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Perfluoroalkyl substance excretion: Effects of organic anion-inhibiting and resin-binding drugs in a community setting

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ABSTRACT

Background: Longer serum half-lives of perfluoroalkyl substances (PFAS) in humans compared to other species has been attributed to differences in the activity of organic anion transporters (OAT).
Methods: Among 56,175 adult participants in the community-based C8 Health Project, 23 subjects were taking the uricosuric OAT-inhibitor probenecid, and 36 subjects were taking the bile acid sequestrant cholestyramine. In regression models of log transformed serum PFAS, medication effects were estimated in terms of mean ratios, adjusting for age, gender, BMI, estimated glomerular filtration rate (eGFR) and water-district of residence.
Results: Probenecid was associated with modest, but not statistically significant increases in serum PFAS concentrations. In contrast, cholestyramine significantly lowered serum PFAS concentrations, notably for perfluorooctane sulfonic acid (PFOS).
Conclusions: The effectiveness of cholestyramine in a community setting supports the importance of gastrointestinal physiology for PFAS excretion kinetics, especially for PFOS. We did not find clear evidence that probenecid, an inhibitor of OAT, affects PFAS clearance.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic and environmentally persistent compounds. Several of these, including perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) are nearly ubiquitous in the environment and can be detected in the blood of most inhabitants of industrialized or industrializing nations (K. H. Harada et al., 2010). The presence of PFAS in inhaled air, or ingested food and water, may be explained by pollution related to their widespread use in fire-fighting foams, the manufacture of fluoropolymers and in other industrial processes, as well as to their residual presence in building structural materials, outdoor soil, drinking water, indoor dusts, food packaging materials, and a wide variety of consumer products (Gluge et al., 2020; Lindstrom et al., 2011; Liu et al., 2013; Strynar and Lindstrom, 2008).

PFAS are well absorbed by humans (Kudo and Kawashima, 2003), with average serum half-lives estimated to be about 3 years for PFOA (Bartell et al., 2010; Li et al., 2018; Seals et al., 2011) and approximately 4–5 years for PFOS (Li et al., 2018; Olsen et al., 2007). Inter-individual variation in elimination rates and half-life values for PFOA, PFOS and PFHxS ranging from 1 to 10 years have been noted (Li et al., 2018). Important routes of elimination include urinary and biliary excretion, with urinary excretion generally considered to be predominating for most PFAS compounds (Kudo and Kawashima, 2003). Compared to animal species, longer serum PFAS half-lives in humans (Kudo and Kawashima, 2003) are potentially explained by differences in secretion and reabsorption in kidney and gut, mediated by active renal tubular organic anion transporters (OATs) (K. Harada et al., 2005; Kudo et al., 2002; Kudo and Kawashima, 2003; Nakagawa et al., 2009) and organic anion transporter polypeptides (OATp) (Yang et al., 2009, 2010; Zhao

Abbreviations: ng/mL, nanograms/milliliter; OAT, organic anion transporter; PFAS, Per- and polyfluoroalkyl substances; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate; PFNA, Perfluorononanoic acid; eGFR, estimated glomerular filtration rate; BMI, body mass index.

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et al., 2017), although the interactions of renal secretion, reabsorption, and total renal excretion of PFAS by OATs in humans are complex and incompletely understood (Fabrega et al., 2014; Han et al., 2012; Loccisano et al., 2013). Two OATs (OAT1, OAT3) are involved in renal tubular secretion, and have been reported to have high affinity for PFOA in humans and in rats (Nakagawa et al., 2008). Nevertheless, poor renal excretion by OATs has been implicated as a likely contributor to the human species-specific slow excretion of PFAS such as PFOA (K. Harada et al., 2005). In contrast, OAT4 and OATp are considered to be involved in tubular reabsorption of PFAS (Andersen et al., 2008; Nakagawa et al., 2009), as well as PFAS membrane effects in the gut (Kimura et al., 2020) and in other organs (Sabovic et al., 2019). Highly active renal (and gut) reabsorption might therefore represent a fundamental mechanism of the long human half-life of the long-chain PFAS such as PFOA and PFOS (Han et al., 2012).

