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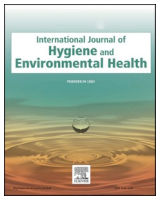

Biochemical and haematological effects of serum PFOA, ADV and cC_6O_4 in workers of a chemical company producing fluoropolymers, Italy, 2013–2022

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ABSTRACT

Introduction: Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are widely used in the manufacture of fluoropolymers. We evaluated biochemical and haematological effects of three PFAS, serum perfluorooctanoic acid (PFOA), ADV, and cC_6O_4 in workers of a fluoropolymer company.

Methods: Using data (2013–2022), we fitted random intercept regression models adjusted for several covariates and reciprocal adjustment between the three PFAS.

Results: We analysed data of 814 workers (698 men, 116 women), 607 from the chemical plant, 207 from the research centre, for a total of 4912 blood samples (2065 with all three PFAS measured). Median levels of PFOA and ADV were 21.3 and 120 μg/L. Most (65.5%) cC_6O_4 measurements were below the limits of quantification (which varied over time from 5 to 0.1 μg/L). For PFOA, we observed positive associations with total cholesterol (+1.1% increase per ln(PFOA) increase) and apolipoprotein B (+1.4%) and negative associations with alkaline phosphatase (−1.5%); suggestive associations were also found with RBC (−0.4%), IgA (−1.5%), IgM (−1.4%). ADV was positively associated with total and LDL cholesterol (+1.0% and +1.6% per ln(ADV) increase), apolipoprotein B (+1.0%), GGT (+2.1%), IgM (+1.4%), and WBC (+1.5%) and negatively associated with direct bilirubin (−2.3%) and alpha-2-globulins (−0.7%); suggestive associations were found for indirect bilirubin (−2.0%), oestradiol (−2.1%), ad CRP (+6.0%). For samples with detectable cC_6O_4 levels we observed higher values of ALP (+2.3%), proteins (+0.5%), IgG (+0.7%) and platelets (+1.6%) and suggestively increased total bilirubin (+3.9%), RBC (+0.6%), and oestradiol (+5.8%). Some associations (total cholesterol, apolipoprotein B, WBC, total bilirubin, and alkaline phosphatase showed reverse time trends in parallel with the strong decrease of serum PFOA and ADV over the study period.

Discussion: We found associations of serum PFOA and ADV with lipid metabolism, liver function, and immunoglobulins. The reverse time trends of some endpoints in parallel with decrease of serum PFOA and ADV reinforce causal interpretation of results. cC_6O_4 showed a different pattern of associations.

1. Introduction

Fluoropolymers are an important class of chemical substances that are virtually chemically inert, non-wetting, non-stick, and highly resistant to temperature, fire, and weather. They include polytetrafluroethylene (PTFE) and fluoroelastomers. used as non-stick coatings on cookware, membranes for clothing that are both waterproof and breathable, electrical-wire casing, fire- and chemical-resistant tubing, and plumbers thread-seal tape. Several perfluoroalkyl and polyfluoroalkyl substances (PFAS) are used as surfactants for the synthesis of fluoropolymers (IARC, 2017).

Among PFAS, perfluorooctanoic acid (PFOA, CAS 335-67-1, also known as C8, molecular formula: C8HF15O2) and its ammonium salt (ammomium perfluorooctanoate, APFO), has been the single mostly used surfactant agent. PFOA is highly persistent in the environment and in living organisms (estimated half-life in humans 2.3–3.5 years) (Fustinoni and Consonni, 2023) and has been associated with several effects, including dyslipidemia, altered liver and immune function, and

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endocrine disruption (Fenton et al., 2021; Steenland et al., 2020). The International Agency for Research on Cancer (IARC) very recently upgraded classification of PFOA from Group 2B (possibly carcinogenic to humans) to Group 1 (carcinogenic to humans) based on limited evidence in humans for renal cell carcinoma and testicular cancer, sufficient evidence in experimental animals, and strong mechanistic evidence in exposed humans (epigenetic alterations and immunosuppressive effects) (IARC, 2017; Zahm et al., 2023). PFOA, its salts and related compounds have been banned since 2020 under the Persistent Organic Pollutants (POPs) Regulation No 2019/1021 (Regulation (EU) No 2019/1021 and Commission Delegated Regulation (EU) 2020/784).

In Italy, a chemical plant located in Spinetta Marengo (Alessandria, Piedmont Region) used PFOA from the beginning of the sixties until 2013; in the same years, PFOA was also used in the companion research centre in Bollate (Lombardy region). Both belonged to Solvay Specialty Polymers Italy from 2011 until the end of 2023. The plant also produced and used two other PFAS, ADV (CAS 330809-92-2) and cC_6O_4 (CAS 1190931-27-1). ADV is a polymerisation reaction mass of perfluoropolyether carboxylic acids containing multiple isomers produced in the same plant and applied in the synthesis of plastomers and elastomers since 1996. cC_6O_4 (molecular formula: $C_6H_4F_9NO_6$) is a "new generation" PFAS that was developed as a new polymerisation adjuvant with short half-life (7 days) (Fustinoni et al., 2023). cC_6O_4 has been produced in the plant since 2012 and increasingly used in the synthesis of plastomers and elastomers. To our knowledge, there are no studies on the effects of ADV(which is produced and used only by Solvay Specialty Polymers Italy) and cC_6O_4.

Details on the company and these three compounds can be found in a recent paper, where we documented marked decreases of serum PFOA (phased out in 2013), and ADV (due to implementation of preventive measures) concentrations over time (Fustinoni and Consonni, 2023). Workers of this plant had been included in a multicentre mortality study originally focused on tetrafluoroethylene (TFE) effects (Consonni et al., 2013).

