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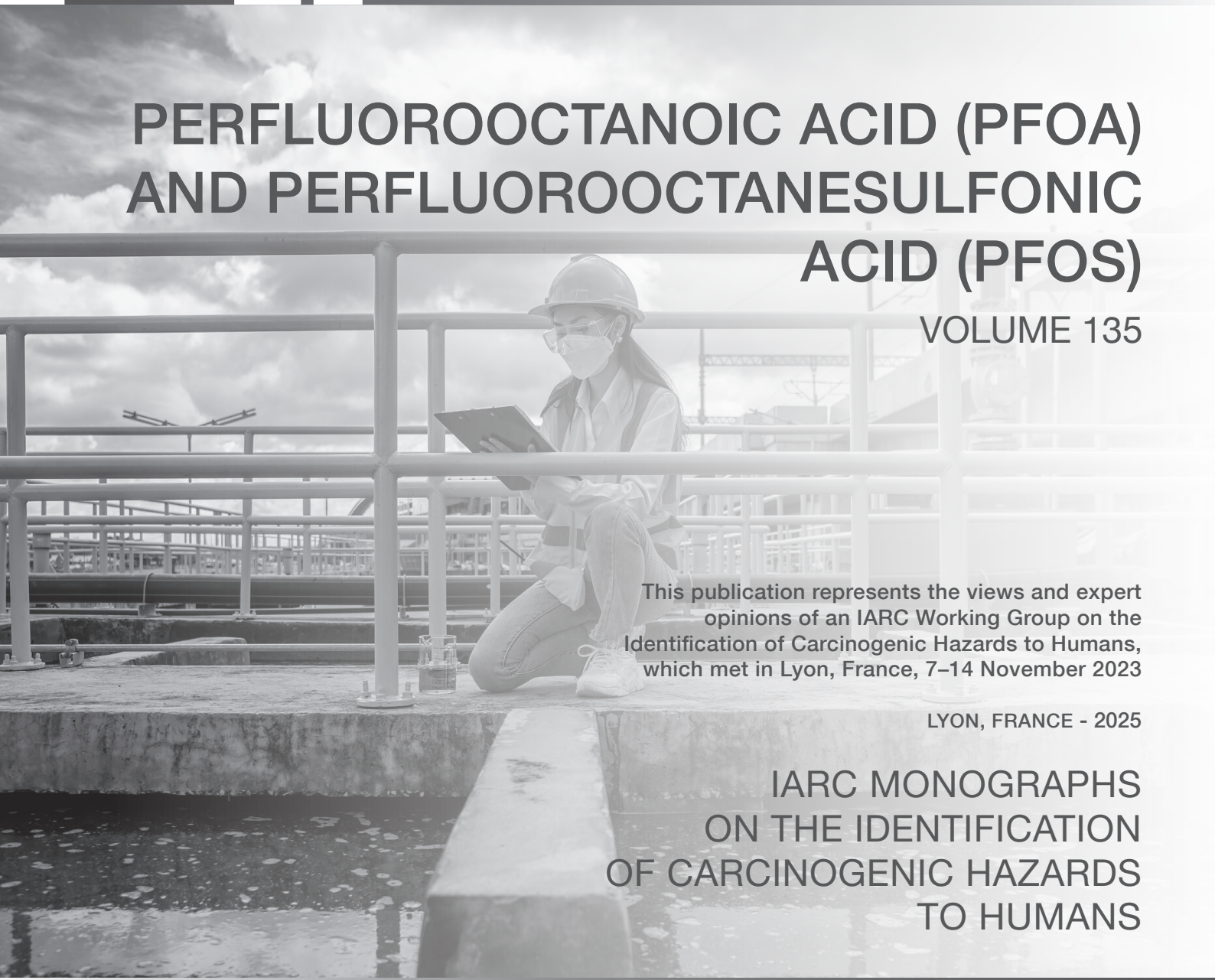

PERFLUOROOCTANOIC ACID (PFOA) AND PERFLUOROOCTANESULFONIC ACID (PFOS)

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ANNEX 3. SUPPLEMENTARY ANALYSES USED IN REVIEWING EVIDENCE ON CANCER IN HUMANS

A3.1 How well does the PFOA concentration in a single serum sample represent long-term exposure in a population with low exposure?

Introduction

Several of the epidemiology studies on perfluoroalkyl and polyfluoroalkyl substance(s) (PFAS) and cancer were cohort studies in the general population, or nested case–control studies within such cohorts, and used a single serum sample per participant to assess exposure. There was little information on how well the PFAS measurement in a single serum sample (typically at baseline) represents longer-term exposure, which is important for studying chronic diseases. In this analysis, summary statistics from two cohorts with repeated measurements of serum perfluorooctanoic acid (PFOA) for the participants in the control groups were used to evaluate the potential impacts of using a single serum sample to represent chronic exposure for each participant.

