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TITLE: A Nested Case-Control Study of Serum Levels of Polyfluoroalkyl Chemicals (PFCs) and Kidney Cancer Among Participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

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<b>6. AUTHOR(S)</b> Dr. Jonathan Hofmann  <a href="mailto:hofmannjn@mail.nih.gov">hofmannjn@mail.nih.gov</a>	<b>5d. PROJECT NUMBER</b>
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<b>13. SUPPLEMENTARY NOTES</b>
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<b>14. ABSTRACT</b> Objective: We hypothesize that perfluorooctanoic acid (PFOA), and possibly other per- and polyfluoroalkyl substances (PFAS) are human carcinogens, and that exposure to these substances is associated with increased risk of renal cell carcinoma (RCC). Our study is, to our knowledge, the first to assess RCC risk in relation to direct measurements of PFOA and other PFAS at levels of exposure comparable to those seen in the general population. Primary Aim: Prospectively investigate the relationship between serum concentrations of eight PFAS and RCC risk among 324 cases and 324 individually matched controls in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Major findings: We observed positive exposure-response associations with PFOA, perfluorooctane sulfonic acid (PFOS), and perfluorohexane sulfonic acid (PFHxS). The association with PFOA was similar after adjustment for other PFAS, and remained apparent in analyses restricted to individuals without evidence of diminished kidney function and in cases diagnosed $\geq 8$ years after phlebotomy. Impact: Our findings add substantially to the weight of evidence that PFOA is a renal carcinogen and may have important public health implications for the many individuals exposed to this ubiquitous and highly persistent chemical.
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<b>15. SUBJECT TERMS</b> Oncology, Cancer, Prevention
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## 1. INTRODUCTION:

Per- and polyfluoroalkyl substances (PFAS) are common industrial chemicals used in non-stick cookware, textiles, and firefighting foams. Many PFAS are resistant to environmental and biological degradation, with serum elimination half-lives exceeding 3-5 years. Nearly 98% of the U.S. adult population have detectable serum concentrations of certain PFAS. Recent evidence has suggested exposure to certain PFAS (e.g., perfluorooctanoic acid) may be associated with risk of kidney cancer; however, these results were based upon relatively small numbers of cancer cases in highly exposed populations. To further examine the carcinogenic potential of PFAS within the general population, we are evaluating the relationship between pre-diagnostic serum concentrations of 8 specific PFAS and risk of renal cell carcinoma (RCC), the most common form of kidney cancer, in a nested case-control study within the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Our results add substantially to the body of evidence suggesting a relationship between perfluorooctanoic acid and risk of RCC, and provide new insights regarding associations with additional PFAS that have not been evaluated in relation to RCC risk in previous epidemiologic studies.

## 2. KEYWORDS:

Per- and Polyfluoroalkyl Substances (PFAS)  
Perfluorooctanoic acid (PFOA)  
Renal Cell Carcinoma (RCC)  
Kidney cancer  
Environmental Contamination  
Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO)

## 3. ACCOMPLISHMENTS

**Primary Aim: Prospectively investigate the relationship between serum concentrations of eight per- and polyfluoroalkyl substances (PFAS) and renal cell carcinoma risk among participants in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial.**

**Major Task 1:** Generate the PFAS dataset, including regulatory review and approval by the USAMRMC Human Research Protection Office (HRPO), measurements of serum PFAS concentrations, and measurements of serum kidney function biomarkers.

**Major Task 2:** Analyze the PFAS dataset and prepare the results of the project for publication in peer-reviewed journals.

### **Summary of the key research accomplishments:**

Major Tasks 1 and 2 were completed during the Award period.

For Major Task 1, we received HRPO Concurrence with the “Determination or Research Not Involving Human Subjects” for the study protocol. Cases of renal cell carcinoma (RCC) and individually matched controls with available pre-diagnostic serum samples were selected from the

Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. De-identified serum samples were sent to the analytic laboratories at the Centers for Disease Control and Prevention (CDC) and the Brigham and Women's Hospital (BWH) for measurements of serum PFAS concentrations and kidney function biomarkers, respectively. Measurements of eight PFAS were performed using on-line solid phase extraction liquid chromatography isotope dilution tandem mass spectrometry. For the kidney function biomarkers, serum cystatin C and kidney injury molecule-1 (KIM-1) were measured using a microbead-based assay, and serum creatinine was measured using a clinical chemistry analyzer.

For Major Task 2, we combined the results from laboratory assays at the CDC and BWH with study questionnaire data to create an analytic dataset. Measurements of cystatin C and creatinine were used to calculate estimated glomerular filtration rate (eGFR) for each of the cases and controls based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. We conducted statistical analyses using conditional logistic regression to compute odds ratios (OR) and 95% confidence intervals (95% CI) estimating the risk of RCC in relation to serum concentrations of each PFAS. The results of our analyses are summarized in the attached article (Shearer et al, *JNCI* 2020).

**What opportunities for training and professional development did the project provide?**

Drs. Joseph Shearer and Catherine Callahan worked on this project during their postdoctoral fellowship training; they were also co-first authors of the resulting paper (listed below under publications).

**How were the results disseminated to communities of interest?**

Nothing to Report

**What do you plan to do during the next reporting period to accomplish the goals and objectives?**

Nothing to Report

**4. IMPACT:**

**What was the impact on the development of the principal discipline(s) of the project?**

Our results may have important implications for evaluations of the carcinogenicity of these ubiquitous and highly persistent chemicals. In 2014, the International Agency for Research on Cancer (IARC) classified perfluorooctanoic acid (PFOA), the most studied PFAS, as a possible carcinogen (group 2B) based in part on limited epidemiologic evidence of associations with kidney cancer from small studies of exposed workers and communities near a PFAS producing plant. More recently, an Advisory Group for IARC recommended that PFOA and other PFAS should be a high priority for future evaluations of carcinogenicity.

**What was the impact on other disciplines?**

Nothing to Report

**What was the impact on technology transfer?**

Nothing to Report

**What was the impact on society beyond science and technology?**

These results may provide regulatory and public health agencies with important epidemiologic evidence that they can utilize to help develop and implement regulatory policies aimed at mitigating exposure to certain potentially harmful substances.

**5. CHANGES/PROBLEMS:**

**Changes in approach and reasons for change**

Nothing to Report

**Actual or anticipated problems or delays and actions or plans to resolve them:**

Nothing to Report

**Changes that had a significant impact on expenditures**

Nothing to Report

**Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:**

Nothing to Report

**Significant changes in use or care of human subjects:**

Nothing to Report

**Significant changes in use or care of vertebrate animals:**

Nothing to Report

**Significant changes in use of biohazards and/or select agents:**

Nothing to Report

## 6. PRODUCTS:

### Publication:

Shearer JJ, Callahan CL, Calafat AM, Huang WY, Jones RR, Sabbisetti VS, Freedman ND, Sampson JN, Silverman DT, Purdue MP, Hofmann JN. Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma. *Journal of the National Cancer Institute* 2020 Sep 18 (Epub ahead of print).

### Books or other non-periodical, one-time publications.

Nothing to Report.

### Other publications, conference papers, and presentations.

Shearer JJ, Callahan CL, Calafat AM, Huang WY, Jones RR, Sabbisetti VS, Freedman ND, Sampson JN, Silverman DT, Purdue MP, Hofmann JN. Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma: A nested case-control study in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Oral presentation at the 32<sup>nd</sup> Annual Conference of the International Society for Environmental Epidemiology (ISEE). August 2020 (Virtual Meeting).

### Website(s) or other Internet site(s)

Nothing to Report

### Technologies or techniques

Nothing to Report

### Inventions, patent applications, and/or licenses

Nothing to Report

### Other Products

Nothing to Report

## 7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name:	Jonathan Hofmann
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-1043-5812

Nearest person month worked:	3.0 calendar months
Contribution to Project:	Project management
Funding Support:	NCI Intramural Research Program

Name:	Antonia Calafat
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	0000-0002-9796-7482
Nearest person month worked:	2.0 calendar months
Contribution to Project:	Measurements of serum PFAS concentrations in de-identified samples
Funding Support:	Serum PFAS measurements were supported by the NCI Intramural Research Program and this award.

Name:	Venkata Sabbiseti
Project Role:	Collaborator
Researcher Identifier (e.g. ORCID ID):	0000-0001-8696-2726
Nearest person month worked:	2.0 calendar months
Contribution to Project:	Measurements of kidney function biomarkers in de-identified samples
Funding Support:	Kidney function biomarker measurements were supported by this award.

**Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report

**What other organizations were involved as partners?**

Nothing to Report

**8. SPECIAL REPORTING REQUIREMENTS:**

**COLLABORATIVE AWARDS:** Nothing to Report

**QUAD CHARTS:** Attached

**9. APPENDICES**

Shearer JJ, Callahan CL, Calafat AM, Huang WY, Jones RR, Sabbisetti VS, Freedman ND, Sampson JN, Silverman DT, Purdue MP, Hofmann JN. Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma. *Journal of the National Cancer Institute* 2020 Sep 18 (Epub ahead of print).

Shearer JJ, Callahan CL, Calafat AM, Huang WY, Jones RR, Sabbisetti VS, Freedman ND, Sampson JN, Silverman DT, Purdue MP, Hofmann JN. Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma: A nested case-control study in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Oral presentation at the 32<sup>nd</sup> Annual Conference of the International Society for Environmental Epidemiology (ISEE). August 2020 (Virtual Meeting).