Pharmaceutical agents prescribed for many reasons may interact with excretion of PFAS in community settings. Consideration of their activity provides a practical, observational opportunity to address knowledge gaps concerning excretion. Probenecid inhibits OAT1 and OAT3. It is prescribed primarily because it competitively interferes with renal tubular transport of uric acid, leading to increased uric acid excretion and thus lower serum uric acid levels. OAT1 and OAT3 have also been hypothesized to play a role in the renal excretion of PFAS (Nakagawa et al., 2008). Probenecid-induced inhibition of OAT1 and OAT3 therefore suggests the *a priori* hypothesis that those taking probenecid could have slower renal excretion and higher PFAS, opposite to what is known about probenecid's effect on uric acid. Rodents exposed to probenecid show reduced renal clearance and higher serum PFOA concentrations, consistent with a role of OAT1 and OAT3 in rodent PFAS excretion (Kudo et al., 2002), although there are also interspecies and reabsorption reasons to question how important such a role could be in humans (Nakagawa et al., 2008; Yang et al., 2009, 2010).

PFAS also enter the intestine as a component of bile; where they can be reabsorbed and returned to the liver through enterohepatic circulation, in a repeating cycle of biliary excretion and reabsorption (Jandacek and Tso, 2007; Lau et al., 2007). Cholestyramine, a bile acid sequestrant resin historically used in the treatment of lipid disorders, increases the excretion of cholesterol in the form of bile acids rather than allowing for enterohepatic circulation. Cholestyramine also promotes the removal of anionic contaminants, such as the obsolete pesticide chlordecone, through fecal excretion (Cohn et al., 1978). Johnson et al. demonstrated that feeding cholestyramine to rats, following administration of either PFOA or PFOS, increased PFAS fecal elimination (Johnson et al., 1984). Cholestyramine treatment has been shown to result in increased fecal PFAS excretion under short-term observed conditions in a small human population (n = 8) (Genuis et al., 2013). An earlier case report also showed that more prolonged treatment resulted in diminished serum concentrations, notably for PFOS, in a highly exposed individual (Genuis et al., 2010).

The C8 Health Project enrolled 69,030 participants of all ages from six water districts with varying degrees of PFOA water contamination, and it measured a suite of PFAS compounds in most of them (Frisbee et al., 2009). The large population size provided an opportunity to investigate the effects of relatively uncommon drugs such as the uricosuric drug probenecid and the bile acid sequestrant cholestyramine on serum concentrations of PFAS in an unsupervised population setting. We are not aware of an existing investigation of the influence of OAT on PFAS excretion in a community exposure population, nor of a published study that investigates the influence of chronically prescribed pharmaceutical medications on enhancing or delaying excretion.

2. Methods

2.1. Study population

The C8 Health Project arose from a court-directed settlement following the discovery of PFOA contamination of six water districts in the mid-Ohio Valley region of Ohio and West Virginia. The circumstances of the settlement and the independently administered population survey methodology have been described (Frisbee et al., 2009). Briefly, participants filled out an extensive health survey in 2005–6 including socioeconomic, employment, and personal health information, diagnoses, and prescriptions as well as over-the-counter drugs. Survey participation has been estimated to represent 80–81 % of adult residents then residing in the six affected water districts (Frisbee et al., 2009; Steenland et al., 2009a). This study of deidentified participant data was conducted under a West Virginia University IRB.

The following PFAS were detected in the serum of the vast majority of 56,554 adult survey participants for the initial survey: PFOA, PFOS, PFHxS (perfluorohexane sulfonate), and PFNA (perfluorononanoic acid). We included 56,175 participants who had no missing values for all four analytes as the final study population. Data for six other PFAS species, each with numerous values below the limit of detection, were not considered to be suited for the purpose of this study. Because our outcome variable is related to excretion kinetics, and the proportion below the LOD is small, and the population is large, we excluded rather than used surrogate variables for the very rare serum concentrations below the level of detection for the four PFAS considered. No takers of either drug were excluded by this strategy. Participants providing ambiguous responses concerning present use of the medications of interest were excluded.

2.2. Laboratory methods

PFAS serum concentrations were determined by a modification to a previously described procedure combining protein precipitation extraction with reverse-phase high-performance liquid chromatography/tandem mass spectrometry. A triple quadrupole mass spectrometer in selected reaction monitoring mode detected the M/Z transitions of individual perfluorocarbon compounds and the appropriate species of ^{13}C-PFAS surrogate. Additional details of the technique and quality assurance protocols have been published elsewhere (Frisbee et al., 2009).