Methods

The first study, by Rhee et al. (2023a), was a nested case–control study on prostate cancer, with 675 cases and 675 controls from within the Prostate, Lung, Colorectal, and Ovarian Cancer (PLCO) cohort. In this study, three repeat serum samples were collected from 60 control participants at baseline, 1 years, and 5 years (in 1996, 1997, and 2001). The second study, by Purdue et al. (2023), was a nested case–control study on testicular cancer among Air Force servicemen, with 530 cases and 530 controls. Purdue et al. (2023) had available a second prediagnostic serum sample from 187 case–control pairs. Of these, summary statistics for repeat samples were available from 84 controls for which the dates of first and repeat sampling were the furthest apart (collected ≥ 4.7 years apart) (mean for years of sampling, 1999 and 2007). Serum PFOA concentrations in these populations were similar to those in the general US population as measured by the US National Health and Nutrition Examination Survey (NHANES). The actual analysis by Rhee et al. (2023a) and the main analysis by Purdue et al. (2023) used only the single (or first) sample for each subject.

The summary statistics from these repeat samples are posted on a National Cancer Institute GitHub project (NCI, 2024). Summary statistics for the repeated serum PFOA measurements for the controls in two cohorts were used to generate plausible serum concentrations for each participant at each time point, taking within-subject correlations into account. Five data sets of controls were generated for each cohort, with

Table A3.1 Descriptive statistics for participants with repeated samples (*n* = 60) from the study by Rhee et al. (2023a)[a]

Parameter[b]	Sample 1 (T0)			Sample 2 (T1)			Sample 3 (T5)			All samples (T0, T1, T5)		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Observed PFOA concentration (ng/mL)	3.88	3.63	1.8	3.87	3.53	1.99	4.69	4.53	2.43	4.15	NR	1.91
Simulated PFOA concentration (ng/mL)	3.85	3.88	1.82	3.82	3.81	1.96	4.63	4.61	2.4	4.1	4.09	1.81

NR, not reported; PFOA, perfluorooctanoic acid; SD, standard deviation.

[a] An analysis of prostate cancer in the PLCO study. The data reported here are posted in the National Cancer Institute GitHub project (NCI, 2024).

[b] Spearman correlations: T0–T1 observed, 0.78; simulated, 0.77; T1–T5 observed, 0.60; simulated, 0.60; T0–T5 observed, 0.62; simulated, 0.60.

the same number of controls as in the original studies for each simulated data set. The statistics used to generate simulated data were the mean and standard deviation (SD) for the PFOA serum concentrations among controls at each time point, as well as the Spearman correlation between each set of samples (three correlations for Rhee et al. (2023a) for three samples, one for Purdue et al. (2023) for two samples).

The samples appeared to have an approximately normal distribution, judging by the small differences between means and medians. From the three samples from the PLCO study used in Rhee et al. (2023a), the means and medians were 3.88 ng/mL and 3.63 ng/mL, 3.87 ng/mL and 3.53 ng/mL, and 4.69 ng/mL and 4.53 ng/mL, respectively (SDs, 1.8, 1.99, and 2.43) (Table A3.1). For the samples from Air Force servicemen in Purdue et al. (2023), the mean and median for the first sample were 6.8 ng/mL and 6.1 ng/mL (SD, 3.0), respectively, while for the second sample they were 5.5 ng/mL and 5.1 ng/mL (SD, 2.3), respectively (Table A3.2). For normally distributed data, Pearson and Spearman correlations are similar (de Winter et al., 2016), and we used Spearman correlation coefficients between samples to generate the simulated data, as an approximation of the Pearson correlations (Rhee et al., 2023a; ρ for sample T0–T1, 0.78; ρ for samples T1–T5, 0.60; ρ for samples T0–T5, 0.62); (Purdue et al., 2023; ρ for samples 1 and 2, 0.32) (Table A3.1, Table A3.2).

The distributions were generated using an R package (mvtnorm library) for generating multivariate normal samples with known means, standard deviations, and (Pearson) correlations between different sets of samples (Genz and Bretz, 2009). The mean across the five simulations for each control was then used to represent the simulated data for each sample.

Having generated simulated serum PFOA concentrations for each control at each time point (three time points for Rhee et al., 2023a, two time points for Purdue et al., 2023), mean concentrations across samples for each control in each study were used as an estimate of long-term exposure. For the controls in each study, the first samples were then compared with the long-term average exposure, the latter taken as the "true" exposure and the former as the "misclassified" exposure.

Exposures were categorized into quintiles, as in the original published analysis of Rhee et al. (2023a), and used to determine the extent of misclassification across exposure categories using serum concentrations at the first time point versus the long-term exposure. "True" or long-term exposure values were then also generated

Table A3.2 Descriptive statistics for participants with repeated samples (*n* = 84) from the study by Purdue et al. (2023) [a]

Parameter[b]	Sample 1			Sample 2			Both samples
	Mean	Median	SD	Mean	Median	SD	Mean[c]
Observed PFOA concentration (ng/mL)	6.8	6.1	3.0	5.5	5.1	2.3	6.1
Simulated PFOA concentration (ng/mL)	6.8	6.8	2.9	5.5	5.5	2.3	6.1

PFOA, perfluorooctanoic acid; SD, standard deviation.

[a] A study of testicular cancer in the United States Air Force cohort. The data reported here are posted in the National Cancer Institute GitHub project (NCI, 2024).

[b] Spearman correlation between samples 1 and 2: observed, 0.32; simulation, 0.30.

