1), had a higher hazard of prostate cancer, although only Q2 reached statistical significance ($HR_{Q2vsQ1}$ = 1.36, 95% CI: 1.01, 1.83) [Table 2]. Non-parametric analyses using restricted cubic splines showed similar trends, in which the hazard of prostate cancer appeared to increase per doubling of PFHxS concentrations until it plateaued at approximately 1.7 (corresponding to a value of 3.2 ng/mL in untransformed PFHxS concentration; Supplemental Fig. 1A). However, the confidence intervals were wide and results of the likelihood ratio test comparing the model with only the linear term to the model with the restricted cubic spline models indicated that inclusion of the cubic splines did not significantly improve the fit of the model and our data were also compatible with a linear relationship, if any existed. When modeled continuously, the HR per doubling of PFHxS concentration was 1.06 (95% CI: 0.96, 1.17).

In multivariable models, relative to men with PFOS concentrations in Q1, men with concentrations in Q3–Q4 had a slightly higher hazard of prostate cancer (e.g., $HR_{Q4vsQ1}$ = 1.18, 95% CI: 0.89, 1.58). Non-parametric models using restricted cubic splines revealed a mostly linear relationship between PFOS concentrations and prostate cancer risk, although there was some suggestion of a possible plateau at 4.2 (corresponding to a value of 18.4 ng/mL in untransformed PFOS concentration), after which the risk of prostate cancer appeared to decrease with increasing PFOS concentrations (Supplemental Fig. 1B). However, this should be interpreted with caution as confidence intervals were wide. When modeled continuously, the HR per doubling of PFOS concentration was 1.02 (95% CI: 0.90, 1.16). We did not observe any meaningful associations or trends with prostate cancer for PFNA or PFOA.

### 3.3. PFAS and prostate cancer by grade and stage

Among case-participants, the number of men diagnosed with low-, intermediate-, and high-grade tumors was 814 (33 of whom were also in the sub-cohort), 490 (12 of whom were also in the sub-cohort), and 278 (12 of whom were also in the sub-cohort), respectively. We excluded 27 case-participants not in the sub-cohort for whom grade category could not be inferred from grade-specific analyses. One case-participant in the sub-cohort did not have information available to infer grade and was included in the sub-cohort only. In multivariable models, we observed some heterogeneity in PFAS-prostate cancer associations according to grade. The hazard of low- and high-grade prostate cancer, but not intermediate-grade prostate cancer, was higher among individuals with higher PFHxS and PFOS concentrations (Table 3).

Among case-participants, the number of men diagnosed with non-advanced and advanced stage prostate cancer were 1456 (50 of whom were also in the sub-cohort) and 132 (6 of whom were also in the sub-cohort), respectively. We excluded 20 case-participants who were missing information on stage from stage-specific analyses (2 case-participants who were also in the sub-cohort and missing information on stage were included in the sub-cohort only). We did not observe any clear patterns in PFAS-prostate cancer associations by stage (Table 3). However, there was some suggestion that men with the highest concentrations of PFNA (Q4), relative to those with the lowest concentrations (Q1), had a higher hazard of advanced stage prostate cancer (HR = 1.52, 95% CI: 0.83, 2.78) but not non-advanced stage prostate cancer (HR = 1.01, 95%CI: 0.74, 1.37).

### 3.4. Interaction with family history of prostate cancer

A first-degree relative with prostate cancer was reported by 270 case-participants and 72 sub-cohort participants and was positively associated with prostate cancer (HR = 1.42, 95% CI: 1.07, 1.88) in models adjusting for age and year at blood sample collection, education, alcohol consumption, and race/ethnicity. In interaction models, we found evidence of a statistically significant interaction between PFHxS and family history on the multiplicative scale ($p = 0.0007$ for the $\log_2$-PFAS measure) but not the additive scale (e.g., the RERI comparing men who had a family history of prostate cancer and PFAS concentrations in the highest tertile to men without a family history and concentrations in the lowest tertile was −0.77, 95% CI: −2.0, 0.45; Table 4). Specifically, PFHxS was positively associated with prostate cancer among men without a family history (HR per doubling of concentration = 1.10, 95% CI: 0.99, 1.23) but was inversely associated with prostate cancer among men with a family history (HR per doubling of concentration = 0.87, 95% CI: 0.70, 1.09). We did not observe meaningful differences in associations among men with and without a family history of prostate cancer for the other PFAS.

### 3.5. Interaction with covariates

Associations of PFHxS, PFNA, PFOA, and PFOS with prostate cancer were generally stronger among men aged ≥70 years at the time of blood sample collection (Table 5). For example, among men aged ≥70 years, the hazard of prostate cancer increased with doubling concentrations of PFHxS (HR = 1.13, 95% CI: 0.99, 1.29), PFNA (HR = 1.11, 95% CI: 0.95, 1.30), PFOA (HR = 1.23, 95% CI: 0.98, 1.54); and PFOS (HR = 1.25, 95% CI: 1.05,

1.49). However, among men aged <70 years, associations of PFHxS, PFNA, and PFOS with prostate cancer were largely null or tended to be inverse (e.g., PFOS HR = 0.86, 95% CI: 0.72, 1.03). The higher hazard of prostate cancer among older men was even more apparent when quartiles of PFAS concentrations were considered. For example, among men aged ≥70 years, the HRs (95% CIs) comparing the highest to lowest concentration quartiles were 1.54 (1.02, 2.31) for PFHxS, 1.51 (0.98, 2.31) for PFNA, and 1.62 (1.08, 2.44) for PFOS. While there were some differences observed in interaction models with other sociodemographic and lifestyle characteristics, few patterns emerged. For example, the hazard of prostate cancer increased with doubling concentrations of PFHxS among men who reported not consuming alcohol (HR = 1.20, 95% CI: 1.00, 1.43) but the association among men who consumed alcohol was less clear. In addition, there was some suggestion of an interaction between PFNA (and possibly PFHxS and PFOS) and education. For example, the hazard of prostate cancer increased with doubling concentrations of PFNA among men with a college graduate or higher education (HR = 1.15, 95% CI: 0.99, 1.33) but decreased among men with less than a college education (HR = 0.87, 95% CI: 0.72, 1.04).

In a post-hoc analysis to further explore the interactions between PFAS and age, we categorized age into 4 levels: <65 years, 65–69 years, 70–74 years, and 75+ years. The higher HRs observed among men aged ≥70 years appeared primarily driven by the 70–74-year age group (Supplemental Table 2). For example, among men aged 70–74 years, the HRs (95% CIs) comparing the hazard of prostate cancer per doubling concentrations of PFAS were 1.30 (1.10, 1.5) for PFHxS, 1.21 (1.00, 1.46) for PFNA, 1.33 (0.98, 1.80) for PFOA, and 1.26 (1.01, 1.58) for PFOS. Due to the concern that sparse data could bias these findings, we also conducted unadjusted models investigating the association between $\log_2$-transformed PFAS concentrations and prostate cancer risk, by only including the PFAS of interest, dummy variables representing the product terms between the PFAS of interest and each of the dummy coded age categories, and stratifying the baseline hazard on category of age (<65 years, 65–69 years, 70–74 years, and 75+ years). Results from unadjusted models yielded largely similar findings (Supplemental Table 3).

## 4. Discussion

In summary, findings from our large, prospective, case-cohort study do not provide strong, consistent evidence that higher serum concentrations of PFAS are associated with a higher risk of prostate cancer in a cohort of U.S. men with PFAS exposure levels similar to the general population. In addition, we found limited evidence that PFAS associations with prostate cancer vary by tumor characteristics like grade and stage. Nonetheless, our results provide some evidence that some types of PFAS might be associated with the risk of prostate cancer, particularly in certain sub-populations, including men without a family history of prostate cancer (PFHxS), men who did not consume alcohol (PFHxS), and possibly men with a higher education (strongest evidence for PFNA but also possibly PFHxS and PFOS) and men aged 70–74 years (PFHxS, PFNA, PFOA, and PFOS), which warrant further investigation. While it is unclear whether the observed positive associations in these subgroups represent true effects or if they are a product of bias or chance, our findings suggest that these subgroups warrant further investigation, especially for PFHxS given the consistent positive findings across multiple subgroups. This information is important as

researchers continue efforts to better understand the relationship between PFAS exposure and prostate cancer incidence, particularly among men in the general population who typically have lower exposure levels.

In our main analysis investigating *overall* prostate cancer risk (regardless of grade or stage), we observed an 18–36% higher hazard among men with PFHxS concentrations >2.00 ng/mL (Q2–Q4), relative to men with concentrations ≤2.00 ng/mL (Q1), although HRs ranging from 0.88 to 1.83 also appeared reasonably compatible with our data. It was not clear whether this relationship was approximately linear, plateaued (suggesting a threshold effect), or if the lower hazard observed in the lowest quartile was due to chance. Our findings are consistent with those from a previous retrospective case-control study among a general population of Swedish men, where the odds of prostate cancer were 30% higher (95% CIs ranged from 20% lower to 90% higher odds) among men with PFHxS concentrations above the median (0.87 ng/mL) relative to those at or below it (Hardell et al., 2014). Similarly, findings from a prospective nested case-control study suggested that PFHxS may be associated with a higher risk of aggressive prostate cancer (defined as stage III or IV tumors, or Gleason score ≥8, or Gleason 7 and death from prostate cancer) (Rhee et al., 2023), though confidence intervals were wide and encompassed the null. Interestingly, when analyses were restricted to men with blood draws during 1998–2004 (similar to the time period in which blood draws were conducted in our study, 1998–2001), the authors reported 21% higher odds of aggressive prostate cancer per doubling concentration of PFHxS (95% CI: 1%–46%), whereas associations were null for earlier calendar years. While our study differed in that we investigated the risk of prostate cancer of all grades and stages, our subgroup analyses that restricted the case definition to include only stage III or IV tumors, or tumors with a Gleason score ≥8, revealed similar findings to our main analysis.