Solvay workers undergo yearly occupational health examinations, including biomonitoring of PFOA, ADV, and cC_6O_4 and measurement of 38 clinical biochemistry and haematological (CBH) variables. In this work we exploited those data to perform a panel (longitudinal or repeated cross-sectional) study (Checkoway et al., 2004) to evaluate associations between PFAS and CBH data covering the period 2013–2022.

2. Methods

From 2014 to 2022, we received each year anonymised data from the Occupational Health Service of the company containing data on workers of both the chemical plant and the research centre. Health surveillance, including serum PFAS biomonitoring, was performed according to the Italian law for the occupational safety and health (DLgs 81/2008) under the responsibility of the occupational physicians. Workers signed an informed consent.

2.1. Demographics and clinical data

Information on age, body mass index (BMI), and self-reported lifestyle habits (alcohol consumption and tobacco smoking), was updated each year by the occupational physicians during the annual visits.

2.2. Serum PFAS

Biological monitoring of PFAS had started in 2004 for PFOA, in 2011 for ADV and in 2013 for cC_6O_4. Blood drawing was performed in the two infirmaries of the chemical plant and the research centre. Plasma samples were refrigerated at −20 °C and sent to a laboratory Medizinisches Labor Bremen, Germany for analysis of PFAS in the serum fraction (Fustinoni and Consonni, 2023). The lower limits of quantification (LLOQ) for PFOA and ADV was 5 μg/L; for cC_6O_4 the LLOQ was 5 μg/L in 2013, 2.5 μg/L from 2014 to 2017, 1 μg/L from 2018 to 2021, and 0.1 μg/L in 2022. In the first years (until 2017) the number of workers undergoing PFAS measurements in each year depended on the decision of the occupational physicians. In the last years we advised the company to extend biomonitoring to as many workers as possible and to measure all the three PFAS in order to have more data for statistical analysis.

2.3. Clinical biochemistry and haematological data

The following CBH variables were dosed in the serum by two different laboratories, one for workers in the chemical plant and one for workers of the research centre: total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides, apolipoproteins A and B, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), bilirubin (total, direct, and indirect), alkaline phosphatase (ALP), proteins, albumin, alpha-1-, alpha-2-, beta-, and gamma-globulins, immunoglobulins A (IgA), immunoglobulins G (IgG), immunoglobulins M (IgM), amylase, glucose, blood urea nitrogen (BUN), creatinine, uric acid, red blood cells (RBC), white blood cells (WBC), platelets, free triiodothyronine (FT3), free thyroxine (FT4), thyroid-stimulating hormone (TSH), testosterone, 17-beta-oestradiol (E2), prostate-specific antigen (PSA, men), and C-reactive protein (CRP). The two laboratories regularly participate to external verification exercises organized at regional level to ensure the quality of services and comparability of results.

2.4. Statistical analysis

Although data covered the period 2004–2022, we restricted analyses to years 2013–2022 because in this period all three PFAS have been measured. Workers had only one blood sample collected in the same year. Measurements of ADV and PFOA below the LLOQ were assigned the value LLOQ/2. ADV and PFOA were approximately log-normally distributed and thus were ln-transformed to approach Gaussian distributions. Measurements with detectable levels of cC_6O_4 were the minority before 2022, hence cC_6O_4 measurements were dichotomized as 0 (undetectable) and 1 (detectable) using year-specific LLOQ (5 μg/L in 2013, 2.5 μg/L from 2014 to 2017, 1 μg/L from 2018 to 2021, and 0.1 μg/L in 2022).

Correlation between PFAS. To evaluate the correlation between the three xenobiotics, we used the Pearson's r correlation coefficient on ln-transformed data.

Dose-response analyses. We fitted random-intercept regression models to study the association between independent (ADV and PFOA, both ln-transformed, and cC_6O_4, detectable vs undetectable) and dependent (CBH) variables measured on the same serum sample, in order to take into account within-subject correlation (Rabe-Hesketh and Skrondal, 2008). All CBH dependent variables (except albumin, beta-globulins, and uric acid, which showed approximately normal distributions) were positively skewed and thus were ln-transformed.

Beyond univariate (M0) models, we fitted two types of multivariable models. M1 models contained one PFAS at a time and the following fixed or time-varying covariates as potential confounders (because they may affect both serum PFAS levels and the outcomes): year of blood collection/measurement (treated as a dummy variable, because laboratory instruments and methods may change over time), centre (chemical plant = 0, research centre = 1, because served by two different laboratories making CBH analyses), gender (male = 0, female = 1), age (years), alcohol consumption (no, occasional, daily), and smoking (no/yes). The random-intercept linear regression formula for each of the 38 outcome (CNH) variable is:

$$CBH_{it} = alpha_i + PFAS_{kit} + year_t + centre_{it} + gender_i + age_{it} + alcohol_{it} + smoking_{it}$$

where i indicates the individual (with $alpha_i$ indicating the individual intercepts assumed to have a Gaussian distribution), t (t = 1, 2, …, 10) the blood sampling, and k one of the three PFAS, ln(PFOA), ln(ADV), or cC_6O_4, detected (1) vs undetected (0)). Given the large number of analyses, for simplicity we used this set of covariates in all models.