[c] Restricted to those with a second sample collected > 4.7 years (the median for controls sampled twice) after the first sample. The mean time between first and second samples for these 84 subjects was 7.8 years.

for a set of hypothetical cases for each study, such that there was a monotonic increasing trend across quintiles, and an approximate rate ratio of 1.5 for the highest versus the lowest quintile, using long-term exposure. The misclassification rates used for the hypothetical cases were the same as those observed in the controls (i.e. assuming non-differential exposure misclassification) to simulate "misclassified" exposure at the first time point for the hypothetical cases. Finally, epidemiological effect estimates (odds ratios) were computed across quintiles for cases and controls, using the long-term ("true") versus first sample ('misclassified') data.

Results

The simulated data corresponded well with the observed means and standard deviations for the original data, and the Spearman correlations between repeated samples in each study from the simulated data closely resembled the same correlation from the observed data. For example, for Rhee et al. (2023a), the observed Spearman correlations (ρ) between samples T0–T1, T1–T5, and T0–T5 were 0.78, 0.60, and 0.62, respectively, while the Spearman correlations in the simulated data were 0.77, 0.60, and 0.60 (Table A3.1). The Spearman correlations between first and second samples for Purdue et al. (2023) in the simulated and observed data were 0.30 and 0.32, respectively (Table A3.2).

Comparing long-term "true" exposure (the mean across samples) with "misclassified" exposure (for the first sample alone), epidemiological results were quite similar, with a relatively small bias to the null when using only a single serum sample per participant (bias to the null is expected for non-differential misclassification, see Weinberg et al., 1994). For the data from Rhee et al. (2023a), the odds ratios (ORs) by quintile, using the long-term average, or "true", data were 1.00, 1.14, 1.29, 1.43, and 1.57 (*P* for trend, 0.007), while the odds ratios by quintile using the first serum sample only were 1.00, 1.12, 1.24, 1.35, and 1.42 (*P* for trend, 0.007) respectively, indicating only a slight bias to the null (Table A3.3). Similarly for the data from Purdue et al. (2023), odds ratios by quintile using the long-term average were 1.00, 1.12, 1.23, 1.35, and 1.47 (*P* for trend, 0.005), whereas odds ratios by quintile using the first sample only were 1.00, 1.07, 1.13, 1.17, and 1.31 (*P* for trend, 0.02), again indicating only a slight bias towards the null (Table A3.4).

Table A3.3 Hypothetical "true" (long-term) and "misclassified" (first sample only) for cases (*n* = 675) and controls (*n* = 675) with a positive exposure–response relation for PFOA, based on the PLCO data in Rhee et al. (2023a)

Analysis	Exposure metric	PFOA quintile (ng/mL)	Cases	Controls	Odds ratio
True	Mean (T0, T1, T5) [a]	≤ 3.45	105	135	1.00
		> 3.45 to ≤ 3.90	120	135	1.14
		> 3.90 to ≤ 4.31	135	135	1.29
		> 4.31 to ≤ 4.77	150	135	1.43
		> 4.77	165	135	1.57
					Trend-test *P* value, 0.007
Misclassified	T0 only	≤ 3.45	177	210	1.00
		> 3.45 to ≤ 3.90	123	130	1.12
		> 3.90 to ≤ 4.31	145	138	1.24
		> 4.31 to ≤ 4.77	124	109	1.35
		> 4.77	105	88	1.42
					Trend-test *P* value, 0.007

OR, odds ratio; PFOA, perfluorooctanoic acid; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.
[a] The quintile cut-points used here stem from the data generated via multivariate normal distributions based on summary statistics from the controls in each study (posted in the National Cancer Institute GitHub project; NCI, 2024) with repeat samples, and differ from the cut-points in the original papers, which are based on all cases and controls in the original studies.

Table A3.4 Hypothetical "true" (long-term) and "misclassified" (first sample only) cases and controls with positive exposure–response relation for PFOA, based on the Air Force data in Purdue et al. (2023)

Analysis	Exposure metric	PFOA quintile (ng/mL)	Cases	Controls	Odds ratio
True	Mean (samples 1, 2)[a]	≤ 5.38	86	106	1.00
		> 5.38 to ≤ 5.93	96	106	1.12
		> 5.93 to ≤ 6.39	106	106	1.23
		> 6.39 to ≤ 6.81	116	106	1.35
		> 6.80	126	106	1.47
		Totals	530	530	Trend-test *P* value, 0.005
Misclassified	Sample 1 only	≤ 5.38	58	70	1.00
		> 5.38 to ≤ 5.93	47	53	1.07
		> 5.93 to ≤ 6.39	61	65	1.13
		> 6.39 to ≤ 6.81	70	72	1.17
		> 6.80	294	270	1.31
		Totals	530	530	Trend-test *P* value, 0.02

[a] The quintile cut-points used here stem from the data generated via multivariate normal distributions based on summary statistics from the controls in each study (posted in the National Cancer Institute GitHub project; NCI, 2024) with repeat samples, and differ from the cut-points in the original papers, which are based on all cases and controls in the original studies.