In our study, we observed an estimated 18% higher hazard of prostate cancer among men with PFOS concentrations in the highest relative to the lowest quartile. However, HRs ranging from a 11% lower hazard (modest negative association) to a 58% higher hazard (substantial positive association) were also reasonably compatible with our data. While our results from continuous models were largely null, in non-parametric models, the risk of prostate cancer appeared to increase with increasing $\log_2$-transformed PFOS concentrations until it plateaued at 4.2, after which the risk of prostate cancer appeared to decrease, which could account for the null findings observed in continuous models. Alternatively, the association between PFOS and prostate cancer could truly be null, and the slightly higher risk observed in the highest quartile of exposure might be due to chance. Two previous studies investigated the link between PFOS and risk of prostate cancer (of any grade or stage) in men with general population-level exposures. The first study, a case-cohort study by Eriksen and colleagues (Eriksen et al., 2009), found a 35%–38% higher risk of prostate cancer among individuals with PFOS concentrations in quartiles 2–4, relative to quartile 1, suggesting a potential threshold effect. The HRs observed in the study by Eriksen and colleagues were somewhat higher than those found in our study, potentially due to higher exposure levels (e.g., the median PFOS concentration was 35 ng/mL compared to a median of 18 ng/mL in our sub-cohort). However, it is important to note that our measurements may not be directly comparable, as ours only included the linear isomers, and others may have also included branched isomers, which could account for the differences in exposure levels.

In contrast, in the second study, a case-control study by Hardell and colleagues (Hardell et al., 2014), the authors observed a null association between PFOS exposure and prostate cancer risk. However, the median PFOS value among controls in the aforementioned study (8.3 ng/mL) was lower than that found among the sub-cohort in our study (18 ng/mL), which may have contributed to their null findings. In addition, the Hardell study was limited in that it was based on few prostate cancer cases (n = 252) and a retrospective study design (PFAS were measured after prostate cancer diagnosis). The PFOS findings reported by Rhee and colleagues in relation to aggressive prostate cancer were similar to those described above for PFHxS, in which associations were largely null, except when restricted to men who had their blood draw during 1998–2004 (OR = 1.20, 95% CI: 0.97,1.49) (Rhee et al., 2023). Two previous studies investigated occupational levels of PFOS exposure and the risk of prostate cancer, both of which provide some support for a positive relationship (Grice et al., 2007; Olsen et al., 2004), although both studies were based on <25 prostate cancer cases.

Three studies investigated exposure to PFAS-contaminated water containing multiple PFAS types and the risk of prostate cancer (Li et al., 2022; Mastrantonio et al., 2018; Messmer et al., 2022). In the first, an ecological study among residents in Italy that compared mortality in municipalities with and without PFAS concentrations (PFOS, PFOA, or “other PFAS”) exceeding the set limits in their drinking water, the authors found no association with prostate cancer mortality (Mastrantonio et al., 2018). In the second, Messmer and colleagues found 10-year cancer incidence rates in a New England town with PFAS-contaminated water (primarily PFOA but also PFOS and PFHxS) were higher than those in other demographically similar towns but lower than the US national average (Messmer et al., 2022). In the third, Li and colleagues compared prostate cancer incidence rates in a Swedish community with drinking water highly contaminated with mostly PFHxS and PFOS, but also PFOA, to those in other towns in the same county and found a largely null association (Li et al., 2022). Li and colleagues also compared various levels of exposure to the PFAS-contaminated drinking water among residents in the exposed community and found largely null or inverse associations between higher exposures to the contaminated water and risk of prostate cancer.

Our results provided limited support that PFAS-prostate cancer associations varied by tumor characteristics, like grade or stage, with some exceptions. For grade, while we did observe some heterogeneity across grade categories, it does not appear biologically plausible that PFAS would impact both low- and high-grade prostate cancer similarly but have no impact on intermediate-grade prostate cancer. Therefore, it is likely that the differences observed in the intermediate-grade prostate cancer group were due to chance. For stage, there was some suggestion that PFNA may be more strongly associated with advanced stage prostate cancer compared to non-advanced stage, but we did not observe this trend for other PFAS types. Only two previous studies investigated heterogeneity in PFAS-prostate cancer associations by tumor characteristics (Hardell et al., 2014; Rhee et al., 2023), one of which was limited in that it only looked at heterogeneity within aggressive prostate cancers (not all prostate cancers) (Rhee et al., 2023). In the first, Hardell and colleagues observed somewhat stronger associations for several PFAS types, including PFHxS and PFNA, among men with low-grade prostate cancer (defined as Gleason’s scores of 2–6) compared to high-grade prostate cancer (defined as Gleason’s scores 7–10), although confidence intervals were wide and

overlapping (Hardell et al., 2014). It is possible that the weaker associations observed by Hardell and colleagues in the high-grade group are the result of including men with a Gleason's score of 7, a group in which we tended to observe null associations. In the second, there was limited evidence that associations with PFAS varied by grade or stage, similar to our current findings, with some exceptions (Rhee et al., 2023). The authors observed somewhat stronger associations with PFHxS among men with a Gleason's score of 7 who died from prostate cancer (compared to men with a Gleason's score ≥8, regardless of whether they died from prostate cancer) and among men diagnosed with advanced stage prostate cancer (compared to non-advanced stage).

In our study, PFHxS was positively associated with prostate cancer among men without a family history of prostate cancer, but not among men with a family history. We found that while individually, both PFHxS (among those without a family history) and family history (among those with concentrations in the lowest PFHxS tertile) appeared associated with prostate cancer, their additive interaction was negative, with the implication being that any intervention on PFHxS (as it relates to prostate cancer) would be more effective among men without a family history. This contrasts with findings by Hardell and colleagues, in which the estimated single effects of family history and PFHxS on prostate cancer were approximately null, but the odds ratio representing their estimated joint effects was 4.4 (suggesting a positive additive interaction and a more effective intervention among those with a family history) (Hardell et al., 2014). While the study by Rhee and colleagues did not examine the interaction between PFAS and family history of prostate cancer on the risk of aggressive prostate cancer on the additive scale, their findings provided limited support of an interaction on the multiplicative scale, with a slight positive association among those without a family history and a slight negative association among those with a family history, which is somewhat similar to our findings (Rhee et al., 2023).

Unexpectedly, we found that PFHxS, PFNA, PFOA, and PFOS were associated with a higher risk of prostate cancer among men aged ≥70 years at blood sample collection, but not among men aged <70 years. Our post-hoc analysis suggested the positive associations were driven by men aged 70–74 years. There are several possible explanations for these findings. First, given that men born in different calendar years likely have different exposure histories (e.g., age when they were first exposed, age at peak exposure), it is possible that men in the 70–74-year age group (born during 1929–1936) were uniquely susceptible to the effects of PFAS on prostate cancer when they were first exposed. However, this seems unlikely, as men in this age group were likely young adults when they were first exposed to PFAS, and we would expect to see greater susceptibility among men first exposed during more critical periods of development (such as in utero or during childhood (Olshan et al., 2000)). Second, the effects of PFAS may truly be stronger among older men, but biases with a stronger presence in the 75+ year group might have precluded our ability to detect associations in this oldest age group. For example, men in our study were likely exposed to several types of PFAS for many years prior to meeting the eligibility criteria for this study. Therefore, those who were most susceptible to the effects of PFAS on prostate cancer might have encountered an event (such as a prostate cancer diagnosis or other cancer diagnosis) that made them ineligible or unable to participate in this study, leaving a population of men who were less susceptible to the effects of PFAS on prostate cancer. This can introduce a

type of selection bias due to left truncation, resulting in a downward bias in measures of association that can make a harmful exposure appear protective (Applebaum et al., 2011; Hernán, 2010). We would expect this to be especially apparent among the oldest men. A stronger effect of PFAS on prostate cancer among older men is biologically plausible. Older age is associated with reduced immune function (Weyand and Goronzy, 2016), potentially making older men more susceptible to the impacts of PFAS on prostate cancer (Corthay, 2014; Palmer et al., 2018). In addition, mounting evidence suggests that PFAS exposure is also linked to decreased immune function (Granum et al., 2013; Grandjean et al., 2020; Risk to human health related, 2020). Therefore, it is possible the combined impacts of age and PFAS exposure on immune function might act synergistically to facilitate prostate cancer development. Third, it is also possible that there is no true association between PFAS and prostate cancer, and that our observed positive findings in the 70–74-year group were due to bias or chance. For example, it is possible that men in the 70–74-year group were uniquely exposed to another potential prostate cancer risk factor (e.g., Agent Orange (Chamie et al., 2008; Ansbaugh et al., 2013)) that is also associated with PFAS exposure (e.g., through military operations like fire training exercises) that we were unable to control for in analyses, and could explain the positive findings in this group. Given that similar associations were observed in our unadjusted models, bias due to sparse data is unlikely to account for our findings.