We expect that serum levels of the three PFAS are correlated (because determined by department, occupation, and tasks) and therefore may confound each other (Weisskopf et al., 2018). Thus, we fitted M2 models containing all M1 covariates and all the three PFAS, according to the formula:

$$CBH_{it} = alpha_i + \ln(PFOA)_{it} + \ln(ADV)_{it} + cC_6O_{4it} \ (+M1\text{-covariates}).$$

Therefore, M2 models are based on blood samples in which with all the three PFAS have been measured. In sensitivity analyses we calculated tests for linear trend across deciles of PFOA and ADV in M2 models by treating the 10-category variables as ordinal in the models; for brevity we present only results with $P < 0.20$. For testosterone and oestradiol we also stratified M2 analyses by sex. For oestradiol in women we further stratified age at blood sampling using 50 years as cut-off (a rough approximation of age at menopause). Serum proteins (albumin and globulins) bind to PFAS, thus affecting their unbound fraction: for this reason it has been suggested to consider them as covariates in studies of PFAS effects (Fischer et al., 2024). We do not expect they have a confounding effect because they probably do not affect the 38 outcomes under study. However, for the sake of completeness, we fitted M2 sensitivity analyses in which we additionally adjusted for serum albumin. In a further sensitivity analysis we fitted random intercept and random slope linear regression models in which year of blood collection was treated as a random quantitative variable (M3 models).

In case of non-transformed dependent variables (albumin, beta-globulins, and uric acid) the regression slopes represent absolute changes in the original measurement units for every unit increase in ln (ADV) or ln(PFOA); for cC_6O_4 the slope is the difference between detectable and undetectable categories. For ln-transformed variables the association was expressed as percent change (positive or negative) using the formula: Change (%) = [exp(slope) - 1)] x 100] for every unit increase in ln(ADV) or ln(PFOA). A unit increase in ln(ADV) or ln(PFOA) is equivalent to an approximate three-fold increase of ADV and PFOA (exactly: 2.718 times). For cC_6O_4 the percent change is for detectable category vs the undetectable. We present results of M0, M1, and M3 models in Supplementary material but we give more weight to findings from fully adjusted M2 models, presented in the main body of the paper.

Time trends of selected clinical biochemistry and haematological variables. In a previous paper we showed that serum levels of PFOA and ADV strongly decreased over time (Fustinoni and Consonni, 2023). In order to verify if some of the outcome variables positively or negatively associated with serum PFOA or ADV showed a reverse trend, we fitted multiple random intercept linear regression models adjusted for worksite, gender, age, BMI, alcohol consumption, and current smoking, We then estimated the percent change (per year) with the formula above and presented graphs of the adjusted average changes over time.

In interpreting results we did not stick to the usual (alpha = 0.05) statistical threshold (Sterne and Davey Smith, 2001; Wasserstein et al., 2019) and always provided 95% percent confidence intervals (CI) for all results. Statistical analyses were performed with Stata 18 (StataCorp. 2023).

3. Results

3.1. Study subjects and biomonitoring data

In 2013–2022 814 workers underwent blood drawing for PFAS measurements, 607 in the chemical plant (546 men, 61 women) and 207 in the research centre (152 men, 55 women) (Table 1). The number of measurements per year were above 400 in all years except in 2013 and 2016. The number of workers with at least 5 yearly measurements was 422/607 (69.5%) in the chemical plant, 72/207 (34.8%) in the research centre, and 494/814 (60.7%) overall. The number of serum measurements available for M1 models were 3315 for PFOA, 4609 for ADV, and 2650 for cC_6O_4. In 2065 samples all three PFAS were measured and thus available for multivariable analyses with reciprocal adjustment between PFAS (M2 models).

Table 1
Characteristics of workers producing fluoropolymers in the chemical plant in Spinetta Marengo and in the research centre in Bollate, Italy, 2013–2022.

	Chemical plant		Research center		Total
	Men	Women	Men	Women	
Workers with blood drawing (n)	546	61	152	55	814
Age at first blood drawing, mean (min-max)	36.7 (19–63)	37.5 (21–61)	39.2 (20–63)	40.5 (25–60)	37.5 (19–63)
Age at last blood drawing, mean (min-max)	42.9 (19–71)	44.3 (23–63)	42.8 (21–68)	44.5 (27–65)	43.1 (19–71)
Age at any blood drawing, mean (min-max)	41.2 (19–71)	41.0 (21–63)	41.3 (20–68)	44.4 (25–65)	41.4 (19–71)
Blood samples (n)	3625	380	642	265	4912
2013	274	32	55	21	382
2014	394	48	100	36	578
2015	417	52	108	43	620
2016	251	18	36	9	314
2017	354	36	44	19	453
2018	363	41	40	22	466
2019	371	44	59	22	496
2020	401	37	62	29	529
2021	402	37	69	32	540
2022	398	35	69	32	534
Number of workers with:					
1 measurement	46	5	29	7	87
2 measurements	52	5	33	12	102
3 measurements	36	4	31	9	80
4 measurements	32	5	12	2	51
5 measurements	23	3	5	5	36
6 measurements	23	3	7	4	37
7 measurements	33	4	2	3	42
8 measurements	75	8	4	3	90
9 measurements	91	21	11	6	129
10 measurements	135	3	18	4	160
Blood samples with PFOA measured (n)	2597	275	310	133	3315
Blood samples with ADV measured (n)	3422	369	585	233	4609
Blood samples with cC_6O_4 measured (n)	1836	223	396	195	2650
Blood samples with all the three PFAS measured (n)	1597	197	176	95	2065

The majority of serum PFOA measurements were above the LLOQ in workers of the chemical plant, especially in men, while they were a small minority in the research centre (Table 2 and Supplementary Fig. 1). ADV measurements were above the LLOQ in most workers (Table 2 and Supplementary Fig. 2), with higher proportions of detected levels in men and in the chemical plant. Levels of cC_6O_4 were mostly below the LLOQ, and detected levels were lower than PFOA and ADV levels, with slightly higher levels in men (Table 2 and Supplementary Fig. 3). The proportion of detected cC_6O_4 values was quite low in 2013 (LLOQ 5 μg/L) and in the years 2014–2017 (LLOQ 2.5 μg/L), increased (but remained below 50%) in the years 2018–2021 (LLOQ 1 μg/L), and further increased in 2022 (LLOQ 0.1 μg/L) (Table 2, bottom).