Discussion

Single (first) samples represented rather well the mean of repeated samples taken an average of 5 and 8 years apart in two cohort studies of populations with background levels of exposure to PFOA (Spearman correlations, 0.87 and 0.83, for PLCO and Air Force data respectively). Others have demonstrated that changes in PFOA exposure estimates after correcting for measurement error cause little change in epidemiological findings in a high-exposure population in which the rank order of exposure among participants changes little with modest group-level misclassification (Avanasi et al., 2016). The same results were found here, for individual-level misclassification in low exposure populations more typical of the general population. Individual serum PFOA concentrations changed somewhat over time in these two cohorts, but the reported within-subject correlations were high, so the relative ranking of exposure remained approximately the same over time. This implied that tests for trend in disease risk in studies relying on only one serum sample might not differ markedly from those using a more accurate estimate of long-term exposure, i.e. the average for repeated samples over time. Such relative rankings might remain relatively constant in the case of the legacy PFAS, such as PFOA, because these chemicals have relatively long half-lives, and because local sources in the environment where the participants live (e.g. drinking-water, consumer products, and diet) may be relatively stable over time, at least during the decade of serum sampling represented by these two cohorts, despite longer-term secular trends in environmental levels and serum concentrations.

The limitations of these findings were the restriction to only a few repeated samples over relatively short time periods, before the production of PFOA and perfluorooctanesulfonic acid (PFOS) was phased out in the USA. However, another study with five repeated samples collected over almost 40 years in a general population sample in Norway also reported a relatively high within-subject correlation between samples over time (Nøst et al., 2014). Another limitation here was that all the data in the studies by Rhee et al. (2023a) and Purdue et al. (2023) came from men. However, while there are important differences between men and women, e.g. women's serum levels of PFAS change during pregnancy and after menopause (Dhingra et al., 2017; Steenland et al., 2018), there is no a priori reason to think that the findings regarding the consistency of relative rankings across time would be radically different for men and women. Other limitations included the assumption of normality, the use of Spearman instead of Pearson coefficients, and a relatively small number of simulations. However, it is not expected that changing these assumptions would have a substantial impact on the findings of the present analysis.

A3.2 Summary of the Working Group's ecological analysis of PFOA and orchiectomy among men aged 15–54 years in 21 municipalities of the Veneto region in Italy, 1997–2014

A3.2.1 Background on PFOA exposure in the Veneto region

The Trissino factory in the Veneto region, Italy, produced PFOA from 1968 to 2014 (Girardi and Merler, 2019). When PFOA production started in 1968, production was estimated to be about 12 tonnes per year in 1968–1970 and then increased over time until the 2000s, when the annual production of PFOA and its ammonium salt was on average 250 tonnes, peaking at 460 tonnes in 2007. PFOS production also occurred at the site but at much lower volumes, with an average of 36.6 tonnes per

year in 2001–2011, peaking at 88 tonnes in 2004 (Girardi and Merler, 2019). As reported by Pitter et al. (2020):

> "…based on general information on production practices, it is believed that the plant produced long-chain PFAS only, particularly PFOA and PFOS, from 1968 until 2001. PFOA reached the highest concentrations both in drinking-water and serum, consistent with previous reports from the Mid-Ohio Valley (Frisbee et al., 2009). PFBA and PFBS were found in high concentrations in drinking-water but were detected only in a minority of serum samples at relatively low concentration, whereas PFOS and PFHxS, which were scarcely represented in drinking-water, were detected in almost 100% of serum samples. This discrepancy may be explained by the exposure to PFOS and PFHxS from other sources, as demonstrated for the general population".

Releases of PFOA from the factory resulted in contamination of ground and surface water used for drinking in the region. The groundwater contamination plume extended over an area of 190 km^2 and affected both public waterworks and private wells. The municipalities in the area of maximum exposure (referred to as the "red area") are further divided into "red area A", which includes municipalities served by the contaminated waterworks that are also located on the groundwater contamination plume; and "red area B", which includes municipalities served by the contaminated waterworks but not located on the groundwater contamination plume. Initially, the red area was composed of 21 municipalities, with 126 000 inhabitants. In 2018, nine additional municipalities were added, some of which were only partially supplied by the contaminated waterworks. Currently, the red area is 595 km^2 wide and has a total population of approximately 140 000 people.

Biomonitoring has been conducted in this community since 2015 (Ingelido et al., 2018; Pitter et al., 2020). In 2015–2016, Ingelido et al. (2018) measured PFOA and PFOS and other PFAS in the serum of 257 individuals, aged 20–51 years, residing in municipalities in the affected areas (Altavilla, Brendola, Creazzo, Lonigo, Montecchio Maggiore, Sarego, and Sovizzo) and in 250 individuals living in uncontaminated areas. In each area, participants were selected and stratified by sex and age. Each participant had resided in an area for at least 10 years. Serum levels of PFOA were much higher in the contaminated areas (median, 13.77 ng/g; maximum, 754.50 ng/g) than in uncontaminated areas (median, 1.64 ng/g; maximum, 27.88 ng/g); similarly, PFOS levels were higher in the exposed group (median, 8.69 ng/g; maximum, 70.27 ng/g) than in the non-exposed (median, 5.84 ng/g; maximum, 118.58 ng/g). The Spearman correlation for PFOA and PFOS in serum was 0.743 in the exposed and 0.619 in the unexposed. In 2015–2016, Pitter et al. (2020) conducted a larger study of 18 345 participants aged 14–39 years at recruitment; 63.5% agreed to participate in the surveillance programme; serum results for people who had lived in the red area for < 1 year were excluded. The PFAS with the highest serum concentrations were PFOA (median, 44.4 ng/mL; interquartile range, IQR, 19.3–84.9 ng/mL), PFOS (median, 3.9 ng/mL; IQR, 2.6–5.8 ng/mL), and PFHxS (median, 3.9 ng/mL; IQR, 1.9–7.4 ng/mL). Within the red areas, median PFOA levels varied by community, ranging from 10.9 ng/mL in Terrazo to 73.3 ng/mL in Asigiliano-Veneto.