Although not directly comparable to the present study, findings by Rhee and colleagues suggested no substantial heterogeneities in PFAS associations with aggressive prostate cancer according to age (Rhee et al., 2023), contrasting with our study results. However, Rhee and colleagues did report borderline positive findings for PFNA (and MeFOSAA, which was not considered in this study) among the oldest age group (aged >75 years), but largely null findings for younger age groups, providing some (although weak) support for the idea that older men may be more susceptible to the effects of PFAS on prostate cancer. We believe that these findings, combined with the very strong associations observed in the 70–74-year age group in our study warrants further investigation of the potential interaction between PFAS and age in future studies.

We observed positive relationships between certain PFAS types and prostate cancer among men who reported not consuming alcohol (PFHxS) and possibly among those with a college graduate education or higher (PFNA, PFHxS, PFOS). While it is possible that these were chance findings due to multiple subgroup comparisons, the consistent positive findings for PFHxS in multiple subgroups (e.g., men without a family history, older men) warrants further consideration. It is possible that those who do not consume alcohol represent a group more, or less, impacted by bias due to unmeasured/residual confounding from other health behaviors (e.g., diet) that could be risk factors for prostate cancer, as health behaviors are often correlated (Noble et al., 2015) and could be associated with PFAS concentrations. It is also possible that those with a lower education represent a group more impacted by unmeasured confounding through occupation. For example, those without a college degree may be more likely to have jobs (e.g., firefighters) that put them at risk for PFAS exposure and other prostate cancer risk factors (e.g., radiation) (Sritharan et al., 2017). The biological mechanisms through which PFAS may affect cancer development, and specifically prostate cancer development, remain unclear. A recent review of the potential

mechanisms proposed three major interdependent pathways that likely work together to promote cancer development and progression following PFAS exposure, including through alterations in cellular metabolism, endocrine homeostasis, and the epigenome (Boyd et al., 2022).

Our findings should be considered in context with the study's limitations. First, PFAS exposure prior to enrollment in any of the CPS-II cohorts may have led to health effects affecting study participation, leading to potential selection bias. If underlying prostate cancer symptoms occurring prior to a prostate cancer diagnosis also impacted study participation, or if there are any uncontrolled factors affecting both participation and prostate cancer, our results could be biased. In the specific example of selection bias due to left truncation (mentioned in the paragraph discussing our findings by age), we would expect the direction of the bias to be downward, and it is unlikely that this would account for any observed positive findings. However, the expected direction of bias due to other uncontrolled factors (e.g., socioeconomic status) would likely depend on the factor and is difficult to determine. Second, in our study, PFAS serum concentrations were only available at a single timepoint and may not be representative of a person's cumulative exposure. However, the PFAS examined in our study, especially PFHxS and PFOS, have long half-lives (Agency for Toxic Substances and Disease Registry (ATSDR), 2021), and the ranking of exposures is likely consistent over time. In a previous study that examined multiple PFAS measurements over a 6-year time period, the authors observed strong correlations across samples over time for most PFAS, suggesting single-sample measurements may be informative surrogates of long-term exposure (Rhee et al., 2023). Another study examined five repeated PFAS measurements over a 28-year period (1979–2007) among a cohort of Norwegian men and found robust correlations (Spearman's $\rho > 0.6$) for PFOS and PFOA between successive measurements (Nøst et al., 2014). Third, given that many types of PFAS are correlated with one another, it is possible that associations with prostate cancer for a given type of PFAS may represent the effect of other types of PFAS on prostate cancer. In the absence of unmeasured confounding, more complex mixture models that include multiple PFAS in a single model can theoretically isolate the independent effects of specific PFAS and avoid confounding by the other PFAS in the model (Weisskopf et al., 2018). However, if an unmeasured confounder (e.g., diet) of the association between some components of the mixture, but not others, with the outcome exists, including correlated exposures can often result in more biased estimates (sometimes called "coexposure amplification bias") (Weisskopf et al., 2018). Therefore, we did not consider mixture models in this analysis. Fourth, we conducted multiple statistical tests, amplifying the probability of one or more statistically significant results. Therefore, it is important to consider the overall patterns of our results and not interpret any single estimate in isolation. Future studies are needed to confirm our findings. Last, given that most of our study population were older men who were white, our results may not be generalizable to younger, nonwhite populations.

The current study has several noteworthy strengths. To our knowledge, our study of PFAS and prostate cancer is the largest of its kind, with over 1600 prostate cancer cases, enabling us to investigate a variety of potential differences by medical, demographic, and lifestyle-related characteristics. The prospective nature of this study is also a notable strength. In addition, after accounting for differences in PFAS measurements (i.e., our study only

measured linear isomers whereas the U.S. National Health and Nutrition Examination Survey (NHANES) measured linear and branched isomers together), the concentrations of PFAS in our study were similar to those observed among men in the 1999–2000 NHANES (Centers for Disease Control and Prevention). Thus, this study provides important evidence regarding the relationship between PFAS exposures at levels seen in the general population and prostate cancer, an understudied area of research.

In conclusion, our study of general population-level PFAS exposures and the risk of prostate cancer does not provide strong, consistent evidence that PFAS are associated with a higher risk of prostate cancer. However, the positive associations observed in some subgroups, like older men, and the consistently positive associations observed for PFHxS warrant further investigation. Additional studies are still needed to better understand this relationship, especially large, well-designed studies that can further investigate interactions with medical, sociodemographic, and lifestyle-related factors that may help explain discrepancies in the literature.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Availability of data and materials

Data are available from the American Cancer Society by following the ACS Data Access Procedures (https://www.cancer.org/content/dam/cancer-org/research/epidemiology/cancer-prevention-study-data-access-policies.pdf) for researchers who meet the criteria for access to confidential data. Please email cohort.data@cancer.org to inquire about access.

## Data availability

Data are available from the American Cancer Society to researchers who meet the criteria for access to confidential data. Please email cohort.data@cancer.org to inquire about access.

## List of Abbreviations

| | |
|---|---|
| **ACS** | American Cancer Society |
| **AJCC** | American Joint Committee on Cancer |

| | |
|---|---|
| **BMI** | Body mass index |
| **CI** | Confidence interval |
| **CPS-II** | Cancer Prevention Study-II |
| **FOSA** | Perfluorooctane sulfonamide |
| **HR** | Hazard Ratio |
| **NHANES** | National Health and Nutrition Examination Survey |
| **PFAS** | Per- and polyfluoroalkyl substances |
| **PFBS** | Perfluorobutane sulfonic acid |
| **PFHpA** | Perfluoroheptanoic acid |
| **PFHxS** | Perfluorohexane sulfonic acid |
| **PFNA** | Perfluorononanoic acid |
| **PFOA** | Perfluorooctanoic acid |
| **PFOS** | Perfluorooctane sulfonate |
| **PSA** | Prostate-specific antigen |
| **RERI** | Relative excess risks due to interaction |
| **Q1** | Quartile 1 |
| **Q2** | Quartile 2 |
| **Q3** | Quartile 3 |
| **Q4** | Quartile 4 |
| **U.S.** | United States |

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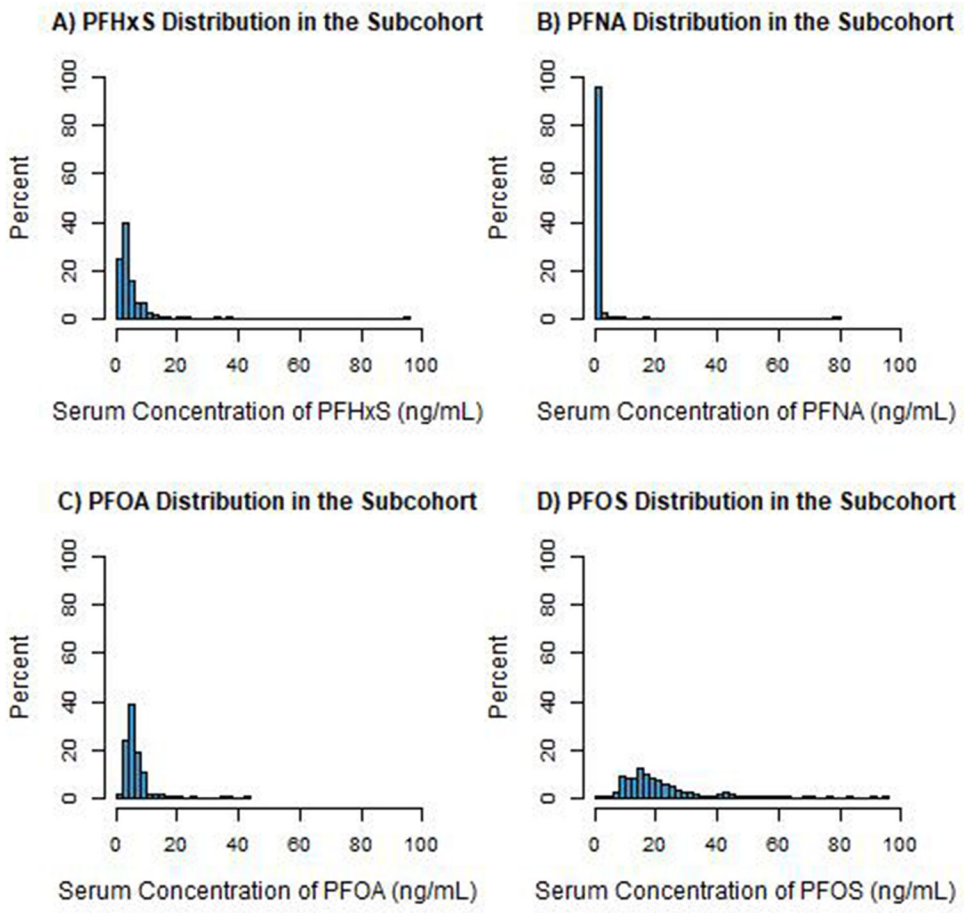


**Fig. 1.**

Baseline (1998–2001) Serum Concentrations (ng/mL) of PFAS Among Male Sub-cohort Participants in the Case-Cohort Study of PFAS and Cancer Incidence in the Cancer Prevention Study-II LifeLink Cohort, United States. Abbreviations: PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate.