We estimated the following correlation coefficients on ln-transformed data: PFOA and ADV: r = 0.44 (3129 measurements); PFOA and cC_6O_4: r = 0.35 (864 measurements); ADV and cC_6O_4: r =

Table 2
Serum levels (μg/L) of perfluorooctanoic acid (PFOA), ADV, and cC_6O_4 in workers producing fluoropolymers in the chemical plant in Spinetta Marengo and in the research centre in Bollate, Italy, 2013–2022. Values ≥ 100 rounded to integers. For cC_6O_4 descriptive statistics are based on detected values (≥5 μg/L in 2013, ≥2.5 μg/L in 2014–2017, ≥1 μg/L in 2018–2021, and ≥0.1 μg/L in 2022).

Variable	Chemical plant		Research center		Total
	Men	Women	Men	Women	
PFOA					
n measurements	2597	275	310	133	3315
n (%) detected (≥5 μg/L)	2256 (86.9)	169 (61.5)	43 (13.9)	24 (18.0)	2492 (75.2)
Minimum	2.5	2.5	2.5	2.5	2.5
First quartile	10.1	2.5	2.5	2.5	5.0
Median	34.8	7.5	2.5	2.5	21.3
Geometric mean	40.9	9.2	3.6	3.4	26.1
Mean	195	23.1	20.1	6.3	157
Third quartile	164	24.6	2.5	2.5	108
Maximum	4020	370	940	114	4020
Standard deviation	407	43.6	95.2	14.9	369
ADV					
n measurements	3422	369	585	233	4609
n (%) detected (≥5 μg/L)	3369 (98.5)	342 (92.7)	514 (87.9)	149 (64.0)	4374 (94.9)
Minimum	2.5	2.5	2.5	2.5	2.5
First quartile	64.9	16.9	13.8	2.5	37.9
Median	178	46.2	43.9	8.7	120
Geometric mean	173	42.8	40.3	12.4	112
Mean	482	102	125	48.4	385
Third quartile	513	110	119	39.3	376
Maximum	14386	2010	2213	965	14386
Standard deviation	869	177	228	105	773
cC_6O_4					
n measurements	1836	223	396	195	2650
n (%) detected	765 (41.7)	57 (25.6)	71 (17.9)	20 (10.3)	913 (34.5)
Minimum	0.1	0.1	0.1	0.1	0.1
First quartile	1.3	1.1	1.1	0.6	1.3
Median	2.7	1.7	7.0	2.0	2.6
Geometric mean	2.7	1.7	2.4	1.8	2.5
Mean	7.2	3.4	8.1	4.3	7.0
Third quartile	6.1	3.5	6.9	3.9	5.9
Maximum	873	24.6	164	33.3	873
Standard deviation	33.9	5.1	24.4	7.3	31.8
Effect of varying LLOQ					
n measurements 2013	95	15	44	17	171
n (%) detected (≥5 μg/L)	13 (13.7)	0 (0.0)	2 (4.6)	1 (5.9)	16 (9.4)
n measurements 2014–2017	515	61	167	65	808
n (%) detected (≥2.5 μg/L)	106 (20.6)	6 (9,8)	9 (5.4)	2 (3.1)	123 (15.2)
n measurements 2018–2021	952	119	137	85	1293
n (%) detected (≥1 μg/L)	401 (42.1)	31 (26.0)	36 (26.3)	9 (10.6)	477 (36.9)
n measurements 2022	274	28	48	28	378
n (%) detected (≥0.1 μg/L)	245 (89.4)	20 (71.4)	24 (50.0)	8 (28.6)	297 (78.6)

0.31 (856 measurements).

3.2. Association between clinical biochemistry and haematological data with PFAS dose

Results of crude (M0) and partially adjusted (M1) models are reported in Supplementary Figures 4-6 and 7-9, respectively. Considering PFOA, in fully adjusted M2 models we observed positive associations ($P < 0.05$) with total cholesterol (+1.1% increase per ln(PFOA) increase) and apolipoprotein B (+1.4%) and negative associations with ALP (−1.5%) (Fig. 1). We also found suggestive ($0.05<P < 0.10$) associations with IgA (−1.5%), IgM (−1.4%), and RBC (−0.4%),. Positive associations with ALT and GGT were also observed, but with wide confidence intervals.

Tests for trends across PFOA deciles yielded the following results: total cholesterol (positive, $P = 0.01$), apolipoprotein B (positive, $P = 0.01$), ALP (negative, $P = 0.05$), proteins (positive, $P = 0.09$), IgA (negative, $P = 0.13$), IgM (negative, $P = 0.15$), RBC (negative, $P = 0.06$).

In women, one ln(PFOA) increase was associated with a 24.2% oestradiol decrease (95% CI -38.2 to −7.0, $P = 0.008$, 69 women, 239 samples), with a negative trend across deciles ($P = 0.01$). The association was weaker (−13.4%, $P = 0.11$) below 50 years of age (59 women, 200 measurements), and positive (+22.3%, $P = 0.52$) for measurements performed on or after 50 years of age (based on 39 measurements in 15 women).