Individuals in this contaminated area of the Veneto region are exposed to a mixture of PFAS, including PFOA and PFOS; but information on production volumes, water levels, and biomonitoring data are consistent with PFOA being the PFAS present at highest concentrations throughout the region (Ingelido et al., 2018; Mastrantonio et al., 2018; Girardi and Merler, 2019; Pitter et al., 2020; Giglioli et al., 2023).

Taken as a whole, these data provided extensive evidence for PFOA contamination in the region, with both water and biomonitoring data showing differences in concentrations within the region. While other PFAS, notably PFOS and PFHxS, are correlated with PFOA, they are present at levels that are substantially lower than those of PFOA.

A3.2.2 Working Group analysis of orchiectomy data

The Working Group conducted an ecological analysis comparing biomonitoring data from Pitter et al. (2020) with data from an investigation on the frequency of orchiectomies in 21 municipalities in this region between 1997 and 2014 (Sistema Epidemiologico Regionale, 2016, summarized in English by Saugo et al., 2024). Orchiectomy was used as a proxy for diagnosis of testicular cancer [sensitivity and positive predictive values of 91.7% (95% CI, 88.0–95.4%) and 92.8% (95% CI, 89.3–96.2%), respectively, in this region]. Orchiectomies were ascertained using information in hospital discharge records, including address of residence, which included the main medical procedures from hospital stays and were completed for the purpose of reimbursement from the Italian national health system. As shown in Table A3.5 below, standardized incidence ratios (SIRs) for orchiectomy were estimated for each of the 21 municipalities separately by comparing the observed orchiectomies (n = 70, overall) versus expected numbers based on rates in the region overall that were standardized on age by 5-year age groups from 15 to 54 years (Sistema Epidemiologico Regionale, 2016). A strong correlation was observed between median serum PFOA concentration and the rate of orchiectomy by municipality (Spearman correlation, 0.57; P = 0.006). The Working Group also conducted a Poisson regression of observed orchiectomy counts regressed on median PFOA levels across the 21 municipalities. The Poisson regression was done using the log of expected events as an offset and correcting for dispersion. The rate ratio for each unit (ng/mL) increase of PFOA was 1.018 (95% CI, 1.006–1.031; P = 0.003).

The SAS code used in this analysis is presented in Table A3.6.

A3.3 Working Group meta-analysis of studies on kidney cancer to estimate rate ratio per unit (linear) increase in serum PFOA concentration

The Working Group conducted a meta-analysis that included estimates from the studies of Steenland and Woskie (2012), Barry et al. (2013), Vieira et al. (2013), Shearer et al. (2021), Rhee et al. (2023b), and Winquist et al. (2023). The studies by Barry et al. (2013), Vieira et al. (2013), and Steenland and Woskie (2012) were included, although they overlap to an unknown extent, under the assumptions that: (i) they are largely independent; and (ii) the mortality rate ratio in Steenland and Woskie (2012) is roughly equivalent to what would have been obtained for an incidence rate ratio. The kidney cancer results from Raleigh et al. (2014) were not included, given that the exposure assessment in this study was based on air measurements, nor were those from Consonni et al. (2013), in which there were no serum data to permit the pooling of a comparable cumulative dose–response estimate with the other studies, or from Mastrantonio et al. (2018), because of its ecological design and lack of data on serum levels.

The Working Group used the approach of the meta-analysis by Bartell and Vieira (2021). This approach uses categorical rate ratios based on contrasting the upper category (usually quartiles) with the referent, together with the assumed midpoints of the upper category and referent, to regress the log of the rate ratios on the midpoints to obtain a single linear continuous coefficient that estimates the change in log rate

Table A3.5 Data used by the Working Group for an ecological analysis of PFOA and orchiectomy among men aged 15–54 years in 21 municipalities of the Veneto region, Italy, 1997–2014