## **Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma: A nested case-control study in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial**

*Joseph J. Shearer, Catherine L. Callahan, Antonia M. Calafat, Wen-Yi Huang, Rena R. Jones, Venkata S. Sabbiseti, Neal D. Freedman, Joshua N. Sampson, Debra T. Silverman, Mark P. Purdue, and Jonathan N. Hofmann*

**Background:** Per- and polyfluoroalkyl substances (PFAS) are a diverse class of industrial chemicals used in non-stick cookware, firefighting foams, and textiles. Exposure to PFAS is widespread in the general population, with detectable serum levels in 98% of U.S. adults. The International Agency for Research on Cancer has classified perfluorooctanoic acid (PFOA) as a possible kidney carcinogen; other PFAS have not yet been evaluated. The purpose of this study was to assess whether pre-diagnostic serum concentrations of PFOA and seven additional PFAS are associated with risk of renal cell carcinoma (RCC) in a large population-based cohort.

**Methods:** We conducted a nested case-control study within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial that included 324 histologically-confirmed RCC cases and 324 individually matched controls. Serum concentrations of eight PFAS were quantified using on-line solid phase extraction liquid chromatography isotope dilution tandem mass spectrometry. Multivariable conditional logistic regression analysis was used to estimate RCC risk in relation to each individual PFAS modeled both continuously (Log<sub>2</sub>-transformed) and categorically. All models were implicitly conditioned on matching factors (e.g., age, sex, race, study center) and further adjusted for kidney function (i.e., estimated glomerular filtration rate) and other potential confounders.

**Results:** In preliminary analyses, we observed positive exposure-response associations with RCC risk for several PFAS including PFOA, perfluorooctane sulfonic acid, and perfluorohexane sulfonic acid. However, only the association with PFOA remained apparent after adjustment for all three chemicals.

**Conclusions:** This is, to our knowledge, the first study to investigate the associations between serum concentrations of individual PFAS and risk of RCC in a prospective cohort with PFAS concentrations comparable to the general population. Our findings may have important public health implications for the many individuals exposed to these ubiquitous and highly persistent chemicals.

**Supplementary material for “Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma”**

**Supplementary Table 1.** Quality Control Results for Serum PFAS Measurements in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial <sup>a</sup>

PFAS	<u>Below Limit of Detection</u>		<u>Coefficients of Variation</u>		Overall	ICCs
	Controls	Cases	Intra-batch	Inter-batch		
PFOA	0.0%	0.0%	8.2%	3.2%	8.8%	0.97
PFOS	0.0%	0.0%	7.2%	4.7%	8.6%	0.96
PFHxS	0.0%	0.0%	11.7%	14.1%	18.3%	0.92
PFUnDA	51.2%	49.7%	16.6%	16.2%	23.1%	0.92
PFNA	2.2%	1.5%	10.7%	9.5%	14.3%	0.94
EtFOSAA	0.0%	0.0%	8.5%	11.2%	14.1%	0.97
MeFOSAA	0.0%	0.3%	11.2%	16.5%	20.0%	0.92
PFDA	28.1%	28.4%	10.6%	20.0%	22.6%	0.94

Abbreviations: EtFOSAA, 2-N-ethyl-perfluorooctane sulfonamido acetic acid; ICC, intraclass correlation coefficient PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; MeFOSAA, 2-N-methyl-perfluorooctane sulfonamido acetic acid.

<sup>a</sup> Results were calculated from a total of 59 quality control samples from 22 individuals, with replicate samples from the same individual distributed within and across batches (including within-batch paired samples from all 22 individuals and an additional across-batch sample from 15 individuals).

**Supplementary Table 2.** Spearman Correlation Coefficients of Serum PFAS Concentrations among Control Participants (N=324) in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

PFAS	PFOA	PFOS	PFHxS	PFUnDA	PFNA	EtFOSAA	MeFOSAA	PFDA
PFOA	1.00							
PFOS	0.62	1.00						
PFHxS	0.42	0.45	1.00					
PFUnDA	0.12	0.30	0.21	1.00				
PFNA	0.42	0.54	0.36	0.57	1.00			
EtFOSAA	0.38	0.50	0.02	-0.02	0.07	1.00		
MeFOSAA	0.27	0.32	0.17	0.04	0.29	0.18	1.00	
PFDA	0.25	0.48	0.27	0.81	0.68	0.07	0.15	1.00

Abbreviations: EtFOSAA, 2-N-ethyl-perfluorooctane sulfonamido acetic acid; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; MeFOSAA, 2-N-methyl-perfluorooctane sulfonamido acetic acid.

**Supplementary Table 3.** Adjusted Geometric Mean (95% CI)<sup>a</sup> Serum PFAS Concentrations (µg/L) among Controls (N=324) in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

Characteristic	N	PFOA	PFOS	PFHxS	PFUnDA	PFNA	EtFOSAA	MeFOSAA	PFDA
Age <sup>b</sup> , years									
55-59	95	4.4 (3.4-5.6)	33.8 (25.9-44.2)	3.2 (2.2-4.7)	0.2 (0.1-0.3)	0.6 (0.5-0.9)	0.9 (0.6-1.5)	1.5 (1.1-2.1)	0.2 (0.2-0.4)
60-64	112	4.3 (3.4-5.4)	34.3 (26.5-44.4)	2.5 (1.7-3.6)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.1 (0.7-1.8)	1.5 (1.1-2.1)	0.3 (0.2-0.4)
65-69	80	4.1 (3.3-5.3)	35.6 (27.2-46.5)	2.7 (1.9-4.0)	0.2 (0.1-0.2)	0.7 (0.5-1.0)	1.1 (0.7-1.8)	1.4 (1.0-1.9)	0.2 (0.2-0.4)
70+	37	4.4 (3.3-5.8)	34.4 (25.3-47.0)	2.3 (1.5-3.6)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.2 (0.7-2.1)	1.3 (0.9-1.9)	0.2 (0.2-0.4)
Center <sup>c</sup>									
Upper Midwest Region	130	3.9 (3.0-5.0)	33.3 (25.3-43.9)	2.4 (1.6-3.6)	0.1 (0.1-0.2)	0.5 (0.4-0.8)	1.0 (0.6-1.6)	1.3 (0.9-1.8)	0.2 (0.1-0.3)
Western and Southern Region	102	4.6 (3.7-5.8)	37.6 (29.4-48.1)	2.7 (1.9-3.8)	0.2 (0.1-0.2)	0.7 (0.5-0.9)	1.3 (0.8-2.0)	1.6 (1.2-2.1)	0.2 (0.1-0.3)
Eastern States Region	92	4.3 (3.4-5.6)	32.9 (25.0-43.2)	2.9 (2.0-4.3)	0.2 (0.2-0.4)	0.9 (0.7-1.3)	1.0 (0.6-1.7)	1.4 (1.0-2.0)	0.3 (0.2-0.5)
Gender									
Female	108	4.0 (3.2-5.1)	31.3 (24.0-40.7)	2.3 (1.6-3.3)	0.2 (0.1-0.2)	0.7 (0.5-0.9)	1.1 (0.7-1.8)	1.4 (1.0-2.0)	0.2 (0.1-0.3)
Male	216	4.5 (3.6-5.7)	38.1 (29.5-49.2)	3.1 (2.2-4.5)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.0 (0.6-1.7)	1.4 (1.1-1.9)	0.3 (0.2-0.4)
Race									
White, non-Hispanic	287	4.8 (3.9-5.8)	30.9 (24.7-38.8)	3.3 (2.4-4.5)	0.1 (0.1-0.1)	0.6 (0.5-0.8)	1.1 (0.7-1.7)	1.3 (1.0-1.8)	0.2 (0.1-0.2)
Black, non-Hispanic	21	4.5 (3.4-6.0)	52.3 (38.2-71.6)	3.3 (2.1-5.1)	0.3 (0.2-0.4)	0.9 (0.6-1.3)	1.1 (0.6-2.1)	1.7 (1.2-2.4)	0.4 (0.3-0.7)
Other	16	3.6 (2.6-5.1)	25.4 (17.7-36.6)	1.8 (1.1-3.0)	0.2 (0.1-0.4)	0.6 (0.4-0.9)	0.9 (0.5-1.9)	1.3 (0.8-2.0)	0.2 (0.1-0.4)
Body Mass Index <sup>b</sup> , kg/m <sup>2</sup>									
18.5-<25	83	4.0 (3.3-4.9)	35.2 (28.2-43.9)	2.4 (1.8-3.3)	0.2 (0.1-0.3)	0.8 (0.6-1.0)	1.1 (0.7-1.6)	1.3 (1.0-1.6)	0.3 (0.2-0.4)
25-<30	158	4.5 (3.7-5.3)	35.6 (29.1-43.6)	2.6 (2.0-3.5)	0.2 (0.1-0.2)	0.8 (0.6-1.0)	1.1 (0.7-1.6)	1.5 (1.2-1.9)	0.2 (0.2-0.3)
30+	76	4.5 (3.7-5.6)	36.6 (29.3-45.7)	2.5 (1.8-3.5)	0.2 (0.1-0.2)	0.8 (0.6-1.0)	1.2 (0.8-1.8)	1.5 (1.2-2.0)	0.2 (0.1-0.3)
History of Hypertension <sup>b</sup>									
No	216	4.3 (3.4-5.5)	35.7 (27.5-46.2)	2.8 (1.9-4.0)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.0 (0.6-1.7)	1.5 (1.1-2.0)	0.3 (0.2-0.4)
Yes	108	4.3 (3.4-5.4)	33.4 (25.8-43.3)	2.6 (1.8-3.7)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	1.1 (0.7-1.8)	1.4 (1.0-1.9)	0.2 (0.2-0.3)
Smoking Status <sup>b</sup>									
Never	155	4.4 (3.5-5.6)	36.3 (28.0-47.2)	3.0 (2.1-4.3)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	1.0 (0.6-1.6)	1.5 (1.1-2.0)	0.3 (0.2-0.4)
Current	35	4.0 (3.1-5.3)	30.8 (22.8-41.5)	2.3 (1.5-3.5)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	1.1 (0.6-1.9)	1.4 (1.0-2.1)	0.2 (0.1-0.3)
Former	134	4.4 (3.5-5.5)	36.8 (28.5-47.5)	2.8 (1.9-4.0)	0.2 (0.1-0.3)	0.7 (0.6-1.0)	1.1 (0.7-1.8)	1.4 (1.0-1.9)	0.2 (0.2-0.4)
eGFR <sup>d</sup> , mL/min/1.73 m <sup>2</sup>									
90+	109	4.5 (3.5-5.7)	34.6 (26.7-44.9)	2.7 (1.9-3.9)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.0 (0.6-1.7)	1.4 (1.0-1.9)	0.3 (0.2-0.4)
<90-60	197	4.4 (3.5-5.5)	34.8 (27.1-44.7)	2.8 (2.0-4.0)	0.2 (0.1-0.3)	0.7 (0.5-0.9)	1.1 (0.7-1.8)	1.3 (1.0-1.8)	0.2 (0.2-0.3)
<60	18	4.0 (2.9-5.4)	34.2 (24.3-48.1)	2.5 (1.6-4.1)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.1 (0.6-2.1)	1.5 (1.0-2.3)	0.2 (0.1-0.4)