## Table 1

Baseline characteristics of sub-cohort participants and prostate cancer cases in the case-cohort study of PFAS and cancer incidence in the cancer prevention Study-II LifeLink cohort, United States, 1998–2001.

| Variable | Categories | Subcohort[a,b] | Prostate Cancer Cases[c] |
|---|---|---|---|
| | | **N = 500** | **N = 1610** |
| ***General Characteristics*** | | **N (%)** | **N (%)** |
| Year of Blood Sample | 1998 | 43 (8.6) | 147 (9.1) |
| | 1999 | 152 (30.4) | 477 (29.6) |
| | 2000 | 280 (56.0) | 905 (56.2) |
| | 2001 | 25 (5.0) | 81 (5.0) |
| Race/Ethnicity | Non-Hispanic white | 487 (98.2) | 1580 (98.5) |
| | Other | 9 (1.8) | 24 (1.5) |
| Education | High school or less | 101 (20.4) | 252 (15.7) |
| | Some college or trade | 132 (26.6) | 379 (23.6) |
| | College Graduate | 124 (25.0) | 439 (27.3) |
| | Graduate School | 139 (28.0) | 537 (33.4) |
| Smoking Status | Nonsmoker | 182 (36.4) | 635 (39.4) |
| | Current Smoker | 18 (3.6) | 64 (4.0) |
| | Former Smoker | 288 (57.6) | 894 (55.6) |
| | Ever Smoker (unknown if current or former) | 12 (2.4) | 16 (1.0) |
| Alcohol Consumption | Non-drinker | 160 (32.1) | 507 (31.5) |
| | Less than daily | 233 (46.7) | 728 (45.2) |
| | 1 drink/day or more | 106 (21.2) | 374 (23.2) |
| Family History of PC | No | 428 (85.6) | 1340 (83.2) |
| | Yes | 72 (14.4) | 270 (16.8) |
| ***Age and PFAS Concentrations*** | | **Median (25th–75th)** | **Median (25th–75th)** |
| Age at blood collection | | 70 (66–74) | 69 (65–73) |
| PFHxS | | 3.3 (2.1–5.3) | 3.3 (2.2–5.1) |
| PFNA | | 0.7 (0.5–1.0) | 0.7 (0.5–1.0) |
| PFOA | | 5.2 (4.0–7.0) | 5.3 (4.0–6.9) |
| PFOS | | 18.0 (13.5–25.5) | 19 (14.0–26.0) |
| ***Tumor Characteristics Among Cases*** | | | **N (%)** |
| Grade[d] | Low-grade | | 814 (51.5) |
| | Intermediate-grade | | 490 (31.0) |
| | High-grade | | 278 (17.6) |
| Stage at Diagnosis[e] | Non-advanced | | 1456 (91.7) |
| | Advanced | | 132 (8.3) |

[a] n = 58 participants in the sub-cohort were also case-participants.

[b] In the sub-cohort, the following variables had missing values: race/ethnicity (n = 4, 0.8%); education (n = 4, 0.8%); alcohol consumption (n = 1, 0.2%).

[c] Among cases, the following variables had missing values: race/ethnicity (n = 6, 0.4%); education (n = 3, 0.2%); smoking status (n = 1, 0.1%); alcohol consumption (n = 1, 0.1%); grade (n = 28, 1.7%); stage at diagnosis (n = 22, 1.4%).

[d] Grade was defined according to Gleason's score as follows: low-grade (Gleason's score <7), intermediate-grade (Gleason's score = 7), and high-grade (Gleason's score >7). Missing Gleason's scores were inferred based on degree of differentiation and year of diagnosis outlined in Supplemental Table 1.

[e] Stage was defined according to the American Joint Committee on Cancer (AJCC) stage as follows: non-advanced (AJCC stage 1–2), advanced (AJCC stage 3–4).

**Table 2**

Associations of per- and polyfluoroalkyl substance concentrations and incident prostate cancer in the case-cohort study of PFAS and cancer incidence in the cancer prevention Study-II lifelink cohort, United States, 1998–2015 (n = 2110).

| | | Model 1[a] | | | | Model 2[b] | | | |
|---|---|---|---|---|---|---|---|---|---|
| **PFAS Type** | **Category** | **Cases** | **Sub-cohort** | **Sub-cohort person-years** | **HR (95%CI)** | **Cases** | **Subcohort** | **Sub-cohort person-years** | **HR (95%CI)** |
| PFHxS | Q1: 0.14–2.00 ng/mL | 346 | 124 | 1370 | 1.00 (−) | 345 | 120 | 1342 | 1.00 (−) |
| | Q2: 2.10–3.20 ng/mL | 445 | 121 | 1270 | 1.39 (1.03, 1.87) | 442 | 119 | 1250 | 1.36 (1.01, 1.83) |
| | Q3: 3.30–5.20 ng/mL | 437 | 129 | 1477 | 1.20 (0.90, 1.61) | 432 | 126 | 1442 | 1.18 (0.88, 1.59) |
| | Q4: 5.30–95.0 ng/mL | 382 | 126 | 1342 | 1.16 (0.86, 1.56) | 380 | 126 | 1342 | 1.18 (0.88, 1.59) |
| | Continuous[c] | | | | 1.05 (0.95, 1.16) | | | | 1.06 (0.96, 1.17) |
| PFNA | Q1: 0.06–0.48 ng/mL | 392 | 118 | 1287 | 1.00 (−) | 389 | 115 | 1263 | 1.00 (−) |
| | Q2: 0.49–0.66 ng/mL | 337 | 130 | 1514 | 0.72 (0.54, 0.97) | 337 | 127 | 1474 | 0.75 (0.56, 1.01) |
| | Q3: 0.67–0.98 ng/mL | 434 | 124 | 1278 | 1.08 (0.80, 1.44) | 430 | 121 | 1259 | 1.03 (0.76, 1.39) |
| | Q4: 0.99–80.0 ng/mL | 447 | 128 | 1380 | 1.06 (0.79, 1.42) | 443 | 128 | 1380 | 1.05 (0.77, 1.41) |
| | Continuous[c] | | | | 1.03 (0.92, 1.16) | | | | 1.03 (0.92, 1.16) |
| PFOA | Q1: 0.35–3.90 ng/mL | 401 | 116 | 1229 | 1.00 (−) | 398 | 112 | 1194 | 1.00 (−) |
| | Q2: 4.00–5.10 ng/mL | 355 | 124 | 1333 | 0.89 (0.66, 1.19) | 351 | 121 | 1300 | 0.89 (0.65, 1.20) |
| | Q3: 5.20–6.90 ng/mL | 455 | 135 | 1490 | 0.97 (0.73, 1.30) | 454 | 134 | 1485 | 1.01 (0.75, 1.35) |
| | Q4: 7.00–90.0 ng/mL | 399 | 125 | 1408 | 0.92 (0.68, 1.23) | 396 | 124 | 1397 | 0.93 (0.69, 1.25) |
| | Continuous[c] | | | | 0.94 (0.81, 1.09) | | | | 0.95 (0.82, 1.10) |
| PFOS | Q1: 0.35–13.0 ng/mL | 391 | 125 | 1349 | 1.00 (−) | 389 | 123 | 1333 | 1.00 (−) |
| | Q2: 14.0–17.0 ng/mL | 304 | 105 | 1157 | 0.99 (0.73, 1.34) | 301 | 103 | 1135 | 1.01 (0.75, 1.37) |
| | Q3: 18.0–25.0 ng/mL | 504 | 145 | 1612 | 1.15 (0.87, 1.53) | 501 | 141 | 1579 | 1.17 (0.88, 1.56) |
| | Q4: 26.0–160 ng/mL | 411 | 125 | 1341 | 1.10 (0.82, 1.47) | 408 | 124 | 1329 | 1.18 (0.89, 1.58) |
| | Continuous[c] | | | | 1.00 (0.88, 1.13) | | | | 1.02 (0.90, 1.16) |

[a] Adjusts for age and year of blood sample collection. Age violated the proportional hazards assumption, so models adjust for age using a stratified Cox procedure, stratifying the baseline hazard on single year of age.

[b] Adjusts for age, year of blood sample collection, education, alcohol use, smoking status, race. Age and smoking status violated the proportional hazards assumption, so models adjust for these variables using a stratified Cox procedure, stratifying the baseline hazard on single year of age and smoking status (ever smoker, never smoker). A total of 20 participants were missing information on one or more covariates and dropped from the model.