2.3. Study design and statistical analysis

The aim of the study was to separately compare serum levels of four PFAS compounds between users vs. nonusers of probenecid and cholestyramine in a survey containing self-reported information about pharmaceutical use. All names of pharmaceuticals containing probenecid, commercial names and possible misspellings were searched across the medications database of the C8 Health Project to identify probenecid users in the study. This process was repeated for cholestyramine. The distribution of the four PFAS studied was skewed; therefore, we log transformed these variables. We performed linear regression of natural log-transformed PFAS level as the outcome variable and probenecid or cholestyramine use as the main exposure, additionally adjusting for covariates including age, gender, socioeconomic status (SES), estimated glomerular filtration rate (eGFR), water district, and BMI in the full model. Water district was also included in the event that prescribing habits varied within the narrow geography of six water districts within the Ohio River Valley; the water districts definitely varied substantially in their historic exposures to PFOA (Steenland et al., 2009a).

The statistical analysis plans for probenecid and cholestyramine were similar, but the analyses were conducted separately. We exponentiated the coefficients from the regression models to calculate the ratio of geometric mean of serum PFAS and the 95 % confidence interval in the probenecid or cholestyramine users, relative to their respective comparison groups, i.e., the comparison population not taking these pharmaceuticals. The power to detect one standard deviation (SD) difference in log-transformed PFAS between the case and control populations was more than 95 % for the probenecid analysis and more than

99 % for the cholestyramine analysis (e.g., for log-transformed PFOS, one SD is 0.83 for probenecid analysis and 0.76 for cholestyramine analysis). All statistical analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

2.4. Sensitivity analysis

To address the possibility of over-adjustment, we performed sensitivity analyses for the same comparisons without adjustment for eGFR, and also without adjustment for water district. We also carried out a 1:3 case-control comparison matched on gender and birth year, in order to reassure that the age adjustment had been complete in the regression analysis.

3. Results

3.1. Probenecid and PFAS

Demographic data are presented for the probenecid comparisons in Table 1. Probenecid users (mean age 64.1) were substantially older than the entire adult population with serum PFAS concentrations (45.3). The male predominant 3:1 gender ratio was also expected because the usual indication, gout, is more common in older males, as was the lower eGFR in older individuals with indications for using a uricosuric medication.

Probenecid users had higher geometric mean PFAS than nonusers (Table 2) in the unadjusted comparisons, and PFAS levels were previously known to be higher for males and to rise with age in this population (Frisbee et al., 2009). However, none of the probenecid-related differences approached statistical significance in a multivariable adjusted model which included age, gender, BMI, water district, and eGFR. A sensitivity analysis showed that this finding was not due to overadjustment for eGFR, with geometric mean ratio of 1.23 (C.I. 0.85−1.78) for PFOA (adjusted for age, gender, BMI, and water district), and still smaller mean ratios were noted for other analytes.

3.2. Cholestyramine and PFAS

Table 3 provides demographic data for the cholestyramine comparisons. The population of medication takers (mean age 54.6) is again older, as expected. The male predominance seen for probenecid does not apply to cholestyramine, also as expected.

In contrast to findings for probenecid, cholestyramine users had unequivocally different (lower) PFAS serum concentrations than nonusers (Table 4) in the multivariable adjusted models. This included the striking difference for PFOS (PFOS serum concentration 0.07 of the serum concentrations in non-users 95 %, C.I. 0.05−0.08. p = 0.00), a finding consistent with a previously reported results of short-term trial by Genuis et al. (2010). Only PFOA results failed to be highly significant in the unadjusted models, and they were significant following adjustment (0.55 lower, 95 % C.I. 0.41−0.74, p = 0.00). Sensitivity testing without eGFR made no difference to the cholestyramine findings. Comparisons with a 3:1 case-control comparison matched on age and sex revealed identical findings, except for wider confidence intervals (and therefore even less apparent differences for probenecid comparisons), reflecting the smaller reference population size. This similarity is expected since cases and controls came from the same population.

Table 1
Baseline characteristics of the study population*.

Characteristics	Whole cohort (n = 56,554)	Probenecid users (n = 23)
Age (years)	45.30 ± 16.28	64.15 ± 11.28
Female (%)	29,675 (52.47)	6 (26.09)
Body mass index (kg/m^2)	28.67 ± 7.65	30.49 ± 3.98
Estimated glomerular filtration rate[a]	79.44 ± 18.38	59.29 ± 19.59
Serum uric acid (mg/dL)	5.58 ± 1.55	6.70 ± 1.57
PFHxS (C6s, ng/mL)	2.97 ± 2.27	3.88 ± 2.67
PFOA (C8, ng/mL)	32.91 ± 3.51	90.47 ± 5.02
PFOS (C8s, ng/mL)	19.09 ± 2.02	28.24 ± 2.25
PFNA (C9, ng/mL)	1.36 ± 1.65	1.53 ± 1.85

* Data presented are number (percentages) or mean values ± standard deviation (SD), as appropriate for the variable.
[a] ml/min/1.73 m^2.