Calendar Year <sup>d</sup>										
1993-1995	84	4.0 (3.1-5.1)	33.1 (25.2-43.6)	2.7 (1.8-4.0)	0.2 (0.1-0.3)	0.6 (0.5-0.9)	1.2 (0.7-2.0)	1.3 (0.9-1.8)	0.2 (0.1-0.3)	
1996-1997	116	4.3 (3.4-5.5)	35.0 (26.8-45.7)	2.5 (1.7-3.6)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	1.1 (0.7-1.9)	1.4 (1.0-1.9)	0.2 (0.2-0.4)	
1998-2002	124	4.6 (3.6-5.8)	35.5 (27.4-46.0)	2.8 (2.0-4.1)	0.2 (0.1-0.3)	0.7 (0.5-1.0)	0.9 (0.6-1.5)	1.6 (1.2-2.1)	0.3 (0.2-0.4)	

Abbreviations: eGFR, estimated glomerular filtration rate; EtFOSAA, 2-N-ethyl-perfluorooctane sulfonamido acetic acid; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; MeFOSAA, 2-N-methyl-perfluorooctane sulfonamido acetic acid.

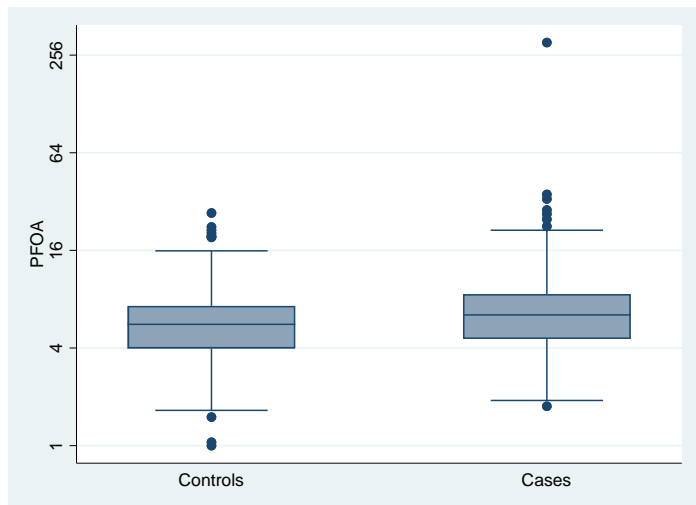
<sup>a</sup> Geometric LS-MEANS among the 324 controls were estimated after adjusting for age category (55-59, 60-64, 65-69, 70+), center, gender (male, female), race (white, black, other), body mass index (underweight, normal, overweight, obese, missing), hypertension (no/missing, yes), smoking status (never, former, current), eGFR (90+, <90-60, <60), previous freeze-thaw cycle, calendar year of blood draw (1993-1995,1996-1997,1998-2002), blood collection (baseline, follow up visit).

<sup>b</sup> Self-reported at study baseline.

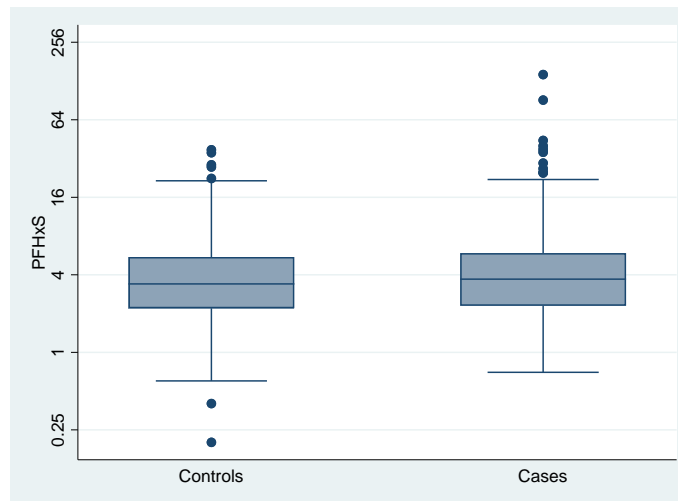
<sup>c</sup> Upper Midwest Region: Minnesota or Marshfield; Western and Southern Region: Colorado, Hawaii, Washington University, University of Utah, or University of Alabama; Southern Region: Georgetown, Henry Ford, or University of Pittsburgh).

<sup>d</sup> At blood draw.