[c] PFAS were log2-transformed and modeled as a continuous variable.

**Table 3**

Multivariable associations[a] of Per- and Polyfluoroalkyl Substance Concentrations and Incident Low-Grade[b] (n = 806), Intermediate-Grade[c] (n = 488), High-Grade[d] (n = 277), Non-Advanced Stage[e] (n = 1445)[e] and Advanced Stage[f] (n = 132) Prostate Cancer in the Case-Cohort Study of PFAS and Cancer Incidence in the Cancer Prevention Study-II Lifelink Cohort, United States, 1998–2015.

| | | | PFAS Concentration Quartile | | | | |
|---|---|---|---|---|---|---|---|
| **PFAS** | **Case-Definition** | | **Q1** | **Q2** | **Q3** | **Q4** | **Continuous[k]** |
| PFHxS[g] | Low-Grade[b] | Cases, n | 162 | 219 | 227 | 198 | |
| | | Sub-cohort, n | 120 | 119 | 126 | 126 | |
| | | Sub-cohort, Person-years | 1342 | 1250 | 1442 | 1342 | |
| | | HR (95% CI) | 1.00 (−) | 1.51 (1.07, 2.12) | 1.32 (0.94, 1.84) | 1.37 (0.97, 1.92) | 1.11 (1.00, 1.24) |
| | Intermediate-Grade[c] | Cases, n | 118 | 136 | 120 | 114 | |
| | | Sub-cohort, n | 120 | 119 | 126 | 126 | |
| | | Sub-cohort, Person-years | 1342 | 1250 | 1442 | 1342 | |
| | | HR (95% CI) | 1.00 (−) | 1.11 (0.76, 1.64) | 0.96 (0.66, 1.39) | 0.96 (0.65, 1.40) | 1.00 (0.88, 1.14) |
| | High-Grade[d] | Cases, n | 59 | 81 | 74 | 63 | |
| | | Sub-cohort, n | 120 | 119 | 126 | 126 | |
| | | Sub-cohort, Person-years | 1342 | 1250 | 1442 | 1342 | |
| | | HR (95% CI) | 1.00 (−) | 1.53 (0.97, 2.42) | 1.22 (0.79, 1.90) | 1.23 (0.78, 1.92) | 1.06 (0.91, 1.23) |
| | Non-Advanced Stage[e] | Cases, n | 313 | 398 | 391 | 343 | |
| | | Sub-cohort, n | 120 | 119 | 126 | 126 | |
| | | Sub-cohort, Person-years | 1342 | 1250 | 1442 | 1342 | |
| | | HR (95% CI) | 1.00 (−) | 1.36 (1.00, 1.84) | 1.17 (0.87, 1.58) | 1.17 (0.87, 1.58) | 1.06 (0.96, 1.17) |
| | Advanced Stage[f] | Cases, n | 28 | 36 | 35 | 33 | |
| | | Sub-cohort, n | 120 | 119 | 126 | 126 | |
| | | Sub-cohort, Person-years | 1342 | 1250 | 1442 | 1342 | |
| | | HR (95% CI) | 1.00 (−) | 1.32 (0.69, 2.51) | 1.26 (0.68, 2.37) | 1.35 (0.73, 2.53) | 1.11 (0.92, 1.35) |
| PFNA[h] | Low-Grade[c] | Cases, n | 183 | 166 | 229 | 228 | |
| | | Sub-cohort, n | 115 | 127 | 121 | 128 | |
| | | Sub-cohort, Person-years | 1263 | 1474 | 1259 | 1380 | |
| | | HR (95% CI) | 1.00 (−) | 0.75 (0.54, 1.06) | 1.11 (0.79, 1.55) | 1.09 (0.78, 1.53) | 1.05 (0.92, 1.20) |

| | | | PFAS Concentration Quartile | | | | |
|---|---|---|---|---|---|---|---|
| **PFAS** | **Case-Definition** | | **Q1** | **Q2** | **Q3** | **Q4** | **Continuous[k]** |
| | Intermediate-Grade[d] | Cases, n | 122 | 109 | 125 | 132 | |
| | | Sub-cohort, n | 115 | 127 | 121 | 128 | |
| | | Sub-cohort, Person-years | 1263 | 1474 | 1259 | 1380 | |
| | | HR (95% CI) | 1.00 (−) | 0.76 (0.52, 1.12) | 0.94 (0.64, 1.38) | 1.00 (0.68, 1.48) | 1.01 (0.86, 1.19) |
| | High-Grade[e] | Cases, n | 78 | 57 | 70 | 72 | |
| | | Sub-cohort, n | 115 | 127 | 121 | 128 | |
| | | Sub-cohort, Person-years | 1263 | 1474 | 1259 | 1380 | |
| | | HR (95% CI) | 1.00 (−) | 0.66 (0.43, 1.03) | 0.94 (0.60, 1.48) | 0.95 (0.61, 1.50) | 1.03 (0.86, 1.23) |
| | Non-Advanced Stage[f] | Cases, n | 349 | 307 | 396 | 393 | |
| | | Sub-cohort, n | 115 | 127 | 121 | 128 | |
| | | Sub-cohort, Person-years | 1263 | 1474 | 1259 | 1380 | |
| | | HR (95% CI) | 1.00 (−) | 0.76 (0.56, 1.03) | 1.05 (0.77, 1.42) | 1.01 (0.74, 1.37) | 1.02 (0.90, 1.15) |
| | Advanced Stage[g] | Cases, n | 36 | 25 | 28 | 43 | |
| | | Sub-cohort, n | 115 | 127 | 121 | 128 | |
| | | Sub-cohort, Person-years | 1263 | 1474 | 1259 | 1380 | |
| | | HR (95% CI) | 1.00 (−) | 0.63 (0.33, 1.19) | 0.77 (0.41, 1.45) | 1.52 (0.83, 2.78) | 1.22 (0.95, 1.57) |
| PFOA[i] | Low-Grade[c] | Cases, n | 194 | 178 | 226 | 208 | |
| | | Sub-cohort, n | 112 | 121 | 134 | 124 | |
| | | Sub-cohort, Person-years | 1194 | 1300 | 1485 | 1397 | |
| | | HR (95% CI) | 1.00 (−) | 0.90 (0.64, 1.28) | 1.03 (0.74, 1.44) | 1.01 (0.72, 1.41) | 1.01 (0.85, 1.19) |
| | Intermediate-Grade[d] | Cases, n | 119 | 102 | 154 | 113 | |
| | | Sub-cohort, n | 112 | 121 | 134 | 124 | |
| | | Sub-cohort, Person-years | 1194 | 1300 | 1485 | 1397 | |
| | | HR (95% CI) | 1.00 (−) | 0.88 (0.59, 1.33) | 1.12 (0.77, 1.62) | 0.82 (0.55, 1.21) | 0.85 (0.69, 1.05) |
| | High-Grade[e] | Cases, n | 80 | 64 | 65 | 68 | |
| | | Sub-cohort, n | 112 | 121 | 134 | 124 | |
| | | Sub-cohort, Person-years | 1194 | 1300 | 1485 | 1397 | |
| | | HR (95% CI) | 1.00 (−) | 0.80 (0.51, 1.26) | 0.74 (0.48, 1.16) | 0.89 (0.58, 1.39) | 0.95 (0.76, 1.18) |
| | Non-Advanced Stage[f] | Cases, n | 355 | 323 | 419 | 348 | |

| | | | PFAS Concentration Quartile | | | | |
|---|---|---|---|---|---|---|---|
| **PFAS** | **Case-Definition** | | **Q1** | **Q2** | **Q3** | **Q4** | **Continuous[k]** |
| PFOS[i] | Low-Grade[c] | Sub-cohort, n | 112 | 121 | 134 | 124 | |
| | | Sub-cohort, Person-years | 1194 | 1300 | 1485 | 1397 | |
| | | HR (95% CI) | 1.00 (−) | 0.90 (0.66, 1.22) | 1.03 (0.77, 1.39) | 0.90 (0.66, 1.22) | 0.94 (0.80, 1.09) |
| | Advanced Stage[g] | Cases, n | 40 | 22 | 31 | 39 | |
| | | Sub-cohort, n | 112 | 121 | 134 | 124 | |
| | | Sub-cohort, Person-years | 1194 | 1300 | 1485 | 1397 | |
| | | HR (95% CI) | 1.00 (−) | 0.68 (0.37, 1.27) | 0.75 (0.42, 1.34) | 1.16 (0.64, 2.10) | 1.03 (0.75, 1.42) |
| | Intermediate-Grade[d] | Cases, n | 184 | 146 | 267 | 209 | |
| | | Sub-cohort, n | 123 | 103 | 141 | 124 | |
| | | Sub-cohort, Person-years | 1333 | 1135 | 1579 | 1329 | |
| | | HR (95% CI) | 1.00 (−) | 1.02 (0.72, 1.45) | 1.29 (0.93, 1.78) | 1.25 (0.90, 1.74) | 1.05 (0.91, 1.21) |
| | High-Grade[e] | Cases, n | 128 | 98 | 138 | 124 | |
| | | Sub-cohort, n | 123 | 103 | 141 | 124 | |
| | | Sub-cohort, Person-years | 1333 | 1135 | 1579 | 1329 | |
| | | HR (95% CI) | 1.00 (−) | 0.98 (0.65, 1.45) | 0.96 (0.66, 1.39) | 1.04 (0.72, 1.50) | 0.94 (0.79, 1.11) |
| | Non-Advanced Stage[f] | Cases, n | 70 | 55 | 86 | 66 | |
| | | Sub-cohort, n | 123 | 103 | 141 | 124 | |
| | | Sub-cohort, Person-years | 1333 | 1135 | 1579 | 1329 | |
| | | HR (95% CI) | 1.00 (−) | 1.11 (0.70, 1.77) | 1.22 (0.79, 1.87) | 1.24 (0.80, 1.93) | 1.06 (0.87, 1.28) |
| | Advanced Stage[g] | Cases, n | 351 | 274 | 448 | 372 | |
| | | Sub-cohort, n | 123 | 103 | 141 | 124 | |
| | | Sub-cohort, Person-years | 1333 | 1135 | 1579 | 1329 | |
| | | HR (95% CI) | 1.00 (−) | 1.01 (0.74, 1.37) | 1.14 (0.86, 1.53) | 1.19 (0.88, 1.59) | 1.02 (0.89, 1.16) |
| | | Cases, n | 36 | 23 | 44 | 29 | |
| | | Sub-cohort, n | 123 | 103 | 141 | 124 | |
| | | Sub-cohort, Person-years | 1333 | 1135 | 1579 | 1329 | |
| | | HR (95% CI) | 1.00 (−) | 0.94 (0.50, 1.73) | 1.28 (0.75, 2.19) | 0.91 (0.51, 1.62) | 0.98 (0.77, 1.25) |