Municipality (red area A or B)	Serum PFOA concentrations, by municipality[a]		Orchiectomy data, by municipality[b]		
	n (%) of samples	Median serum PFOA concentration (ng/mL)	Observed N	SIR	95% CI
Albaredo D'Adige (B)	767 (4.2%)	29	1	0.34	0.01–1.90
Alonte (A)	346 (1.9%)	62.6	1	1.13	0.03–6.27
Arcole (B)	899 (5.0%)	29.5	2	0.58	0.07–2.11
Asigliano Veneto (A)	161 (0.9%)	73.3	1	2.15	0.05–11.98
Bevilacqua (B)	216 (1.2%)	56.2	1	0.97	0.02–5.43
Bonavigo (B)	279 (1.5%)	29.8	1	0.87	0.02–4.85
Boschi Sant'Anna (B)	206 (1.1%)	38.4	0	0	0.00–3.77
Brendola (A)	1007 (5.6%)	41	6	1.60	0.59–3.48
Cologna Veneta (A)	1208 (6.7%)	53.9	2	0.44	0.05–1.60
Legnago (B)	2945 (16.3%)	22.2	11	0.83	0.42–1.49
Lonigo (A)	2569 (14.2%)	61.8	16	1.84	1.05–2.98
Minerbe (B)	628 (3.5%)	55.2	3	1.18	0.24–3.46
Montagnana (A)	1146 (6.3%)	67.6	8	1.54	0.67–3.04
Noventa Vicentina (A)	1410 (7.8%)	46.4	3	0.62	0.13–1.80
Pojana Maggiore (A)	767 (4.2%)	67.5	3	1.18	0.24–3.46
Pressana (A)	365 (2.0%)	58.8	1	0.69	0.02–3.82
Roveredo Di Guà (A)	263 (1.4%)	55.8	0	0	0.00–3.61
Sarego (A)	1124 (6.2%)	47.5	3	0.81	0.17–2.38
Terrazzo (B)	288 (1.6%)	10.9	0	0	0.00–2.51
Veronella (B)	778 (4.3%)	48.2	4	1.58	0.43–4.04
Zimella (A)	750 (4.1%)	49.9	3	1.10	0.23–3.21

CI, confidence interval; PFOA, perfluorooctanoic acid; SIR, standardized incidence ratio.
[a] Data from Pitter et al. (2020).
[b] Data from Sistema Epidemiologico Regionale (2016).

ratio per unit of (linear) PFOA. In addition, for Steenland and Woskie (2012), Barry et al. (2013), and Vieira et al. (2013), which used cumulative exposure, the Working Group divided the midpoints of exposure by the assumed average duration of exposure. In the case of Vieira et al. (2013), there were 10 years of cumulative exposure for cases and controls, so the Working Group divided by 10. In the case of Barry et al. (2013), the Working Group used the average duration of follow-up, which was 33 years (the average length of follow-up in the study), as the divisor. In the case of Steenland and Woskie (2012), the average length of follow-up was 30 years, so the Working Group divided the cumulative exposure by 30. The studies by Steenland and Woskie (2012), Barry et al. (2013), and Rhee et al. (2023b) did not have midpoints for the upper categories. For the studies by Steenland and Woskie (2012) and Barry et al. (2013), we multiplied the upper cut-point by 4, based on the observed midpoint in Vieira et al. (2013) (who studied a similar population), being about 4 times the lower level of the uppermost category. For Rhee et al. (2023b), the Working Group multiplied the upper cut-point by 2.5, based on the observed midpoint for the two other general population studies by Shearer

Table A3.6 SAS code used in the Working Group analysis of orchiectomy data

```
data one;
input medianpfoa sir numsamples obs exp estgeomean;
*estgeomean comes from Pitter Table 2 regression;
logexp=log(exp);
lnmedianpfoa=log(medianpfoa);
lnestgeomean=log(estgeomean);

cards;
29 0.34 767 1 2.94 28.3
62.6 1.13 346 1 0.89 36.6
29.5 0.58 899 2 3.43 31.5
73.3 2.15 161 1 0.47 28.5
56.2 0.97 216 1 1.03 34.4
29.8 0.87 279 1 1.15 29.4
38.4 0 206 0 0.80 32.9
41 1.6 1007 6 3.76 24.5
53.9 0.44 1208 2 4.51 37.4
22.2 0.83 2945 11 13.19 20.4
61.8 1.84 2569 16 8.71 38.4
55.2 1.18 628 3 2.53 46.3
67.6 1.54 1146 8 5.18 39.7
46.4 0.62 1410 3 4.87 29.0
67.5 1.18 767 3 2.53 39.6
58.8 0.69 365 1 1.46 40.6
55.8 0 263 0 0.83 37.4
47.5 0.81 1124 3 3.68 32.4
10.9 0 288  0 1.19 10.9
48.2 1.58 778 4 2.54 48.0
49.9 1.1 750  3 2.73 36.6
;
*proc univariate plot; *var sir medianpfoa estgeomean;

proc corr spearman; var medianpfoa sir; run;
proc freq; tables medianpfoa; run;

proc genmod; model obs=medianpfoa / dist=poisson link=log offset=logexp pscale; *best AIC;
proc genmod; model obs=lnmedianpfoa / dist=poisson link=log offset=logexp pscale;
run;
proc genmod; model obs=estgeomean / dist=poisson link=log offset=logexp pscale;
run;
proc genmod; model obs=lnestgeomean / dist=poisson link=log offset=logexp pscale;
run;
```

et al. (2021) and Winquist et al. (2023) for the upper category.