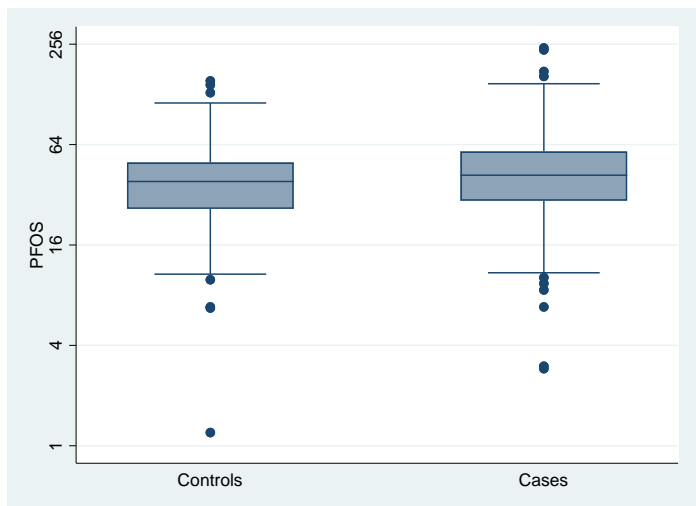
**PFOA**



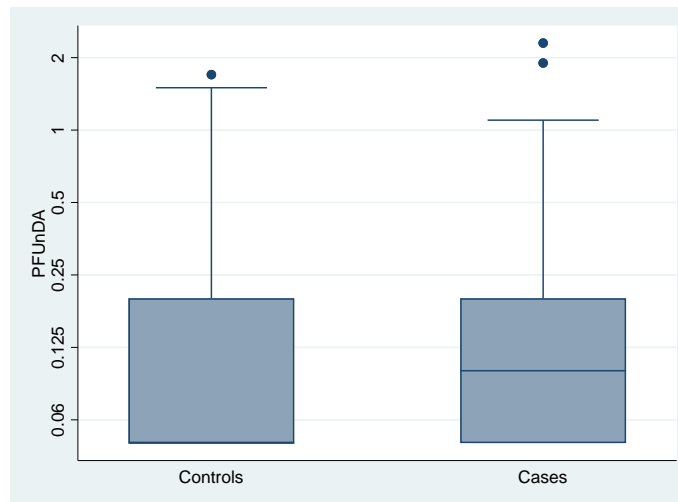
**PFHxS**



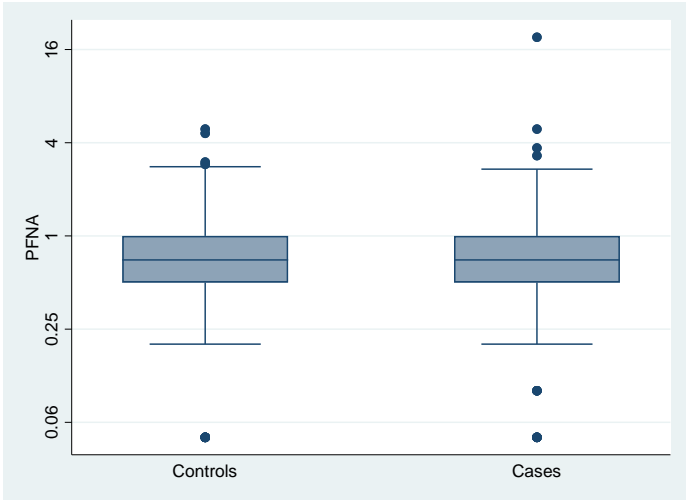
**PFOS**



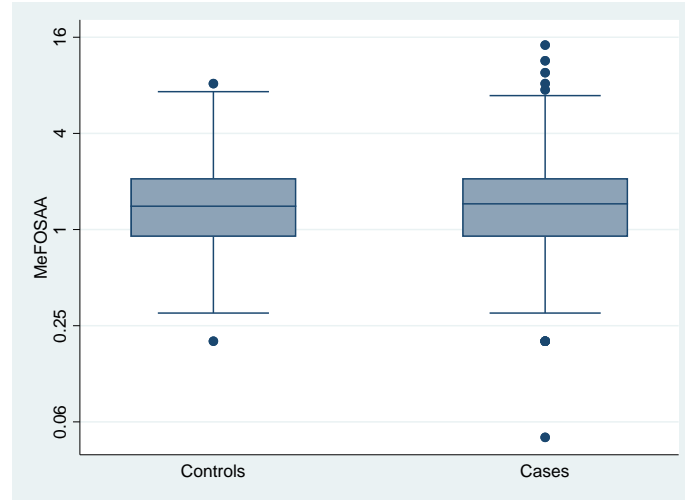
**PFUnDA**



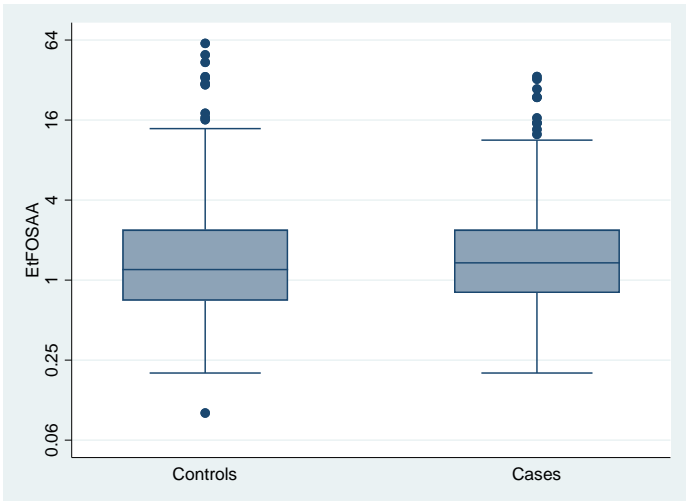
**PFNA**



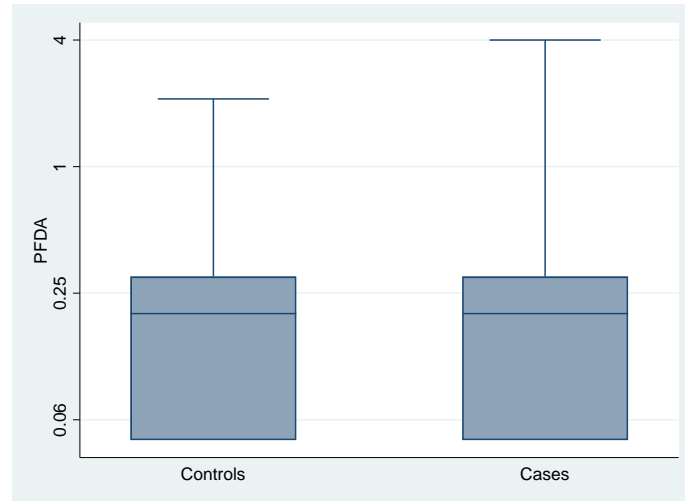
**MeFOSAA**



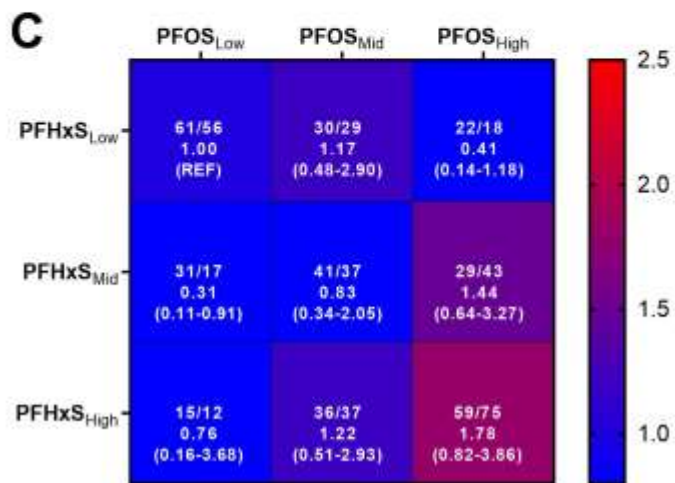
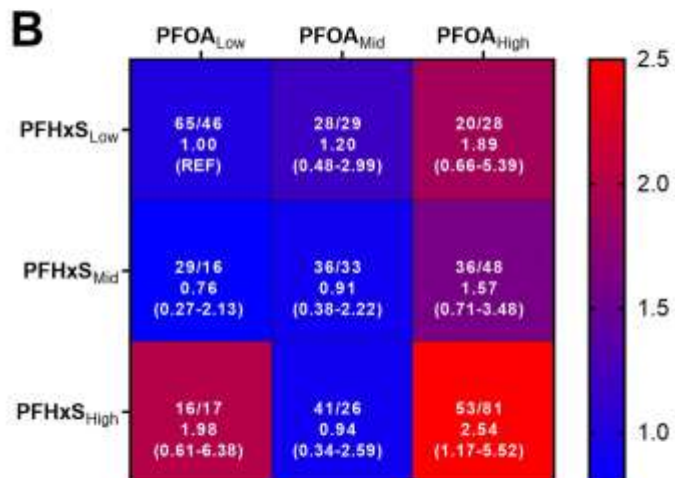
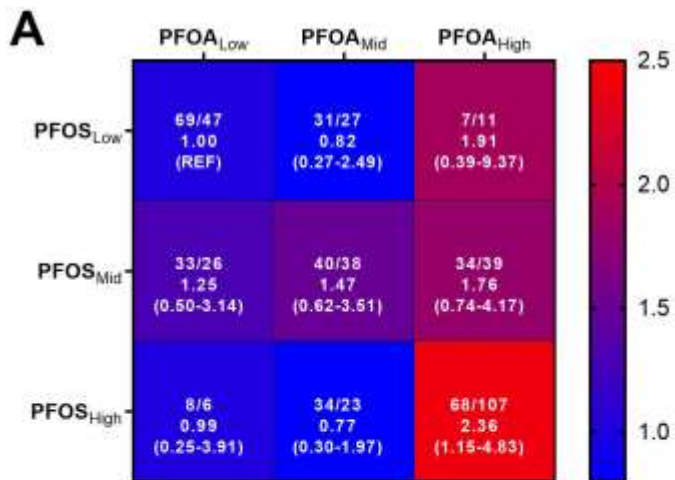
**EtFOSAA**