[a]Adjusts for age, year of blood sample collection, education, alcohol use, smoking status, race. Age and smoking status violated the proportional hazards assumption, so models adjust for these variables using a stratified Cox procedure, stratifying the baseline hazard on single year of age and smoking status (ever smoker, never smoker).



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[b] Low-grade prostate cancer was defined as cases with a Gleason's score <7. Missing Gleason's scores were inferred based on degree of differentiation and year of diagnosis outlined in Supplemental Table 1. The case counts exclude 8 case-participants diagnosed with low-grade prostate cancer who were missing information on one or more covariates and dropped from the model.

[c] Intermediate grade prostate cancer was defined as cases with a Gleason's score of 7. Missing Gleason's scores were imputed based on degree of differentiation and year of diagnosis outlined in Supplemental Table 1. The case counts exclude 2 case-participants diagnosed with intermediate-grade prostate cancer who were missing information on one or more covariates and dropped from the model.

[d] High-grad prostate cancer was defined as cases with a Gleason's score ≥8. Missing Gleason's scores were imputed based on degree of differentiation and year of diagnosis outlined in Supplemental Table 1. The case counts exclude one case-participant diagnosed with high-grade prostate cancer who was missing information on one or more covariates and dropped from the model.

[e] Non-advanced prostate cancer was defined as tumors classified as AJCC stage 1–2. The case counts exclude 11 case-participants diagnosed with non-advanced stage prostate cancer who were missing information on one or more covariates and dropped from the model.

[f] Advanced prostate cancer was defined as tumors classified as AJCC stage 3–4. All case-participants diagnosed with advanced stage prostate cancer had complete information on covariates and were included in the model.

[g] The range of values (in ng/mL) represented for each quartile of PFHxS concentration are as follows: Q1, 0.14–2.00; Q2, 2.10–3.20; Q3, 3.30–5.20; and Q4, 5.30–95.0.

[h] The range of values (in ng/mL) represented for each quartile of PFNA concentration are as follows: Q1, 0.06–0.48; Q2, 0.49–0.66; Q3, 0.67–0.98; and Q4, 0.99–80.0.

[i] The range of values (in ng/mL) represented for each quartile of PFOA concentration are as follows: Q1, 0.35–3.90; Q2, 4.00–5.10; Q3, 5.20–6.90; and Q4, 7.00–90.0.

[j] The range of values (in ng/mL) represented for each quartile of PFOS concentration are as follows: Q1, 0.35–13.0; Q2, 14.0–17.0; Q3, 18.0–25.0; and Q4, 26.0–160.

[k] PFAS were log base 2 transformed and modeled as a continuous variable.

**Table 4**

Multivariable associations[a] of per- and polyfluoroalkyl substance concentrations with prostate cancer according to family history of prostate cancer in the case-cohort study of PFAS and cancer incidence in the cancer prevention Study-II lifeline cohort, United States, 1998–2015 (n = 2090)[b].

| PFAS | PFAS Category | Family History | Cases | Sub-cohort | Sub-cohort person-years | Interaction Model[c] HR (95% C[c]) | Single Referent Group[c] HR (95% C[c]) | RERI[d] (95% CI) |
|---|---|---|---|---|---|---|---|---|
| PFHxS | T1 (0.14–2.4 ng/mL) | No | 407 | 140 | 1586 | 1.00 (−) | 1.00 (−) | |
| | T2 (2.5–4.1 ng/mL) | No | 441 | 137 | 1522 | 1.19 (0.91, 1.57) | 1.19 (0.91, 1.57) | |
| | T3 (4.2–95 ng/mL) | No | 482 | 143 | 1555 | 1.31 (1.00, 1.72) | 1.31 (1.00, 1.72) | |
| | T1 (0.33–2.4 ng/mL) | Yes | 92 | 24 | 226 | 1.00 (−) | 2.04 (1.27, 3.28) | |
| | T2 (2.5–4.1 ng/mL) | Yes | 86 | 23 | 234 | 0.70 (0.37, 1.32) | 1.42 (0.86, 2.34) | −0.81 (−2.0, 0.38) |
| | T3 (4.2–38 ng/mL) | Yes | 91 | 24 | 254 | 0.78 (0.42, 1.45) | 1.59 (0.97, 2.59) | −0.77 (−2.0, 0.45) |
| | P-Interaction (categorical)[e] | | | | | 0.0044 | | |
| | Continuous | No | | | | 1.10 (0.99, 1.23) | | |
| | Continuous | Yes | | | | 0.87 (0.70, 1.09) | | |
| | P-Interaction (continuous)[e] | | | | | 0.0007 | | |
| PFNA[f] | T1 (0.06–0.54 ng/mL) | No | 413 | 135 | 1586 | 1.00 (−) | | |
| | T2 (0.55–0.88 ng/mL) | No | 467 | 142 | 1522 | 1.00 (0.76, 1.31) | | |
| | T3 (0.89–80 ng/mL) | No | 450 | 143 | 1555 | 1.09 (0.82, 1.44) | | |
| | T1 (0.09–0.54 ng/mL) | Yes | 86 | 25 | 226 | 1.00 (−) | | |
| | T2 (0.55–0.87 ng/mL) | Yes | 94 | 23 | 234 | 1.18 (0.68, 2.05) | | |
| | T3 (0.89–10 ng/mL) | Yes | 89 | 23 | 254 | 0.89 (0.51, 1.53) | | |
| | P-Interaction (categorical)[e] | | | | | 0.4681 | | |
| | Continuous | No | | | | 1.02 (0.90, 1.17) | | |
| | Continuous | Yes | | | | 0.93 (0.73, 1.18) | | |
| | P-Interaction (continuous)[e] | | | | | 0.2761 | | |
| PFOA | T1 (0.35–4.3 ng/mL) | No | 422 | 137 | 1586 | 1.00 (−) | 1.00 (−) | |
| | T2 (4.4–6.2 ng/mL) | No | 454 | 146 | 1522 | 1.08 (0.82, 1.43) | 1.08 (0.82, 1.43) | |
| | T3 (6.3–90 ng/mL) | No | 454 | 137 | 1555 | 1.07 (0.81, 1.42) | 1.07 (0.81, 1.42) | |
| | T1 (0.76–4.3 ng/mL) | Yes | 88 | 21 | 226 | 1.00 (−) | 1.52 (0.91, 2.56) | |

| PFAS | PFAS Category | Family History | Cases | Sub-cohort | Sub-cohort person-years | Interaction Model[c] HR (95% C[c]) | Single Referent Group[c] HR (95% C[c]) | RERI[d] (95% CI) |
|---|---|---|---|---|---|---|---|---|
| | T2 (4.4–6.2 ng/mL) | Yes | 94 | 20 | 234 | 1.17 (0.61, 2.23) | 1.78 (1.10, 2.89) | 0.18 (−0.91, 1.27) |
| | T3 (6.3–42 ng/mL) | Yes | 87 | 30 | 254 | 0.82 (0.43, 1.56) | 1.25 (0.78, 2.00) | −0.35 (−1.3, 0.61) |
| | P-Interaction (categorical)[e] | | | | | 0.1122 | | |
| | Continuous | No | | | | 0.96 (0.81, 1.13) | | |
| | Continuous | Yes | | | | 0.91 (0.65, 1.28) | | |
| | P-Interaction (continuous)[e] | | | | | 0.6182 | | |
| PFOS | T1 (0.35–15 ng/mL) | No | 445 | 146 | 1586 | 1.00 (−) | 1.00 (−) | |
| | T2 (16–22 ng/mL) | No | 436 | 135 | 1522 | 1.06 (0.81, 1.39) | 1.06 (0.81, 1.39) | |
| | T3 (23–160 ng/mL) | No | 449 | 139 | 1555 | 1.04 (0.80, 1.37) | 1.04 (0.80, 1.37) | |
| | T1 (2.5–15 ng/mL) | Yes | 89 | 26 | 226 | 1.00 (−) | 1.31 (0.82, 2.10) | |
| | T2 (16–22 ng/mL) | Yes | 92 | 19 | 234 | 1.33 (0.71, 2.48) | 1.74 (1.07, 2.85) | 0.37 (−0.63, 1.37) |
| | T3 (23–130 ng/mL) | Yes | 88 | 26 | 254 | 1.04 (0.56, 1.95) | 1.37 (0.83, 2.25) | 0.01 (−0.87, 0.89) |
| | P-Interaction (categorical)[e] | | | | | 0.3491 | | |
| | Continuous | No | | | | 1.02 (0.89, 1.17) | | |
| | Continuous | Yes | | | | 0.97 (0.70, 1.35) | | |
| | P-Interaction (continuous)[e] | | | | | 0.5942 | | |

[a] All models adjust for age, year of blood sample collection, education, alcohol use, smoking status, race. Age and smoking status violated the proportional hazards assumption, so models adjust for these variables using a stratified Cox procedure, stratifying the baseline hazard on single year of age and smoking status (ever smoker, never smoker).