ADV was positively associated ($P < 0.05$) with total cholesterol (+1.0%), LDL cholesterol (+1.6%), apolipoprotein B (+1.0%), GGT (+2.1%), IgM (+1.4%), and WBC (+1.5%) and negatively associated with direct bilirubin (−2.3%), and alpha-2-globulins (−0.7%) (Fig. 2). We also found suggestive ($0.05<P < 0.10$) associations with indirect bilirubin (−2.0%), oestradiol (−2.1%), and CRP (+6.0%). Positive associations with triglycerides, ALT, IgA, and testosterone were also observed, but with wide confidence intervals.

Tests for trends across ADV deciles yielded the following results: total cholesterol (positive, $P = 0.003$), LDL cholesterol (positive, $P = 0.02$), apolipoprotein B (positive, $P = 0.005$), ALT (positive, $P = 0.09$), GGT (positive, $P = 0.06$), direct bilirubin (negative, $P = 0.01$), indirect bilirubin (negative, $P = 0.19$), alpha-2-globulins (negative, $P = 0.08$), WBC (positive, $P = 0.05$), oestradiol (negative, $P = 0.08$), CRP (positive, $P = 0.20$).

The oestradiol decrease was observed only in men: 2.1% per one ln (ADV) increase (95% CI -3.8 to −0.3, $P = 0.02$, 445 men, 1439 samples), with a negative trend across deciles ($P = 0.10$).

For 913 samples with detectable cC_6O_4 levels vs 1737 undetectable, we observed increases ($P < 0.05$) for ALP (+2.3%), proteins (+0.5%), IgG (+1.7%), and platelets (+1.6%) (Fig. 3). Suggestive ($0.05<P < 0.10$) associations were found for total bilirubin (+3.9%), RBC (+0.6%), and oestradiol (+5.8%). The oestradiol increase was evident in women only: +29.3% (95% CI -5.9 to +77.5, $P = 0.11$, 69 women, 239 measurements).

In sensitivity analyses in which we fitted M2 models additionally adjusted for serum albumin we observed similar patterns (Supplementary Figs. 10–12).

Results obtained with random intercept and random slope models (M3 models) were broadly comparable to those obtained with M2 models, although some differences can be noted. For PFOA (Supplementary Fig. 13), the association with total cholesterol was confirmed, while associations with apolipoprotein B and ALP were statistically weaker; conversely, results for ALT, GGT, proteins, IgA, IgM, and RBC were statistically more robust and a suggestive association with platelets emerged. For ADV (Supplementary Fig. 14), most associations (with total and LDL cholesterol, triglycerides, apolipoprotein B, bilirubin, alpha-2 globulins, IgM. WBC, oestradiol, and CRP) were confirmed. For cC_6O_4 (Supplementary Fig. 15), the associations with ALP and platelets were confirmed, while other associations emerged (HDL cholesterol, apolipoprotein B, AST, albumin, albumin/globulins ratio, FT4, and PSA).

3.3. Time trends of selected clinical biochemistry and haematological variables

Serum PFOA and ADV levels strongly decreased over time (−17.8% and −16.0% per year, respectively) (Supplementary Figs. 16–17), Outcomes variables which were positively associated with PFOA or ADV (total cholesterol, apolipoprotein B, and WBC), showed decreasing trends over time (Supplementary Fig. 15, top and middle panels).

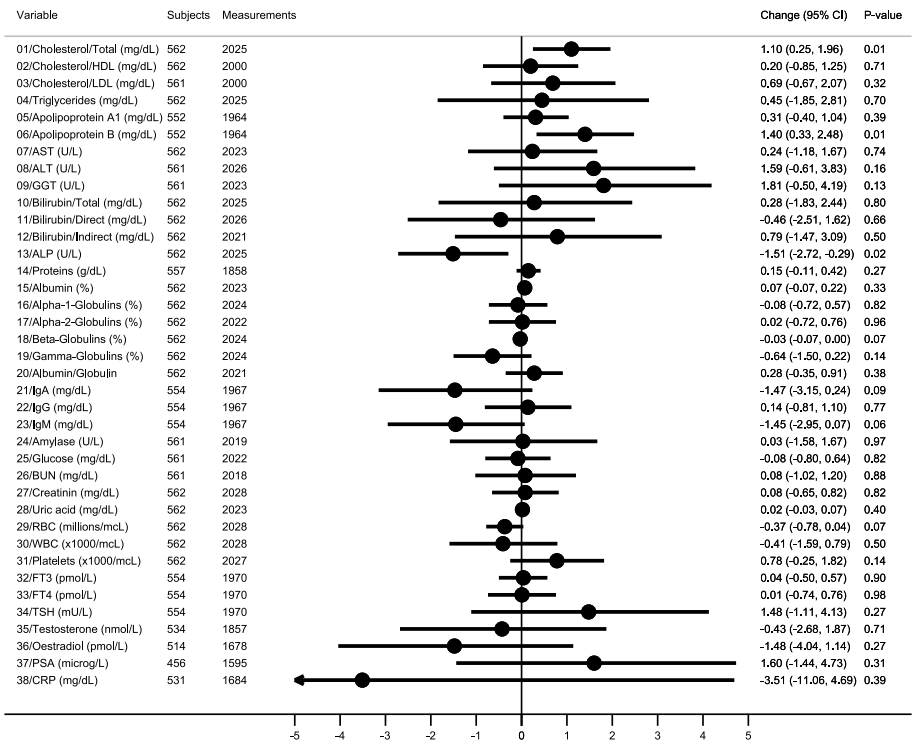

Fig. 1. Association of serum ln(PFOA) with selected clinical biochemistry and haematological data in workers producing fluoropolymers in the chemical plant in Spinetta Marengo and in the research centre in Bollate, Italy, 2013–2022. Change (95% confidence interval (CI)) is percent change (in case of ln-transformed variables) or absolute change (uric acid, albumin, and beta-globulins) per ln(PFOA) increase, estimated from multiple random intercept linear regression models adjusted for worksite (chemical plant, research centre), year of blood collection/measurement (dummy variables), gender, age (years), BMI (kg/m^2), alcohol consumption (no, occasional, daily), current smoking (no/yes) and for ln(ADV) and cC_6O_4 (detected vs undetected) (M2 models).