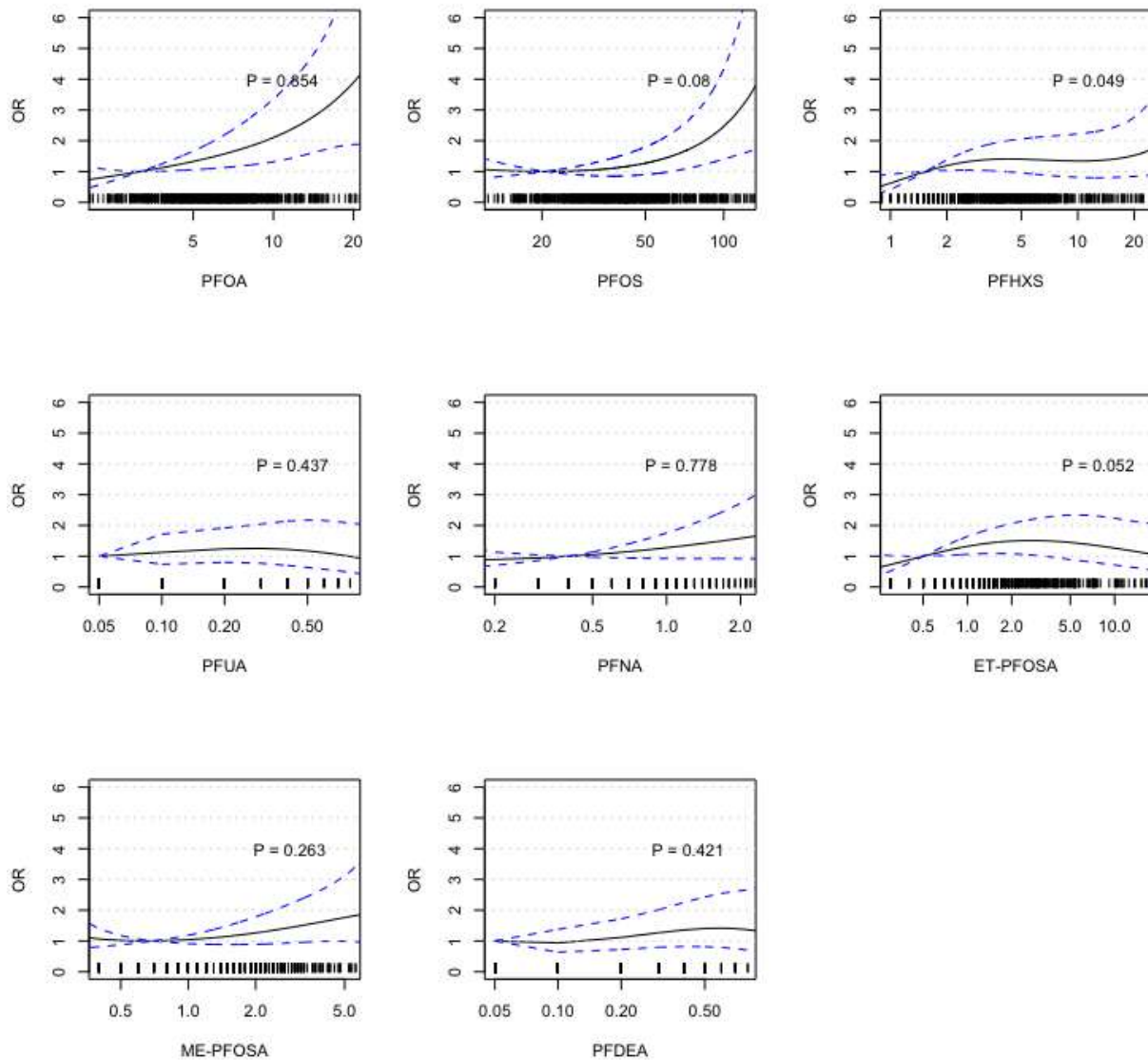
**PFDA**



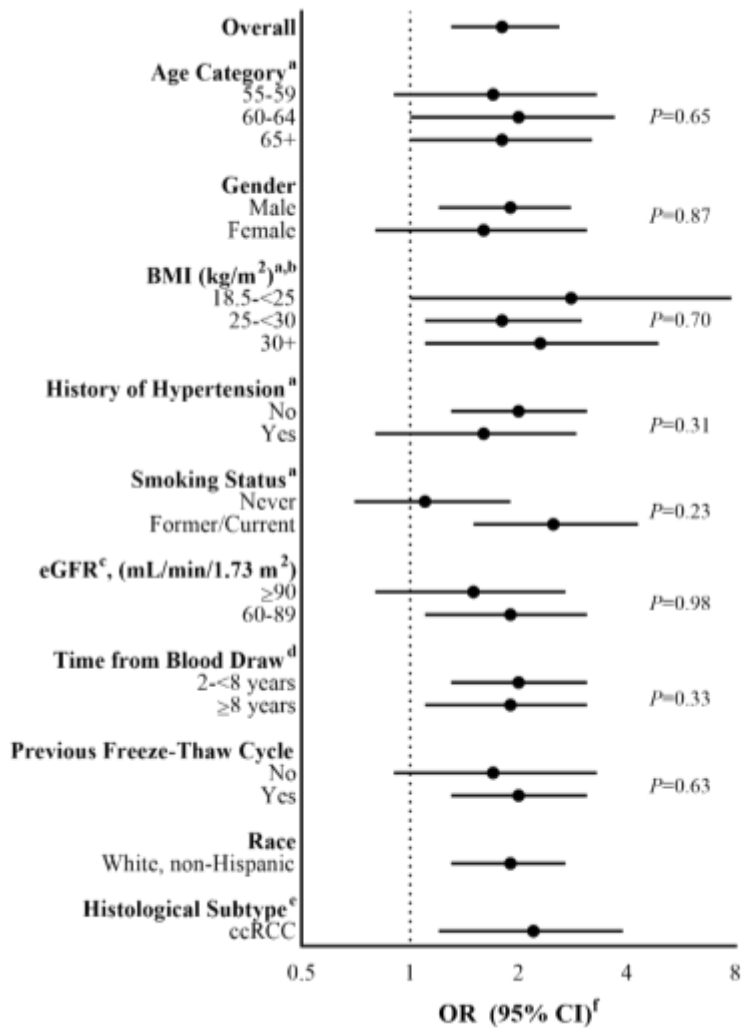
**Supplementary Figure 1.** Box plots for serum PFAS concentrations (ug/L) among RCC cases and controls



**Supplementary Figure 2: Joint Analyses of Select PFAS and RCC Risk in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. (A) PFOA and PFOS, (B) PFOA and PFHxS, and (C) PFOS and PFHxS. Results are shown as the  $N_{\text{controls}}/N_{\text{cases}}$  and ORs (95% CI), adjusted for body mass index (missing, <18.5, 18.5-<25, 25-<30, or  $\geq 30$  kg/m<sup>2</sup>), smoking status (never, former, current), history of hypertension (no, yes), estimated glomerular filtration rate (continuous), previous freeze-thaw cycle, and calendar year of blood draw (1993-1995, 1996-1997, 1998-2002). Tertile values (based on PFAS distributions of controls) are as follows: PFOA<sub>Low</sub>  $\leq 4.5$   $\mu\text{g/L}$ ;  $4.5 < \text{PFOA}_{\text{Mid}} \leq 6.5$   $\mu\text{g/L}$ ; PFOA<sub>High</sub>  $> 6.5$   $\mu\text{g/L}$ ; PFOS<sub>Low</sub>  $\leq 30$   $\mu\text{g/L}$ ;  $30 < \text{PFOS}_{\text{Mid}} \leq 45.6$   $\mu\text{g/L}$ ; PFOS<sub>High</sub>  $> 45.6$   $\mu\text{g/L}$ ; PFHxS<sub>Low</sub>  $\leq 2.5$   $\mu\text{g/L}$ ;  $2.5 < \text{PFHxS}_{\text{Mid}} \leq 4.4$   $\mu\text{g/L}$ ; PFHxS<sub>High</sub>  $> 4.4$   $\mu\text{g/L}$ .**



**Supplementary Figure 3.** The relationship between the PFAS levels (X-axis) and OR (Y-axis). The black and blue curves illustrate the estimated effect and associated 95% confidence interval for a range of PFAS levels. The dark black marks along the x-axis indicate observed serum PFAS concentrations ( $\mu\text{g/L}$ ). Here, we use conditional logistic regression with the  $\log_2$ -transformed PFAS level modeled by a natural spline with three degrees of freedom. We assessed the improvement in the model fit, compared with including only a linear term for the  $\log_2$ -transformed level, using a likelihood ratio test and report the resulting p-value. Note, the figure is trimmed at the 2.5th and 97.5th PFAS percentiles for presentation.



**Supplementary Figure 4.** Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for Stratified and Sensitivity Analyses Evaluating Serum PFOA Concentrations and RCC Risk in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial with Further Adjustment for Other PFAS. Abbreviations: BMI, body mass index; ccRCC, clear cell renal cell carcinoma; eGFR, estimated glomerular filtration rate; PFAS, per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid. <sup>a</sup> Self-reported at study enrollment. <sup>b</sup> BMI specific analyses exclude individuals with missing or <18.5 kg/m<sup>2</sup> BMIs. <sup>c</sup> At blood draw. <sup>d</sup> Time from blood to diagnosis for cases. <sup>e</sup> ICD-O-2 code = 8310. <sup>f</sup> Continuous OR (95% CI), which corresponds to a 1 unit increase on the log<sub>2</sub> scale or an approximate doubling in analyte level, was estimated using unconditional multivariable logistic regression models adjusted for age at enrollment (55-59, 60-64, 65-69, 70+ years), sex (male, female), race/ethnicity (white non-Hispanic, black non-Hispanic, or other), estimated glomerular filtration rate (continuous), body mass index (<18.5 kg/m<sup>2</sup>, 18.5 to <25 kg/m<sup>2</sup>, 25 to <30 kg/m<sup>2</sup>, ≥30 kg/m<sup>2</sup>, missing), history of hypertension (no/missing, yes), smoking status (never, former, current), previous freeze-thaw cycle, calendar year of blood draw (1993-1995, 1996-1997, 1998-2002), study year of blood draw (enrollment, other), and study center ([1]Minnesota or Marshfield; [2] Colorado, Hawaii, Washington University, University of Utah, or University of Alabama; [3] Georgetown, Henry Ford, or University of Pittsburgh), further adjusted for other PFAS (i.e., log<sub>2</sub> transformed concentrations of PFOS and PFHxS). *P*-values represent Wald tests of heterogeneity across strata.

## **Serum concentrations of per- and polyfluoroalkyl substances and risk of renal cell carcinoma**

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## ABSTRACT

**Background:** Per- and polyfluoroalkyl substances (PFAS) are highly persistent chemicals that have been detected in the serum of >98% of the U.S. population. Studies among highly exposed individuals suggest an association with perfluorooctanoic acid (PFOA) exposure and kidney cancer. It remains unclear whether PFOA or other PFAS are renal carcinogens, or if they influence risk of renal cell carcinoma (RCC) at concentrations observed in the general population.

**Methods:** We measured pre-diagnostic serum concentrations of PFOA and seven additional PFAS in 324 RCC cases and 324 individually matched controls within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Multivariable conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CIs) relating serum PFAS concentrations and RCC risk. Individual PFAS were modeled continuously (log<sub>2</sub>-transformed) and categorically, with adjustment for kidney function and additional potential confounders. All statistical tests were two-sided.

**Results:** We observed a positive association with RCC risk for PFOA (doubling in serum concentration, OR<sub>continuous</sub> = 1.71, 95% CI = 1.23 to 2.37; *P* = .002), and a greater than two-fold increased risk among those in the highest quartile vs. the lowest (OR = 2.63, 95% CI = 1.33 to 5.20; *P*<sub>trend</sub> = .007). The association with PFOA was similar after adjustment for other PFAS (OR<sub>continuous</sub> = 1.68, 95% CI = 1.07 to 2.63; *P* = .02), and remained apparent in analyses restricted to individuals without evidence of diminished kidney function and in cases diagnosed ≥8 years after phlebotomy.

**Conclusions:** Our findings add substantially to the weight of evidence that PFOA is a renal carcinogen and may have important public health implications for the many individuals exposed to this ubiquitous and highly persistent chemical.

Per- and polyfluoroalkyl substances (PFAS) are a diverse class of synthetic chemicals that have been used extensively since the 1950s in a wide range of commercial and industrial applications, including non-stick cookware, textiles, and firefighting foams. PFAS are highly persistent in the environment and many can bioaccumulate in humans, with serum elimination half-lives ranging from approximately 3-8 years.<sup>1,2</sup> Exposure to PFAS is widespread in the general population; serum concentrations of four major PFAS were all detectable in >98% of participants in the nationally representative U.S. National Health and Nutrition Examination Survey (NHANES).<sup>3</sup> Elevated concentrations of PFAS have been observed in drinking water supplies near PFAS point sources such as industrial sites, military firefighting training areas, and wastewater treatment plants.<sup>4</sup> In addition, studies of individuals exposed to contaminated drinking water have reported higher than background serum concentrations of certain PFAS.<sup>5-8</sup>

The International Agency for Research on Cancer (IARC) has classified perfluorooctanoic acid (PFOA) as a possible human carcinogen (Group 2B) based in part on limited epidemiologic evidence of associations with kidney cancer; the carcinogenic potential of other PFAS have not yet been evaluated.<sup>9</sup> Higher kidney cancer incidence and mortality were observed among individuals with high PFOA exposures from employment in a PFAS-producing chemical plant or residence in the surrounding community with contaminated drinking water.<sup>10-12</sup> However, to our knowledge, no prospective studies have assessed the relationship between PFOA and kidney cancer risk in the general population, and associations between other PFAS and risk of kidney cancer have not been evaluated. To address these research gaps, we conducted a nested case-control study within the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial to evaluate the risk of renal cell carcinoma (RCC, the most common form of

kidney cancer) in relation to pre-diagnostic serum concentrations of PFOA and seven other PFAS.