[b] Excludes participants missing information on one or more covariates in the model (n = 20).

[c] Results from the interaction model and the single referent group model are from the same model that includes the above covariates, in addition to dummy variables representing the 2nd and 3rd tertiles of PFAS concentrations (i.e., T2, T3), a dummy variable representing whether the participant had a family history of prostate cancer (FAM_HX), and interaction terms between the PFAS dummy variables and the family history of prostate cancer variable (T2*FAM_HX and T3*FAM_HX). For the interaction model, the continuous results are from models that include a continuous variable for the $\log_2$ PFAS concentration, a dummy variable representing whether the participant had a family history of prostate cancer, and a term for the interaction between the two.

[d] Relative excess risk due to interaction (RERI). An assessment of additive interaction, calculated according to methods by Li and Chambless (2007 - PMID: 17320789).

[e] Calculated using a likelihood ratio test.

[f] The proportional hazards assumption for family history of prostate cancer was violated in PFNA models. Therefore, interaction models account for this by including dummy variables representing the product terms between PFNA and family history as model covariates and including family history in the STRATA statement of SAS PROC PHREG. Because the association of family history and prostate cancer appeared to vary over time in PFNA models, we could not provide estimates for the single referent group model.

**Table 5**

Multivariable associations[a] of per- and polyfluoroalkyl substance concentrations with prostate cancer according to strata of selected covariates[b] in the case-cohort study of PFAS and cancer incidence in the cancer prevention Study-II LifeLink cohort, United States, 1998–2015 (n = 2090)[c].

| | | Stratum #1 | | | | Stratum #2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| **PFAS** | **PFAS Category** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** |
| | | **Aged <70 years** | | | | **Aged ≥70 years** | | | |
| PFHxS[e,f] | Q1 | 187 | 45 | 517 | 1.00 (−) | 158 | 75 | 825 | 1.00 (−) |
| | Q2 | 243 | 63 | 728 | 0.96 (0.63, 1.47) | 199 | 56 | 522 | 2.04 (1.34, 3.10) |
| | Q3 | 252 | 70 | 878 | 0.89 (0.60, 1.34) | 180 | 56 | 564 | 1.64 (1.07, 2.51) |
| | Q4 | 208 | 58 | 696 | 0.91 (0.59, 1.40) | 172 | 68 | 646 | 1.54 (1.02, 2.31) |
| | P-Interaction (categorical)[d] | | | | | | | | <0.0001 |
| | Continuous | | | | 1.00 (0.86, 1.15) | | | | 1.13 (0.99, 1.29) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.0159 |
| PFNA[e,g] | Q1 | 203 | 52 | 577 | 1.00 (−) | 186 | 63 | 687 | 1.00 (−) |
| | Q2 | 195 | 58 | 720 | 0.72 (0.47, 1.10) | 142 | 69 | 754 | 0.78 (0.52, 1.18) |
| | Q3 | 260 | 64 | 735 | 0.93 (0.62, 1.40) | 170 | 57 | 524 | 1.17 (0.75, 1.82) |
| | Q4 | 232 | 62 | 787 | 0.81 (0.54, 1.22) | 211 | 66 | 593 | 1.51 (0.98, 2.31) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.0002 |
| | Continuous | | | | 0.96 (0.81, 1.14) | | | | 1.11 (0.95, 1.30) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.0194 |
| PFOA[e,h] | Q1 | 183 | 44 | 480 | 1.00 (−) | 215 | 68 | 714 | 1.00 (−) |
| | Q2 | 202 | 52 | 608 | 0.99 (0.62, 1.58) | 149 | 69 | 693 | 0.77 (0.51, 1.15) |
| | Q3 | 271 | 71 | 867 | 0.93 (0.61, 1.43) | 183 | 63 | 618 | 1.11 (0.74, 1.65) |
| | Q4 | 234 | 69 | 864 | 0.78 (0.51, 1.21) | 162 | 55 | 533 | 1.18 (0.78, 1.79) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.0003 |
| | Continuous | | | | 0.79 (0.64, 0.98) | | | | 1.23 (0.98, 1.54) |
| | P-Interaction (continuous)[d] | | | | | | | | <0.0001 |
| PFOS[e,i] | Q1 | 197 | 53 | 570 | 1.00 (−) | 192 | 70 | 763 | 1.00 (−) |
| | Q2 | 187 | 42 | 504 | 1.21 (0.79, 1.88) | 114 | 61 | 631 | 0.79 (0.51, 1.21) |

| | | Stratum #1 | | | | Stratum #2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| **PFAS** | **PFAS Category** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** |
| | | **Aged <70 years** | | | | **Aged ≥70 years** | | | |
| | Q3 | 281 | 74 | 924 | 1.03 (0.69, 1.53) | 220 | 67 | 655 | 1.36 (0.91, 2.03) |
| | Q4 | 225 | 67 | 821 | 0.94 (0.63, 1.40) | 183 | 57 | 508 | 1.62 (1.08, 2.44) |
| | P-Interaction (categorical)[d] | | | | | | | | <0.0001 |
| | Continuous | | | | 0.86 (0.72, 1.03) | | | | 1.25 (1.05, 1.49) |
| | P-Interaction (continuous)[d] | | | | | | | | <0.0001 |
| | | **College Graduate or Higher Education** | | | | **<College Graduate Education** | | | |
| PFHxS[f] | Q1 | 193 | 61 | 710 | 1.00 (−) | 152 | 59 | 632 | 1.00 (−) |
| | Q2 | 267 | 64 | 677 | 1.42 (0.96, 2.10) | 175 | 55 | 574 | 1.28 (0.82, 1.98) |
| | Q3 | 280 | 76 | 878 | 1.19 (0.81, 1.75) | 152 | 50 | 564 | 1.20 (0.77, 1.89) |
| | Q4 | 231 | 60 | 641 | 1.36 (0.91, 2.04) | 149 | 66 | 701 | 0.98 (0.64, 1.50) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.0985 |
| | Continuous | | | | 1.12 (0.98, 1.29) | | | | 0.99 (0.85, 1.14) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.0143 |
| PFNA[g] | Q1 | 215 | 60 | 676 | 1.00 (−) | 174 | 55 | 587 | 1.00 (−) |
| | Q2 | 191 | 62 | 737 | 0.77 (0.51, 1.16) | 146 | 65 | 736 | 0.71 (0.46, 1.09) |
| | Q3 | 251 | 66 | 700 | 1.03 (0.69, 1.52) | 179 | 55 | 559 | 1.04 (0.67, 1.63) |
| | Q4 | 314 | 73 | 792 | 1.23 (0.84, 1.80) | 129 | 55 | 587 | 0.79 (0.49, 1.25) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.0093 |
| | Continuous | | | | 1.15 (0.99, 1.33) | | | | 0.87 (0.72, 1.04) |
| | P-Interaction (continuous)[d] | | | | | | | | <0.0001 |
| PFOA[h] | Q1 | 233 | 60 | 687 | 1.00 (−) | 165 | 52 | 507 | 1.00 (−) |
| | Q2 | 210 | 66 | 699 | 0.90 (0.60, 1.33) | 141 | 55 | 601 | 0.85 (0.54, 1.34) |
| | Q3 | 289 | 74 | 813 | 1.18 (0.81, 1.72) | 165 | 60 | 672 | 0.77 (0.49, 1.21) |
| | Q4 | 239 | 61 | 707 | 1.05 (0.71, 1.56) | 157 | 63 | 690 | 0.75 (0.48, 1.16) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.0117 |
| | Continuous | | | | 1.01 (0.82, 1.24) | | | | 0.88 (0.72, 1.08) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.0803 |