Once the continuous linear coefficient for each study was obtained, the Working Group then used an R package (metagen) to calculate random weights (inverse variance weights, where the variance is the sum of the within and between variance across studies) using the formulae from restricted maximum likelihood (REML) (Veroniki et al., 2016). The Working Group used random weights, given the high heterogeneity of the linear coefficient across studies (I^2 value, 0.91)

The meta-analysis described above gave the result for an increase in the rate ratio per increase of 10 ng/mL in PFOA as 1.15 (95% CI, 0.97–1.37), with an I^2 value of 91%.

We then also conducted, as a sensitivity analysis, a meta-analysis of Winquist et al. (2023), Shearer et al. (2021), Rhee et al. (2023b), and Barry et al. (2013), to avoid the overlapping nature of Barry et al. (2013) with Steenland and Woskie (2012), and Vieira et al. (2013), and choosing Barry et al. (2013) because it was an incidence study that also had the best exposure estimation.

This sensitivity analysis gave the result for an increase in the rate ratio per increase of 10 ng/mL PFOA as 1.21 (95% CI, 0.94–1.57) with an I^2 value of 95%.

As a general limitation to the meta-analysis, we noted the assumption of a linear exposure–response relation, although we know that, in studies with continuous exposure coefficients (Barry et al., 2013; Shearer et al., 2021; Winquist et al., 2023; Rhee et al., 2023b), a log-linear model (i.e. log-transformed PFOA) seemed to fit the data better than did a linear model (i.e. untransformed PFOA). Other main limitations were: (i) the estimate of the linear coefficient using assumed midpoints of only two categories (uppermost and lowest); (ii) the use of average duration of exposure to transform cumulative exposure in Barry et al. and Viera et al. to an assumed average exposure; and (iii) the assumption in the studies by Rhee et al. (2023b), Shearer et al. (2021), and Winquist et al. (2023) that a single PFOA measurement is a good estimate of long-term lifetime average exposure (beyond a 5–8-year duration, discussed Section A3.1 of the present Annex). Given these limitations, as well as the high heterogeneity across studies with different strengths and weaknesses, the Working Group chose to not rely primarily on the meta-analysis of exposure–response relations to determine the hazard identification for kidney cancer in humans.

The R code used for these estimations is presented in Table A3.7.

Table A3.7 R code[a] used for the Working Group's meta-analysis of kidney cancer to estimate rate ratio per unit (linear) increase in serum PFOA concentration

```
library(meta)
#####Outcome: Summary RR based on 10 ng/mL increase
#function to get increase per unit
trendp = function(datalist){
 lapply(datalist, function(df) {
 se1 = (log(df$upper)-log(df$RR))/qnorm(.975) # se of log RR for each dose category
 se2 = (log(df$RR)-log(df$lower))/qnorm(.975)
 se = (se1 + se2) / 2
 scores = 0:(length(se)-1)
 if(se[1] == 0) {
 lm1 = lm(log(RR) ~ 0 + scores, weights = 1/se^2, data=df, subset=se>0)
 lm2 = lm(log(RR) ~ 0 + mids, weights = 1 / se^2, data=df, subset=se>0)
 p1 = summary(lm1)$coef[1,4]
 p2 = summary(lm2)$coef[1,4]
 slope = summary(lm2)$coef[1,1]
 se = summary(lm2)$coef[1,2]
 } else {
 lm1 = lm(log(RR) ~ scores, weights = 1 / se^2, data=df)
 lm2 = lm(log(RR) ~ mids, weights = 1 / se^2, data=df)
 p1 = summary(lm1)$coef[2,4]
 p2 = summary(lm2)$coef[2,4]
 slope = summary(lm2)$coef[2,1]
 se = summary(lm2)$coef[2,2]
 }
 return(c(p1,p2,slope,se))
 })
}

#####
# Kidney/PFOA (Including Rhee overall)
#per 10 ng/mL serum

kidney = list(
 shearer = data.frame(
 stlab = c("Shearer et al., 2020","","","",""),
 labs = c("0-4 ng/mL","4-5.5 ng/mL", "5.5-7.3 ng/mL", "7.3-27.2 ng/mL"),
 cutpoints = c(0, 4.0, 5.5, 7.3), # max given as 27.2; sub in after lapply
 RR = c(1.0, 1.47, 1.24, 2.63),
 lower = c(1, 0.77, 0.64, 1.33),
 upper = c(1, 2.80, 2.41, 5.20)),
 vieira = data.frame(
 stlab = c("Vieira et al., 2013","","","","",""),
 labs = c("0-3.7 ng/mL-yr","3.8-88 ng/mL-yr", "89-197 ng/mL-yr", "198-599 ng/mL-yr", "600-4679 ng/mL-yr"), #this are
categories cut-points taken from Table S1 (cumulative over 10 years)
 cutpoints = c(0, 3.8, 89, 198, 600) / 10, # max is given as 4679, sub in after lapply #divided by 10 because is 10 cumulative
exposure
 RR = c(1, 0.8, 1.2, 2.0, 2.1),
 lower = c(1, 0.4, 0.7, 1.3, 1.1),
 upper = c(1, 1.5, 2.0, 3.2, 4.2)),
 barry = data.frame(
 stlab = c("Barry et al., 2013","","","",""),
 labs = c("0-219 ng/mL-yr", "219-812 ng/mL-yr", "812-5358 ng/mL-yr", ">5358 ng/mL-yr"),
 cutpoints = c(0, 219, 812, 5358) / 33, # ng/mL-yr / av age diag (divided by 33 because this is average length follow-up)
```