## **METHODS**

### **Study Population**

The design and sample collection procedures for PLCO have been described.<sup>13,14</sup> Briefly, PLCO is a randomized screening trial that recruited ~150,000 adults ages 55-74 years from study centers in 10 U.S. cities between 1993-2001; participants in the screening arm provided non-fasting blood samples. The PLCO Cancer Screening Trial protocol was approved by institutional review boards of the National Cancer Institute and the individual study centers, and all participants provided written informed consent.

Among participants in the screening arm with available pre-diagnostic serum samples, we identified 326 incident RCC cases (ICD-02 C64.9) diagnosed an average of 8.8 years after phlebotomy (range 2-18 years). Controls were individually matched to cases with a 1:1 ratio on age at enrollment (55-59, 60-64, 65-69, or  $\geq 70$  years), sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, Asian, or Native American), study center, and study year of blood draw. All controls were alive and free of RCC as of the diagnosis date of their corresponding matched case.

### **Laboratory Tests**

At the Centers for Disease Control and Prevention (CDC, Atlanta, GA), using on-line solid phase extraction liquid chromatography isotope dilution tandem mass spectrometry as described previously,<sup>15</sup> we quantified serum concentrations of 10 analytes: 2-N-methyl-perfluorooctane sulfonamido acetic acid (MeFOSAA), 2-N-ethyl-perfluorooctane sulfonamido

acetic acid (EtFOSAA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), linear PFOA (n-PFOA), sum of branched PFOA isomers (Sb-PFOA), linear perfluorooctane sulfonic acid (n-PFOS), and sum of perfluoromethylheptane sulfonic acid isomers (Sm-PFOS). The limit of detection (LOD) was 0.1 µg/L for all analytes; concentrations below the LOD were assigned a value of ½ the LOD.

We report results for total PFOA and PFOS, which were calculated by summing the concentrations of their respective isomers (i.e., n-PFOA and Sb-PFOA for total PFOA and n-PFOS and Sm-PFOS for total PFOS).<sup>16</sup> Samples from each matched case-control set were analyzed in the same analytical batch. Intra-assay coefficients of variation (CVs) for individual PFAS were 7.2-16.6%, and overall intraclass correlation coefficients were 0.92-0.97 (Supplementary Table 1). Laboratory staff were blinded to the case/control status of each sample and the quality control replicates among the test samples. CDC determined that analyzing coded specimens at the CDC laboratory did not constitute engagement in human subject research.

Given concerns that diminished kidney function may impact PFAS serum concentrations,<sup>17-20</sup> we measured serum cystatin C and creatinine in all cases and controls and calculated estimated glomerular filtration rate (eGFR) using the CKD-EPI equation.<sup>21</sup> Serum cystatin C was measured using a microbead-based assay on a Luminex system,<sup>22</sup> and serum creatinine was measured using a clinical chemistry analyzer.

## **Statistical Analysis**

Measurements of PFAS concentrations were missing for two RCC cases; we excluded those matched case-control sets, leaving a total of 324 cases and 324 matched controls for

analysis. For our primary analyses, we estimated odds ratios (ORs) and 95% confidence intervals (CIs) using multivariable conditional logistic regression analysis with PFAS concentrations modeled both continuously ( $\log_2$ -transformed) and categorically. Category cut points were assigned based on quartiles of serum concentrations of each PFAS among controls except for PFUnDA and PFDA, for which >25% of measurements were below the LOD (categories for PFUnDA:  $\leq$ LOD, >0.1-0.2  $\mu\text{g/L}$ , >0.2  $\mu\text{g/L}$ ; and PFDA:  $\leq$ LOD, >0.1-0.2  $\mu\text{g/L}$ , >0.2-0.3  $\mu\text{g/L}$ , >0.3  $\mu\text{g/L}$ ). Each model implicitly controlled for matching factors and was further adjusted for eGFR (continuous), body mass index (<18.5  $\text{kg/m}^2$ , 18.5 to <25  $\text{kg/m}^2$ , 25 to <30  $\text{kg/m}^2$ ,  $\geq$ 30  $\text{kg/m}^2$ , or missing), smoking status (never, former, or current), history of hypertension, prior freeze-thaw cycles, and calendar year of blood draw. Wald tests for linear trend were performed by modeling the within-category median of each quartile of exposure as a continuous variable. We also conducted secondary analyses where we flexibly modeled the relationship between the log-OR and the  $\log_2$ -transformed PFAS concentrations using a natural spline with three degrees of freedom, and then used a likelihood ratio test to assess model improvement over the primary model with only the linear term.

To assess the individual effects of specific PFAS, we performed further analyses adjusting for  $\log_2$ -transformed concentrations of PFOA, PFOS, and PFHxS. Then we evaluated the joint effects of PFOA, PFOS, and PFHxS with concentrations of each analyte categorized into tertiles based on the distributions among controls.

We performed secondary stratified analyses using unconditional logistic regression models, adjusting for individual matching factors and other covariates included in the primary analyses noted above, to estimate stratum-specific ORs and 95% CIs for individual PFAS modeled continuously. Because cases and controls within the same matched set may have

differed with respect to some of the stratifying variables, unconditional models were used to reduce the impact of missing data on the stratified analyses. Analyses were stratified by the following: age at enrollment (55-59, 60-64, 65+), sex, body mass index (18.5-<25, 25-<30,  $\geq 30$  kg/m<sup>2</sup>), history of hypertension, smoking history (ever, never), eGFR (60-89,  $\geq 90$  mL/min/1.73m<sup>2</sup>), samples with and without prior freeze-thaw cycles, and years from blood collection to RCC diagnosis (2-<8,  $\geq 8$  years). Wald tests of heterogeneity were performed by including an interaction term in the model. We also conducted sensitivity analyses restricted to non-Hispanic white subjects, those without evidence of diminished kidney function (i.e., eGFR  $\geq 60$  mL/min/1.73m<sup>2</sup>), and RCC cases of clear cell histology (ICD-02 morphology code 8310).

All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC). Results with two-sided *P*-values < .05 were considered statistically significant.

## RESULTS

Based on the matched design, RCC cases and controls had the same distributions for sex, race, age at enrollment, and study center (Table 1). Cases were more likely than controls to report being obese, and to have a history of hypertension at enrollment. A higher proportion of cases had diminished kidney function (eGFR <60 mL/min/1.73 m<sup>2</sup>) compared with controls (9.0% vs 5.6%) but this difference was not statistically significant (*P* = .25). The overall distributions of each PFAS among cases and controls are shown in Supplementary Figure 1. Among the controls, several PFAS were moderately correlated with one another (e.g., Spearman correlation coefficients of 0.62 for PFOA vs. PFOS, 0.42 for PFOA vs. PFHxS, and 0.45 for PFOS vs. PFHxS; Supplementary Table 2). In multivariable analyses, adjusted geometric mean concentrations of PFOS, PFNA, PFDA, and PFUnDA were statistically significantly elevated among African Americans relative to non-Hispanic whites (*P* < .01) (Supplementary Table 3).

These analyses also indicated PFOS, PFHxS, PFDA, and PFUnDA concentrations were statistically significantly lower among women compared with men ( $P < .02$ ).

As shown in Table 2, we observed statistically significant positive trends in RCC risk with increasing pre-diagnostic concentrations of several PFAS including PFOA (highest quartile vs. lowest, OR = 2.63, 95% CI = 1.33 to 5.20;  $P_{\text{trend}} = .007$ ), PFOS (OR = 2.51, 95% CI = 1.28 to 4.92;  $P_{\text{trend}} = .009$ ), and PFHxS (OR = 2.07, 95% CI = 1.06 to 4.04;  $P_{\text{trend}} = .04$ ). No evidence of a gradient in RCC risk with serum concentrations of other PFAS was apparent. When PFAS concentrations were modeled continuously (per a 1-unit increase in  $\log_2$ -transformed concentrations), we observed that a doubling in serum PFOA concentrations was associated with a ~70% increase in the risk of RCC (OR<sub>continuous</sub> = 1.71, 95% CI = 1.23 to 2.37;  $P = .002$ ); the corresponding risk estimates for PFOS and PFHxS were 1.39 (95% CI = 1.04 to 1.86;  $P = .03$ ) and 1.27 (95% CI = 1.03 to 1.56;  $P = .02$ ), respectively. The association with PFOA persisted after adjusting for PFOS and PFHxS concentrations (OR<sub>continuous</sub> = 1.68, 95% CI = 1.07 to 2.63,  $P = .02$ ), whereas the estimates of risk for the other PFAS were attenuated in mutually adjusted analyses. In joint analyses, no statistically significant interactions with PFOA were observed for PFOS ( $P = .71$ ) or PFHxS ( $P = .77$ ) (Supplementary Figure 2). Secondary analyses of  $\log_2$ -transformed concentrations of each PFAS modeled continuously using natural splines found no evidence of a non-linear relationship between PFOA and RCC risk, although there was some suggestion of non-linearity for several other PFAS (Supplementary Figure 3).

Figure 1 shows the results of stratified and sensitivity analyses further assessing the relationship between PFOA concentrations and RCC risk. Notably, our results were unchanged after excluding subjects with diminished kidney function (eGFR <60 mL/min/1.73 m<sup>2</sup>). In addition, associations were similar among subjects with mild loss of kidney function (eGFR 60-

89 mL/min/1.73 m<sup>2</sup>) and those with high function (eGFR  $\geq$ 90 mL/min/1.73 m<sup>2</sup>). Furthermore, the association persisted among cases diagnosed  $\geq$ 8 years after blood collection (OR = 1.66, 95% CI = 1.25 to 2.19), and associations were similar in analyses of samples with and without prior freeze-thaw cycles.