Conversely, outcomes variables which were negatively associated with PFOA or ADV (total bilirubin and ALP) increased over time (Supplementary Fig. 18, bottom panels). No similar "reverse" time changes were found for other variables (results not shown).

4. Discussion

In this study we found moderate effects of PFOA on lipid metabolism (positive association with total cholesterol and apoliporotein B) and liver function (negative association with alkaline phosphatase), immunoglobulins (negative association with IgA and IgM), and hormonal function (negative association with oestradiol in women). Similarly, ADV appeared to affect lipid metabolism (positive association with total and LDL cholesterol and apoliporotein B), liver function (positive association with GGT and negative with bilirubin and alpha-2-globulins). Differently from PFOA, the association between ADV and IgM was positive and a small negative association with oestradiol was found only in men. ADV was also positively associated with WBC. Finally, workers with detectable cC_6O_4 had higher ALP, proteins, IgG, and platelets; positive association with osetradiol was found in women.

Interestingly, for some variables that showed robust positive (total cholesterol, apolipoprotein B, and WBC) or negative (total bilirubin, ALP) associations with serum PFOA or ADV, we found opposite time trends over time. These changes followed the strong reduction over time of both serum PFOA and ADV in workers of the company (Fustinoni and Consonni, 2023); thus, they may be seen as an indication of reversibility of some (but not all) effects. Reversibility of effects is an argument against reverse causation and strengthens a causal interpretation of the associations we found between changes in serum PFOA and ADV and some biochemical variables.

4.1. Strengths and limitations

The main strength of this study is the long period of observation (10 years) and the large number of samples available for analysis regarding more than 800 workers. Moreover, the three PFAS were measured in the same laboratory in Germany. Clinical biochemistry and haematological data were measured in two laboratories (one for workers in the chemical plant and the other for workers in the research centre); possible variations in laboratory instruments and methods over time were accounted for by adjusting for year of sampling (included in models as a fixed covariate using dummy variables). We also presented results of random intercept and random slope models (in which year was treated as a random quantitative variable). However, we feel more confident in results from random intercept models with year treated as a fixed covariate. In fact, if the aim is to completely control confounding it is safer to used fixed dummy (less efficient but unbiased) instead of random variables (more efficient but potentially biased) (Basagana et al., 2018).

The large amount of data allowed us to perform analyses in which we adjusted for potential reciprocal confounding between the three compounds. The positive associations between them were moderate (correlation coefficients below 0.50): therefore, there were no problems of multicollinearity (which occur when correlation coefficients are much larger), as also witnessed by the similar patterns of results obtained with M1 and M2 models.

Some authors cautioned that in some situations reciprocal adjustment between toxicants in analyses of exposure to mixtures may amplify confounding bias (Weisskopf et al., 2018). However, this may happen when unknown or unmeasured confounders differentially affect mixture components. At workplace the main determinant of serum levels of xenobiotics is the work itself (department, job, tasks, etc.), making it difficult to hypothesize other confounders of comparable strength. Moreover, panel studies like this are less affected by this potential