Table A3.7 (continued)

```
#cutpoints are reported in the meta-analysis by Bartell and Vieira (2021), in the R code within the supplement material but not
#in the original publication
  RR = c(1, 1.23, 1.48, 1.58),
  lower = c(1, 0.70, 0.84, 0.88),
  upper= c(1, 2.17, 2.60, 2.84)),
  steenland = data.frame(
  stlab = c("Steenland and Woskie, 2012","","","",""),
  labs = c("0-904 ng/L-years", "904-1520 ng/mL-yr","1520-2700 ng/mL-yr", ">2700 ng/mL-yr"),
  cutpoints = c(0, 904, 1520, 2700) / 30, # Divided by 30 because of average follow-up
  RR = c(1.07, 1.37, 0.005, 2.66), # mortality; 3rd RR is 0 but cannot log
  lower = c(0.02, 0.28, 0.005, 1.15),
  upper= c(3.62, 3.99, 1.42, 5.24)),
  rhee = data.frame(
  stlab = c("Rhee et al., 2023","","","",""),
  labs = c("0-3.27 ng/mL","3.27-4.47 ng/mL", "4.47-6.22 ng/mL", ">6.22 ng/mL"),
  cutpoints = c(0, 3.27, 4.47, 6.22), #
  RR = c(1.0, 1.26, 1.26, 1.04),
  lower = c(1, 0.80, 0.78, 0.60),
  upper = c(1, 1.97, 2.05, 1.81)),
  winquist = data.frame(
  stlab = c("Winquist et al., 2023","","","",""),
  labs = c("0-3.9 ng/mL","3.9-5.2 ng/mL", "5.2-7.3 ng/mL", ">7.3"),
  cutpoints = c(0, 3.9, 5.2, 7.3), #
  RR = c(1.0, 0.93, 0.83, 1.20),
  lower = c(1, 0.56, 0.49, 0.71),
  upper = c(1, 1.56, 1.40, 2.04))
)

#calculate midpoints of the time-averaged serum PFOA categories within each study
kidney2 = lapply(kidney, function(df) {
  cp = df$cutpoints
  l = length(cp) + 1
  cp[l] = 2.5 * cp[l-1] # assume max is 2.5*last cutpoint
  df$mids = apply(rbind(cp[-l],cp[-1]),2,mean)
  return(df)
})
#in this we assumed that maximum is 2.5*last cutpoints, but in reality for some studies maximum is reported
kidney2$vieira$mids[5] = mean(c(600,4679)) / 10
kidney2$shearer$mids[4] = mean(c(7.3,27.2))
kidney2$winquist$mids[4] = mean(c(7.3,54))
kidney2$steenland$mids[4] = mean(c(2700,10800))/30 #assumed a maximum 4 times the highest cutpoint as more similar to
Vieira
kidney2$barry$mids[4] = mean(c(5358,21432))/33 ##assumed a maximum 4 times the highest cutpoint as more similar to Vieira

#apply ktrend function
(ktrend = trendp(kidney2))

# get RR and CI per 10 ng/mL increase in serum PFOA in each study
lapply(ktrend, function(df) round(exp(10*df[3] + 10*c(0,-1,1)*qnorm(.975)*df[4]),2))
klogRR = c(ktrend$vieira[3], ktrend$barry[3], ktrend$shearer[3], ktrend$steenland[3], ktrend$rhee[3], ktrend$winquist[3])
kse = c(ktrend$vieira[4], ktrend$barry[4], ktrend$shearer[4], ktrend$steenland[4], ktrend$rhee[4], ktrend$winquist[4])

(m2 = metagen(klogRR,kse)) # meta-analysis for kidney
round(exp(10*c(m2$TE.fixed,m2$lower.fixed,m2$upper.fixed)),2)
round(exp(10*c(m2$TE.random,m2$lower.random,m2$upper.random)),2)
```

Table A3.7 (continued)

```
m2$pval.Q
m2$tau2
m2$I2


#sensitivity taking out steenland and vieira
# get RR and CI per 10 ng/mL increase in serum PFOA in each study
lapply(ktrend, function(df) round(exp(10*df[3] + 10*c(0,-1,1)*qnorm(.975)*df[4]),2))
klogRR3 = c(ktrend$barry[3], ktrend$shearer[3], ktrend$rhee[3], ktrend$winquist[3])
kse3 = c( ktrend$barry[4], ktrend$shearer[4], ktrend$rhee[4], ktrend$winquist[4])

(m3 = metagen(klogRR3,kse3)) # meta-analysis for kidney
round(exp(10*c(m3$TE.fixed,m3$lower.fixed,m3$upper.fixed)),2)
round(exp(10*c(m3$TE.random,m3$lower.random,m3$upper.random)),2)

m3$pval.Q
m3$tau2
m3$I2
```

[a] Note that, if the code is copied from this document and pasted directly to R, the user may need to retype the quotation marks for the code to run correctly.

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