We observed a stronger association with PFOA in analyses restricted to clear cell RCC (N<sub>cases</sub> = 92; OR = 2.38, 95% = CI 1.51 to 3.74). Associations were also somewhat stronger among those with normal body weight (BMI 18.5-<25 kg/m<sup>2</sup>), those without a history of hypertension, and former/current smokers, although tests of heterogeneity were not statistically significant. We observed similar patterns in stratified and sensitivity analyses of PFOA after simultaneously adjusting for PFOS and PFHxS (Supplementary Figure 4).

## DISCUSSION

In this nested case-control study of 324 cases and 324 matched controls in a general population cohort, we observed a statistically significant increased risk of RCC among participants with higher pre-diagnostic serum concentrations of PFOA based on models adjusted for kidney function and other potential confounding factors. This association persisted in analyses restricted to subjects without evidence of diminished kidney function and among cases diagnosed eight or more years after blood collection. When we restricted the case series to those with confirmed clear cell histology, the association with PFOA was more pronounced. We also observed associations with RCC for PFOS and PFHxS in models unadjusted for other PFAS. However, after mutual adjustment for these three chemicals, only the association with PFOA remained statistically significant.

To our knowledge, this is the first prospective study to investigate the associations between serum concentrations of individual PFAS and kidney cancer risk in a cohort with PFAS concentrations comparable to the general population. The distributions of serum PFAS concentrations among the controls in our study were similar to those observed among adults in the nationally representative NHANES study during the same time period. In particular, participants in the highest quartile of PFOA serum concentrations in our study ( $>7.3$   $\mu\text{g/L}$ ) had concentrations that were comparable to the highest quartile of the distribution among U.S. adults in NHANES in 1999-2000 ( $>7.0$   $\mu\text{g/L}$ ), the earliest NHANES cycle for which such data were available.<sup>23</sup> Notably, quantification of PFAS concentrations for the current study was performed by the same laboratory analyzing NHANES using the same analytical approach.<sup>3</sup>

Moreover, the patterns of PFAS serum concentrations by demographic factors (e.g., sex and race/ethnicity) reflect those observed in NHANES, further supporting the relevance of our results for the general U.S. population. Individuals in the general population can be exposed to PFAS through various sources including food, dust, and contaminated drinking water.<sup>24-26</sup> With an estimated 6 million U.S. residents using public water supplies with PFAS concentrations exceeding the U.S. EPA's lifetime health advisory limit,<sup>4</sup> elucidating the carcinogenic potential of PFAS is a major public health concern.

Our results for PFOA are notable in light of suggestive but somewhat inconsistent prior findings for kidney cancer risk among those with occupational or high environmental PFOA exposure.<sup>10-12,27</sup> In IARC's evaluation of the carcinogenicity of PFOA in 2014,<sup>9</sup> this chemical was classified as possibly carcinogenic to humans (Group 2B) based in part on limited evidence in humans that PFOA causes renal cancer, and on limited evidence of carcinogenicity in experimental animals. The IARC evaluation noted evidence of positive associations with kidney

cancer among individuals highly exposed to PFOA who were working or living near a PFAS-producing facility in the mid-Ohio Valley.<sup>10-12</sup> In an analysis of 5,791 workers from this facility, mortality from kidney cancer was elevated among those with high estimated cumulative serum PFOA concentrations.<sup>11</sup> Two complementary studies of environmentally exposed community members in the mid-Ohio Valley also observed suggestive associations between higher estimated serum PFOA concentrations and increased kidney cancer risk.<sup>10,12</sup> Estimates of lifetime cumulative serum PFOA concentrations in these investigations were based on modeling approaches that have been described in detail and validated previously.<sup>28,29</sup> In contrast, another study of 4,668 workers (including 4,231 who were eligible for cancer follow-up) exposed to ammonium perfluorooctanoate (APFO, the ammonium salt of PFOA) at a facility in Minnesota found no evidence of an excess incidence of kidney cancer.<sup>27</sup> However, the characterization of APFO exposure for this analysis was based on an assessment of inhalation exposure that utilized air monitoring data (in APFO production areas) and expert judgment (in non-APFO production areas). It is possible that this exposure assessment approach, which did not consider other potential routes of exposure, may have resulted in greater exposure misclassification than in the mid-Ohio Valley studies, potentially obscuring an effect. Methodologic advantages of the current study relative to prior work include the direct assessment of serum PFAS concentrations in participants and prospective follow-up.

Serum concentrations of PFOA and other PFAS have been inversely associated with kidney function (i.e., lower eGFR) in cross-sectional analyses among children, adolescents and adults in the mid-Ohio Valley.<sup>17,18</sup> Similar cross-sectional associations have been observed in NHANES,<sup>19,30</sup> although more recent analyses suggest that this relationship may be non-linear in the general population.<sup>20</sup> Researchers have suggested that these inverse associations could be due

to reverse causation as a result of reduced capacity to filter and excrete PFAS among those with diminished kidney function.<sup>18</sup>

Given that lower eGFR has been linked to an increased risk of RCC,<sup>31,32</sup> we assessed kidney function in this investigation and performed multiple sensitivity and stratified analyses to evaluate the potential for confounding and effect modification. We found that the observed association between PFOA and RCC persisted among individuals without evidence of diminished kidney function (i.e., eGFR  $\geq 60$  mL/min/1.73 m<sup>2</sup>) and when restricted to individuals with high kidney function (i.e., eGFR  $\geq 90$  mL/min/1.73 m<sup>2</sup>). Overall, these findings suggest that the relationship between PFOA and RCC observed in our study population is likely to be independent of potential effects related to kidney function.

An increasing number of studies are investigating the biologic plausibility and mechanisms through which PFOA may induce nephrotoxicity and possibly influence renal carcinogenesis.<sup>9,33</sup> While information on the distribution of PFAS in human tissues remains sparse, one study of 20 individuals detected PFOA in 95% of autopsy kidney samples assayed.<sup>34</sup> This finding is consistent with evidence from previous animal studies, which suggest that the distribution of PFOA may be enriched in the kidneys, serum, and liver.<sup>35</sup> Studies of PFAS exposure in animal models have observed evidence of renal tubular hypertrophy or hyperplasia as well as increased kidney weights.<sup>36</sup> In particular, adverse health effects of PFOA and other PFAS in animal studies have been attributed to activation of peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ),<sup>37</sup> which may influence pathways related to oxidative stress and lipid metabolism<sup>36</sup> and has been implicated in RCC development.<sup>38,39</sup>

This study has several important strengths that help advance our understanding of the relationship between exposure to PFAS and risk of kidney cancer. It is, to our knowledge, the

largest investigation of PFOA exposure and RCC risk to date, the first to investigate RCC risk in relation to other PFAS beyond PFOA, and the first to prospectively examine associations with RCC using pre-diagnostic serum PFAS concentrations. We were able to demonstrate that the observed associations are unlikely to be attributable to reverse causation as a result of diminished kidney function among the RCC cases and were able to adjust for other potential confounding factors including obesity and hypertension.

Several limitations of this study should also be noted. Our assessment of PFAS exposure was based on serum concentrations in samples collected from a single point in time.

Nevertheless, the long serum elimination half-lives of many PFAS, including PFOA, PFOS and PFHxS,<sup>1,2</sup> and evidence from other population-based studies of within-subject temporal stability in PFAS concentrations using samples collected multiple years apart,<sup>40</sup> indicate that measured concentrations likely reflect long-term exposures. Also, non-Hispanic whites largely comprised our study population limiting our ability to assess racial/ethnic differences in the relationship between PFAS concentrations and RCC risk. Consistent with findings from NHANES,<sup>3</sup> we observed evidence of higher concentrations of certain PFAS (including PFOS) among African Americans compared with non-Hispanic whites among controls in our study. Future efforts extending this work to more diverse study populations would be informative, given that the incidence of RCC in the U.S. differs by race, with the highest rates among African Americans.<sup>32</sup>

In summary, we observed a statistically significant positive exposure-response association between pre-diagnostic serum PFOA concentrations and subsequent risk of RCC within a population-based U.S. prospective cohort. We also found that this association between PFOA and RCC remained after adjustment for other PFAS. These findings add substantially to the weight of evidence that PFOA is a renal carcinogen and may have important public health

implications for the many individuals exposed to this ubiquitous and highly persistent chemical worldwide.

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## DATA AVAILABILITY STATEMENT

Requests for access to the data underlying this article should be submitted through the PLCO Cancer Data Access System (<https://cdas.cancer.gov/learn/plco/instructions/?type=data>).