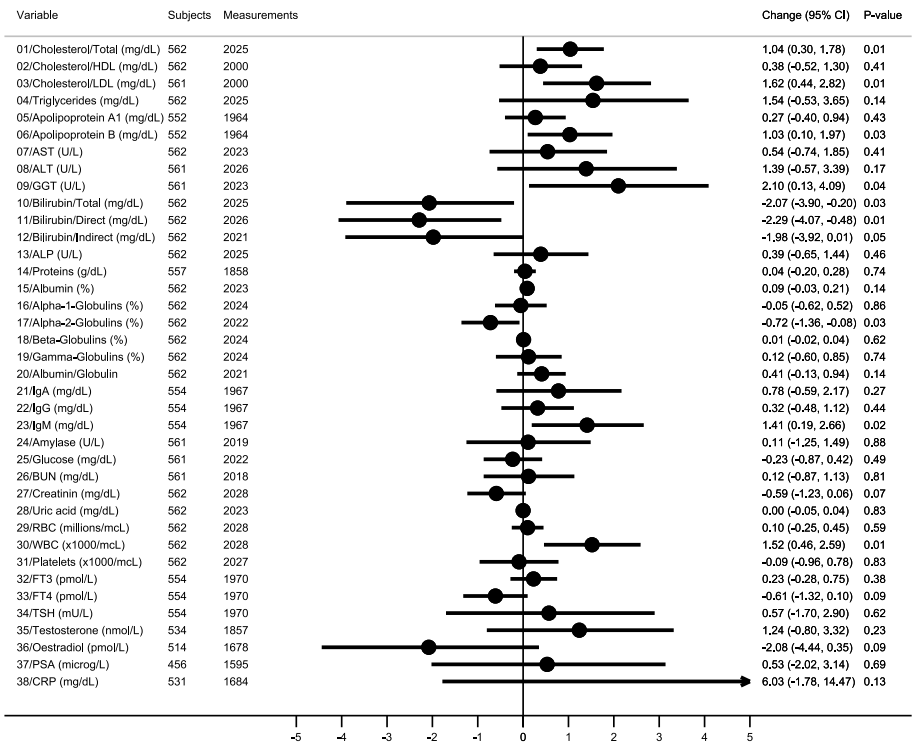

Fig. 2. Association of serum ln(ADV) with selected clinical biochemistry and haematological data in workers producing fluoropolymers in the chemical plant in Spinetta Marengo and in the research centre in Bollate, Italy, 2013–2022. Change (95% confidence interval (CI)) is percent change (in case of ln-transformed variables) or absolute change (uric acid, albumin, and beta-globulins) per ln(ADV) increase, estimated from multiple random intercept linear regression models adjusted for worksite (chemical plant, research centre), year of blood collection/measurement (dummy variables), gender, age (years), BMI (kg/m^2), alcohol consumption (no, occasional, daily), current smoking (no/yes), and for ln(PFOA) and cC_6O_4 (detected vs undetected) (M2 models).

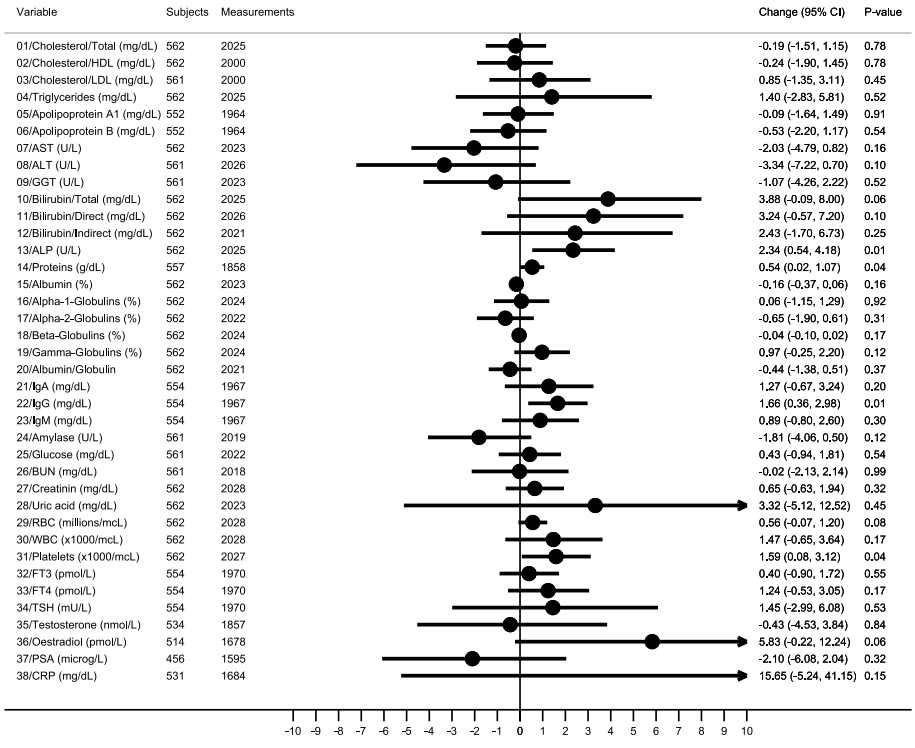

Fig. 3. Association of serum cC_6O_4 (detected vs undetected) with selected clinical biochemistry and haematological data. Change (95% confidence interval (CI)) is percent change (in case of ln-transformed variables) or absolute change (uric acid, albumin, and beta-globulins) for samples with detectable cC6O4 levels (vs undetectable), estimated from multiple random intercept linear regression models adjusted for worksite (chemical plant, research centre), year of blood collection/measurement (dummy variables), gender, age (years), BMI (kg/m^2), alcohol consumption (no, occasional, daily), current smoking, and for ln(PFOA) and ln(ADV) (M2 models).

problem compared with simple (one time) cross-sectional analyses (Fitz-Simon et al., 2013b; Weisskopf et al., 2018).

The major limitations are that only routine clinical biochemistry and haematological data were available for analysis and that not all potential confounding factors (e.g., diet) relevant for each biomarker have been considered in the statistical analysis. A further limit is that for cC_6O_4 we could only perform analyses contrasting detected vs undetected levels because the majority of measurements before 2022 were below the LLOQ.

4.2. Comparison with published research: PFOA

There is abundant literature regarding health effects of PFOA. Therefore, we in general restrict literature evaluation to the most recent years, when several reviews and meta-analyses were published.

Our findings of a positive association between PFOA and total cholesterol are consistent with the conclusions of the C8 Science Panel (Steenland et al., 2020) and of the Society of Environmental Toxicology and Chemistry (SETAC) North America (Fenton et al., 2021), with a recent review (Sunderland et al., 2019), and a recent meta-analysis of 29 studies (Liu et al., 2023). The latter included three Italian studies performed in the Veneto region (North-Eastern Italy), two among workers of a large factory producing PFAS (Batzella et al., 2022; Costa et al., 2009) and one among residents in an area contaminated by PFAS by the same factory (Canova et al., 2020).

There is evidence of associations of serum PFOA with other lipids, mainly LDL cholesterol and triglycerides (Fenton et al., 2021; Liu et al., 2023; Steenland et al., 2020). In our study, we did not find associations between serum PFOA and LDL or triglycerides, but we found a positive association with the concentration of apolipoprotein B, which is involved in the transport of total cholesterol, LDL cholesterol, and triglycerides (Ala-Korpela et al., 2022). These findings support the effect of PFOA on lipid metabolism.