| | | Stratum #1 | | | | Stratum #2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| **PFAS** | **PFAS Category** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** |
| | | **Aged <70 years** | | | | **Aged ≥70 years** | | | |
| PFOS[i] | Q1 | 246 | 66 | 724 | 1.00 (−) | 143 | 57 | 608 | 1.00 (−) |
| | Q2 | 172 | 59 | 672 | 0.87 (0.58, 1.30) | 129 | 44 | 463 | 1.28 (0.81, 2.02) |
| | Q3 | 316 | 82 | 922 | 1.15 (0.80, 1.66) | 185 | 59 | 656 | 1.25 (0.80, 1.94) |
| | Q4 | 237 | 54 | 587 | 1.40 (0.94, 2.07) | 171 | 70 | 742 | 0.98 (0.64, 1.50) |
| | P-Interaction (categorical)[d] | | | | | | | | <0.0001 |
| | Continuous | | | | 1.07 (0.90, 1.26) | | | | 0.95 (0.78, 1.15) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.0727 |
| | | **No Alcohol Consumption** | | | | **Alcohol Consumer** | | | |
| PFHxS[f] | Q1 | 134 | 47 | 454 | 1.00 (−) | 211 | 73 | 888 | 1.00 (−) |
| | Q2 | 146 | 48 | 491 | 1.02 (0.63, 1.66) | 296 | 71 | 760 | 1.60 (1.11, 2.31) |
| | Q3 | 122 | 35 | 347 | 1.24 (0.74, 2.07) | 310 | 91 | 1095 | 1.18 (0.83, 1.68) |
| | Q4 | 102 | 26 | 260 | 1.53 (0.89, 2.62) | 278 | 100 | 1081 | 1.10 (0.77, 1.58) |
| | P-Interaction (categorical)[d] | | | | | | | | <0.001 |
| | Continuous | | | | 1.20 (1.00, 1.43) | | | | 1.02 (0.91, 1.14) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.0058 |
| PFNA[g] | Q1 | 164 | 50 | 490 | 1.00 (−) | 225 | 65 | 773 | 1.00 (−) |
| | Q2 | 111 | 37 | 389 | 0.94 (0.58, 1.53) | 226 | 90 | 1084 | 0.66 (0.45, 0.96) |
| | Q3 | 124 | 41 | 419 | 0.97 (0.60, 1.56) | 306 | 80 | 841 | 1.04 (0.71, 1.51) |
| | Q4 | 105 | 28 | 254 | 1.33 (0.78, 2.27) | 338 | 100 | 1125 | 0.94 (0.66, 1.35) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.0136 |
| | Continuous | | | | 1.12 (0.89, 1.42) | | | | 1.01 (0.88, 1.15) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.1413 |
| PFOA[h] | Q1 | 157 | 38 | 361 | 1.00 (−) | 241 | 74 | 833 | 1.00 (−) |
| | Q2 | 116 | 41 | 390 | 0.82 (0.49, 1.38) | 235 | 80 | 911 | 0.92 (0.64, 1.32) |
| | Q3 | 127 | 35 | 344 | 1.03 (0.61, 1.72) | 327 | 99 | 1140 | 1.01 (0.71, 1.43) |
| | Q4 | 104 | 42 | 458 | 0.67 (0.40, 1.12) | 292 | 82 | 939 | 1.08 (0.75, 1.54) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.009 |

| | | Stratum #1 | | | | Stratum #2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| **PFAS** | **PFAS Category** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** |
| | | **Aged <70 years** | | | | **Aged ≥70 years** | | | |
| | Continuous | | | | 0.89 (0.68, 1.16) | | | | 0.98 (0.82, 1.17) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.2258 |
| PFOS[i] | Q1 | 135 | 43 | 427 | 1.00 (−) | 254 | 80 | 905 | 1.00 (−) |
| | Q2 | 103 | 29 | 305 | 1.16 (0.69, 1.98) | 198 | 74 | 830 | 0.94 (0.65, 1.36) |
| | Q3 | 143 | 42 | 407 | 1.19 (0.72, 1.97) | 358 | 99 | 1171 | 1.16 (0.82, 1.62) |
| | Q4 | 123 | 42 | 413 | 1.15 (0.70, 1.89) | 285 | 82 | 916 | 1.20 (0.85, 1.70) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.4896 |
| | Continuous | | | | 1.05 (0.84, 1.31) | | | | 1.01 (0.87, 1.17) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.5673 |
| | | **Never Smoker** | | | | **Former/Current Smoker** | | | |
| PFHxS[e,f] | Q1 | 135 | 43 | 496 | 1.00 (−) | 210 | 77 | 846 | 1.00 (−) |
| | Q2 | 176 | 45 | 466 | 1.46 (0.90, 2.37) | 266 | 74 | 784 | 1.29 (0.88, 1.89) |
| | Q3 | 164 | 51 | 622 | 1.09 (0.67, 1.77) | 268 | 75 | 820 | 1.25 (0.86, 1.81) |
| | Q4 | 156 | 40 | 422 | 1.66 (1.00, 2.74) | 224 | 86 | 920 | 0.99 (0.68, 1.43) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.0004 |
| | Continuous | | | | 1.10 (0.94, 1.29) | | | | 1.04 (0.92, 1.18) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.3161 |
| PFNA[e,g] | Q1 | 158 | 41 | 460 | 1.00 (−) | 231 | 74 | 803 | 1.00 (−) |
| | Q2 | 129 | 41 | 479 | 0.76 (0.46, 1.26) | 208 | 86 | 995 | 0.74 (0.51, 1.07) |
| | Q3 | 176 | 46 | 509 | 1.01 (0.62, 1.63) | 254 | 75 | 751 | 1.04 (0.71, 1.53) |
| | Q4 | 168 | 51 | 558 | 0.95 (0.58, 1.55) | 275 | 77 | 822 | 1.11 (0.76, 1.62) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.6599 |
| | Continuous | | | | 0.98 (0.80, 1.20) | | | | 1.06 (0.92, 1.23) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.2355 |
| PFOA[e,h] | Q1 | 178 | 47 | 520 | 1.00 (−) | 220 | 65 | 674 | 1.00 (−) |
| | Q2 | 141 | 44 | 485 | 0.90 (0.56, 1.45) | 210 | 77 | 815 | 0.87 (0.58, 1.29) |
| | Q3 | 177 | 46 | 529 | 1.12 (0.70, 1.78) | 277 | 88 | 955 | 0.95 (0.65, 1.38) |

| | | Stratum #1 | | | | Stratum #2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| **PFAS** | **PFAS Category** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** | **Cases** | **Sub-Cohort** | **Sub-cohort person-years** | **HR (95% CI)** |
| | | **Aged <70 years** | | | | **Aged ≥70 years** | | | |
| | Q4 | 135 | 42 | 471 | 0.94 (0.58, 1.53) | 261 | 82 | 925 | 0.91 (0.62, 1.34) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.7162 |
| | Continuous | | | | 1.00 (0.76, 1.30) | | | | 0.93 (0.78, 1.12) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.4393 |
| PFOS[e,i] | Q1 | 155 | 43 | 469 | 1.00 (−) | 234 | 80 | 863 | 1.00 (−) |
| | Q2 | 125 | 37 | 440 | 1.10 (0.67, 1.79) | 176 | 66 | 696 | 0.96 (0.65, 1.42) |
| | Q3 | 207 | 61 | 657 | 1.20 (0.76, 1.91) | 294 | 80 | 921 | 1.15 (0.80, 1.66) |
| | Q4 | 144 | 38 | 440 | 1.32 (0.80, 2.19) | 264 | 86 | 890 | 1.12 (0.79, 1.60) |
| | P-Interaction (categorical)[d] | | | | | | | | 0.7221 |
| | Continuous | | | | 0.99 (0.78, 1.26) | | | | 1.03 (0.89, 1.20) |
| | P-Interaction (continuous)[d] | | | | | | | | 0.6297 |

[a]All models adjust for age, year of blood sample collection, education, alcohol use, smoking status, race. Age and smoking status violated the proportional hazards assumption, so models adjust for these variables using a stratified Cox procedure, stratifying the baseline hazard on single year of age and smoking status (ever smoker, never smoker).

[b]Obtained from an interaction model including dummy variables representing the 2nd through 4th quartiles of PFAS concentrations (i.e., Q2, Q3, Q4), a dummy variable representing the covariate of interest, and their interaction terms (for quartile analyses); or a continuous variable for the $\log_2$ PFAS concentration a dummy variable representing the covariate of interest, and their interaction term (for continuous analyses).

[c]Excludes participants missing information on one or more covariates in the model (n = 20).

[d]Calculated using a likelihood ratio test.

[e]The proportional hazards assumption for age and smoking status was violated. Therefore, interaction models account for this by including dummy variables representing the product terms between the PFAS of interest and the covariate that violated the proportional hazards assumption (e.g., age [<70, ≥70 years] or smoking status [ever, never]) as covariates in the model, and including the covariate the violated the proportional hazards assumption in the STRATA statement of SAS PROC PHREG.

[f]The range of values (in ng/mL) represented for each quartile of PFHxS concentration are as follows: Q1, 0.14–2.00; Q2, 2.10–3.20; Q3, 3.30–5.20; and Q4, 5.30–95.0.

[g]The range of values (in ng/mL) represented for each quartile of PFNA concentration are as follows: Q1, 0.06–0.48; Q2, 0.49–0.66; Q3, 0.67–0.98; and Q4, 0.99–80.0.

[h]The range of values (in ng/mL) represented for each quartile of PFOA concentration are as follows: Q1, 0.35–3.90; Q2, 4.00–5.10; Q3, 5.20–6.90; and Q4, 7.00–90.0.

[i]The range of values (in ng/mL) represented for each quartile of PFOS concentration are as follows: Q1, 0.35–13.0; Q2, 14.0–17.0; Q3, 18.0–25.0; and Q4, 26.0–160.