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**Table 1.** Selected Demographic and Health Characteristics of Renal Cell Carcinoma Cases and Controls in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

Characteristic	Study Participants, No. (%) <sup>a</sup>		P-Value <sup>b</sup>
	Controls (N = 324)	Cases (N = 324)	
Age <sup>c</sup> , years			
55-59	95 (29.3)	95 (29.3)	
60-64	112 (34.6)	112 (34.6)	—
65-69	80 (24.7)	80 (24.7)	
70+	37 (11.4)	37 (11.4)	
Center			
Colorado	20 (6.2)	20 (6.2)	
Georgetown (Washington D.C.)	15 (4.6)	15 (4.6)	
Hawaii	9 (2.8)	9 (2.8)	
Henry Ford (Michigan)	37 (11.4)	37 (11.4)	
Minnesota	84 (25.9)	84 (25.9)	
Washington University (Missouri)	33 (10.2)	33 (10.2)	—
University of Pittsburgh (Pennsylvania)	40 (12.4)	40 (12.4)	
University of Utah	27 (8.3)	27 (8.3)	
Marshfield (Wisconsin)	46 (14.2)	46 (14.2)	
University of Alabama	13 (4.0)	13 (4.0)	
Gender			
Male	216 (66.7)	216 (66.7)	—
Female	108 (33.3)	108 (33.3)	
Race			
White, non-Hispanic	287 (88.6)	287 (88.6)	
Black, non-Hispanic	21 (6.5)	21 (6.5)	—
Other	16 (4.9)	16 (4.9)	
Body Mass Index <sup>c</sup> , kg/m <sup>2</sup>			
<18.5	3 (0.9)	2 (0.6)	
18.5 to <25	83 (25.6)	71 (21.9)	
25 to <30	158 (48.8)	135 (41.7)	.008
30+	76 (23.5)	115 (35.5)	
Unknown	4 (1.2)	1 (0.3)	
History of Hypertension <sup>c</sup>			
No <sup>d</sup>	216 (66.7)	183 (56.5)	.008
Yes	108 (33.3)	141 (43.5)	
Smoking Status <sup>c</sup>			
Never	155 (47.8)	143 (44.1)	
Former	134 (41.4)	148 (45.7)	.54
Current	35 (10.8)	33 (10.2)	
Calendar Year <sup>e</sup>			
1993-1995	84 (25.9)	88 (27.2)	
1996-1997	116 (35.8)	123 (38.0)	.67
1998-2002	124 (38.3)	113 (34.9)	

eGFR <sup>e</sup> , mL/min/1.73 m <sup>2</sup>			
90+	109 (33.6)	106 (32.7)	
<90-60	197 (60.8)	189 (58.3)	.25
<60	18 (5.6)	29 (9.0)	

Abbreviations: eGFR, estimated glomerular filtration rate

<sup>a</sup> Groups may not sum to 100% due to rounding.

<sup>b</sup> Chi-square test, except for body mass index (Fisher's exact test). Not reported for matching factors (age, center, gender, and race).

<sup>c</sup> Self-reported at study baseline.

<sup>d</sup> Includes one case with missing information for history of hypertension.

<sup>e</sup> At blood draw.

**Table 2.** Odds Ratios (ORs) and 95% Confidence Intervals (CIs) Evaluating PFAS Serum Concentrations and Risk of Renal Cell Carcinoma in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial

PFAS	No. of Controls	No. of Cases	µg/L <sup>a</sup>	OR (95% CI) <sup>b</sup>	<i>P</i> <sub>trend</sub> <sup>c</sup>	OR (95% CI) <sup>d</sup>	<i>P</i> <sub>trend</sub> <sup>c</sup>
PFOA	81	47	<4.0	Reference		Reference	
	79	83	≥4.0-5.5	1.47 (0.77 to 2.80)	.007	1.41 (0.69 to 2.90)	.13
	83	69	>5.5-7.3	1.24 (0.64 to 2.41)		1.12 (0.52 to 2.42)	
	81	125	>7.3-27.2	2.63 (1.33 to 5.20)		2.19 (0.86 to 5.61)	
			Continuous <sup>e</sup>	1.71 (1.23 to 2.37)		1.68 (1.07 to 2.63)	
PFOS	81	60	≤26.3	Reference		Reference	
	81	82	>26.3-38.4	1.67 (0.84 to 3.30)	.009	1.24 (0.59 to 2.57)	.64
	81	61	>38.4-49.9	0.92 (0.45 to 1.88)		0.53 (0.22 to 1.24)	
	81	121	>49.9-154.2	2.51 (1.28 to 4.92)		1.14 (0.45 to 2.88)	
			Continuous <sup>e</sup>	1.39 (1.04 to 1.86)		0.92 (0.60 to 1.42)	
PFHxS	88	75	≤2.2	Reference		Reference	
	83	74	>2.2-3.4	1.41 (0.75 to 2.64)	.04	1.28 (0.66 to 2.51)	.40
	76	88	>3.4-5.5	1.14 (0.59 to 2.20)		0.89 (0.43 to 1.85)	
	77	87	>5.5-37.4	2.07 (1.06 to 4.04)		1.46 (0.67 to 3.18)	
			Continuous <sup>e</sup>	1.27 (1.03 to 1.56)		1.12 (0.88 to 1.43)	
PFUnDA	166	161	<LOD	Reference		Reference	
	104	108	≥0.1-0.2	1.29 (0.71 to 2.34)	.09	1.15 (0.62 to 2.16)	.20
	54	55	>0.2-1.7	2.07 (0.90 to 4.76)		1.83 (0.75 to 4.48)	
			Continuous <sup>e</sup>	1.17 (0.93 to 1.47)		1.14 (0.88 to 1.47)	
PFNA	119	95	≤0.5	Reference		Reference	
	79	73	>0.5-0.7	1.43 (0.81 to 2.51)	.08	1.08 (0.57 to 2.07)	.45
	50	78	>0.7-1.0	2.59 (1.30 to 5.15)		2.00 (0.95 to 4.20)	
	76	78	>1.0-4.9	1.81 (0.91 to 3.61)		1.29 (0.58 to 2.89)	
			Continuous <sup>e</sup>	1.19 (0.91 to 1.55)		1.00 (0.73 to 1.37)	
EtFOSAA	90	65	≤0.7	Reference		Reference	
	76	82	>0.7-1.2	1.54 (0.83 to 2.88)	.74	1.37 (0.72 to 2.63)	.63
	79	97	>1.2-2.4	1.69 (0.91 to 3.14)		1.33 (0.69 to 2.58)	
	79	80	>2.4-60.4	1.41 (0.71 to 2.81)		1.04 (0.49 to 2.20)	
			Continuous <sup>e</sup>	1.07 (0.90 to 1.27)		0.97 (0.79 to 1.18)	
MeFOSAA	101	84	≤0.9	Reference		Reference	
	73	78	>0.9-1.4	1.00 (0.53 to 1.89)	.86	0.77 (0.40 to 1.50)	.31
	73	83	>1.4-2.1	1.38 (0.73 to 2.63)		1.00 (0.50 to 2.01)	
	77	79	>2.1-8.2	0.92 (0.48 to 1.76)		0.65 (0.32 to 1.33)	
			Continuous <sup>e</sup>	1.01 (0.80 to 1.29)		0.86 (0.66 to 1.12)	
PFDA	91	92	<LOD	Reference		Reference	
	147	135	≥0.1-0.2	1.01 (0.57 to 1.79)	.20	0.80 (0.42 to 1.51)	.61
	34	40	>0.2-0.3	1.47 (0.62 to 3.45)		1.03 (0.40 to 2.64)	
	52	57	>0.3-2.1	1.70 (0.72 to 4.03)		1.21 (0.44 to 3.31)	
			Continuous <sup>e</sup>	1.19 (0.95 to 1.48)		1.11 (0.85 to 1.44)	

Abbreviations: EtFOSAA, 2-N-ethyl-perfluorooctane sulfonamido acetic acid; LOD, limit of detection; PFAS, per- and polyfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFHxS, perfluorohexane sulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonic acid; PFUnDA, perfluoroundecanoic acid; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; MeFOSAA, 2-N-methyl-perfluorooctane sulfonamido acetic acid.

<sup>a</sup> Category cut points were assigned based on quartiles of serum concentrations of each PFAS among controls except for PFUnDA and PFDA, for which >25% of measurements were below the LOD.

<sup>b</sup> Adjusted for body mass index (missing, <18.5, 18.5-<25, 25-<30, or  $\geq$ 30 kg/m<sup>2</sup>), smoking status (never, former, current), history of hypertension (no, yes), estimated glomerular filtration rate (continuous), previous freeze-thaw cycle, and calendar year of blood draw (1993-1995, 1996-1997, 1998-2002).

<sup>c</sup> Based on intra-quartile median value.

<sup>d</sup> Further adjusted for other PFAS (i.e., log<sub>2</sub>-transformed concentrations of PFOA, PFOS, and PFHxS).

<sup>e</sup> Continuous ORs (95% CI) are a 1 unit increase on the log base 2 scale, corresponding-an approximate doubling in analyte levels.

## FIGURE LEGEND

**Figure 1. Odds Ratios (ORs) and 95% Confidence Intervals (CIs) Evaluating Serum PFOA Concentrations and Risk of RCC in Stratified and Sensitivity Analyses in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial.** Abbreviations: BMI, body mass index; ccRCC, clear cell renal cell carcinoma; eGFR, estimated glomerular filtration rate; PFOA, perfluorooctanoic acid. <sup>a</sup> Self-reported at study enrollment. <sup>b</sup> BMI specific analyses exclude individuals with missing or <18.5 kg/m<sup>2</sup> BMIs. <sup>c</sup> At blood draw. <sup>d</sup> Time from blood draw to diagnosis for cases. <sup>e</sup> ICD-O-2 code = 8310. <sup>f</sup> Continuous OR (95% CI), which corresponds to a 1 unit increase on the log<sub>2</sub> scale or an approximate doubling in analyte level, was estimated using unconditional multivariable logistic regression models adjusted for age at enrollment (55-59, 60-64, 65-69, 70+ years), sex (male, female), race/ethnicity (white non-Hispanic, black non-Hispanic, or other), estimated glomerular filtration rate (continuous), body mass index (<18.5 kg/m<sup>2</sup>, 18.5 to <25 kg/m<sup>2</sup>, 25 to <30 kg/m<sup>2</sup>, ≥30 kg/m<sup>2</sup>, missing), history of hypertension (no/missing, yes), smoking status (never, former, current), previous freeze-thaw cycle, calendar year of blood draw (1993-1995, 1996-1997, 1998-2002), study year of blood draw (enrollment, other), and study center ([1]Minnesota or Marshfield; [2] Colorado, Hawaii, Washington University, University of Utah, or University of Alabama; [3] Georgetown, Henry Ford, or University of Pittsburgh). *P*-values represent Wald tests of heterogeneity across strata.