In this study, we found a clear negative association between ALP and serum PFOA; moreover, we observed positive associations of ALT and GGT with serum PFOA, although with wide confidence intervals. Taken together, these findings suggest that PFOA does affect liver function and are consistent with literature reviews (Ducatman and Fenton, 2022; Fenton et al., 2021; Steenland et al., 2020). A recent meta-analysis using a weighted z-score method applied to epidemiological and animal studies showed positive associations of PFOA with ALT, AST, and GGT in humans and with ALT in rodents (Costello et al., 2022; Ducatman and Fenton, 2022). A reanalysis of the C8 Health Project in the US mid-Ohio valley involving more than 28 thousand people, found positive associations of serum PFOA with abnormally high ALT value, defined as $\geq$35 IU/mL for males and $\geq$25 for females (Ducatman et al., 2023). The Canadian Health Measures Survey (>4500 people) found clear positive associations between PFOA and AST, GGT, and ALP, but only a weak association with ALT (Borghese et al., 2022).

Our findings are suggestive of a slight decrease (<2%) of IgA and IgM in association with exposure to PFOA; although these immunoglobulins are rather unspecific markers of immune function, these results are consistent with recent reviews of epidemiological, animal, and toxicology studies which concluded that PFOA affects the immune system (DeWitt et al., 2019; Fenton et al., 2021; Steenland et al., 2020; Zahm et al., 2023).

In our study we did not find associations with thyroid function. There are contrasting opinions about effects of PFOA on thyroid function: the C8 Science Panel evaluated the available evidence as weak (Steenland et al., 2020), while SETAC concluded that PFOA definitely alters human thyroid hormones (Fenton et al., 2021).

We found a negative association between PFOA and oestradiol in women. The meaning of this observation is unclear as levels of this hormone are highly variable during the menstrual cycle, a factor that was not considered in our analyses. Moreover, in women aged 50+ years the association was in the opposite direction, although based on a few measurements. A recent study using NHANES data 2015–2016 for almost 2000 people reported a negative association but only in young girls (age 12–19 years) (Xie et al., 2021).

Regarding the reverse time trends we observed in parallel with serum PFOA and ADV decrease, analogous findings (reduction of LDL cholesterol following PFOA decrease) has been observed in residents in a contaminated area in Ohio after water filtration systems were introduced to reduce PFOA concentrations in drinking water (Fitz-Simon et al., 2013b).

4.3. Comparison with published research: ADV and cC_6O_4

Considering exposure to ADV, no comparison with previous studies could be performed, as biomonitoring data regarding this chemical are reported for the first time in the present work. In general, we found overlapping effects of ADV compared with PFOA, with a few exceptions. We can at least speculate that ADV probably share a similar toxicological profile compared to other persistent PFAS.

A study in workers of a large factory producing PFAS in Veneto, Italy (see above) examined serum cC_6O_4 concentration but not its effects (Fustinoni and Consonni, 2023). We found few in vitro studies on effects of cC_6O_4 (Coperchini et al., 2021; Coperchini et al., 2023; De Toni et al., 2022; Di Nisio et al., 2023; Minuz et al., 2021; Moro et al., 2022; Pavan et al., 2023) and one in humans (Sabovic et al., 2023). One examined human platelet aggregation induced by agonists after pretreatment with 100 and 200 μg/L of cC_6O_4 and found a positive effect of cC_6O_4 (contrasted by acetylsalicylic acid) at both concentrations (Minuz et al., 2021). These previous evidences seems not to be related with our main result, a positive association of cC_6O_4 with platelet numbers in workers mostly exposed to <10 μg/L. In summary, we found effects of cC_6O_4 quite different from those we observed for PFOA and ADV; this is not surprising, given the difference in the chemical structures of these chemicals as well as the much shorter half-life of cC_6O_4 (Fustinoni et al., 2023).

5. Conclusions

In this 10-year biomonitoring study in workers of a chemical company producing fluoropolymers in Italy we found associations of a few percent changes per ln-increase of PFOA with lipids (increases), liver enzymes (increases), and immunoglobulins A and M (decreases). Some of these findings are in general consistent with the existing literature (Fitz-Simon et al., 2013a). Moreover, like others (Fitz-Simon et al., 2013b), we observed reversibility of these effects over time in parallel, with strong decrease of serum PFOA and ADV. To our knowledge, this is the first epidemiological study of biochemical and haematological outcomes of ADV and cC_6O_4. For ADV, the associations with lipid metabolism and liver function were comparable to those of PFOA, but those with immunoglobulins were in the opposite direction. For cC_6O_4, a different pattern of associations was observed.

In evaluating these findings we should distinguish two levels of interpretation: the group (epidemiological) and the individual (clinical) level (Fitz-Simon et al., 2013a). At a group level we could affirm that PFOA and ADV had similar effects on lipids and liver. At the individual level, given the magnitude of the associations, these results do not suggest particular concerns from a clinical point of view.

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Patient consent for publication

Workers signed informed consents for blood drawing for biomonitoring and for publication of statistical analyses.

Ethics approval

Collection of biomonitoring data is part of the routine occupational health surveillance of workers according to the Italian Law for the safety and health at workplaces (D.Lgs. 81/2008). No ethics approval is required.

Provenance and peer review

Not commissioned; externally peer reviewed.

Data availability statement

Data are not available.

CRediT authorship contribution statement

Dario Consonni: Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Silvia Fustinoni:** Writing – review & editing.

Declaration of competing interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2024.114440.

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