Table 2
Association between Probenecid use and PFAS levels.

	Sample size	Geometric mean (SD)	Unadjusted geometric mean ratio (95 % CI)	Multivariable adjusted* geometric mean ratio (95% CI)
PFHxS				
Probenecid non-users	56152	2.97 (2.27)	1 (referent)	1 (referent)
Probenecid users	23	3.88 (2.67)	1.30 (0.94−1.83)	1.06 (0.76−1.47)
p-value			0.11	0.74
PFOA				
Probenecid non-users	56152	32.90 (3.51)	1 (referent)	1 (referent)
Probenecid users	23	90.47 (5.02)	2.75 (1.65−4.59)	1.23 (0.85−1.78)
p-value			0.00	0.28
PFOS				
Probenecid non-users	56152	19.08 (2.02)	1 (referent)	1 (referent)
Probenecid users	23	28.24 (2.24)	1.48 (1.11−1.97)	1.13 (0.85−1.50)
p-value			0.01	0.39
PFNA				
Probenecid non-users	56152	1.36 (1.65)	1 (referent)	1 (referent)
Probenecid users	23	1.53 (1.85)	1.12 (0.92−1.38)	1.02 (0.83−1.26)
p-value			0.25	0.84

* Adjusted for age, gender, body mass index, water district, and estimated glomerular filtration rate.

Table 3
Baseline characteristics of the study population*.

Characteristics	Whole cohort (n = 56,554)	Cholestyramine users (n = 36)
Age (years)	45.30 ± 16.28	54.68 ± 15.33
Female (%)	29.67 (52.47)	23 (63.89)
Body mass index (kg/m^2)	28.67 ± 7.65	28.33 ± 6.24
Estimated glomerular filtration rate[a]	79.44 ± 18.38	72.96 ± 17.67
Serum uric acid (mg/dL)	5.58 ± 1.55	5.85 ± 1.64
PFHxS (C6s, ng/mL)	2.97 ± 2.27	1.12 ± 2.89
PFOA (C8, ng/mL)	32.91 ± 3.51	23.76 ± 3.70
PFOS (C8s, ng/mL)	19.09 ± 2.02	1.25 ± 5.24
PFNA (C9, ng/mL)	1.36 ± 1.65	0.51 ± 2.03

* Data presented are number (percentages) or mean values ± standard deviation (SD), as appropriate for the variable.
[a] ml/min/1.73 m^2.

4. Discussion

Our study confirms the strong effect of cholestyramine and does not demonstrate a clear effect of OAT inhibition by probenecid on serum PFAS concentrations in a community setting. The negative result for probenecid implies little effect or no effect of OAT1 and OAT3 inhibition on PFAS excretion kinetics. The small population taking probenecid does not allow us to confidently choose between these possibilities. This weak or nil association is physiologically consistent with the observation that PFAS excretion, which is facilitated in other species by OAT, is inefficient in humans (Kudo and Kawashima, 2003) and likely

Table 4
Association between Cholestyramine use and PFAS levels.

	Sample size	Geometric mean (SD)	Unadjusted geometric mean ratio (95 % CI)	Multivariable adjusted* geometric mean ratio (95% CI)
PFHxS				
Cholestyramine non-users	56139	2.97 (2.27)	1 (referent)	1 (referent)
Cholestyramine users	36	1.25 (2.89)	0.38 (0.29−0.49)	0.43 (0.33−0.56)
p-value			0.00	0.00
PFOA				
Cholestyramine non-users	56139	32.92 (3.51)	1 (referent)	1 (referent)
Cholestyramine users	36	23.76 (3.70)	0.723 (0.48−1.09)	0.55 (0.41−0.74)
p-value			0.12	0.00
PFOS				
Cholestyramine non-users	56139	19.12 (2.02)	1 (referent)	1 (referent)
Cholestyramine users	36	1.26 (5.24)	0.07 (0.05−0.08)	0.07 (0.05−0.08)
p-value			0.00	0.00
PFNA				
Cholestyramine non-users	56139	1.36 (1.65)	1 (referent)	1 (referent)
Cholestyramine users	36	0.51 (2.03)	0.38 (0.32−0.44)	0.39 (0.33−0.46)
p-value			0.00	0.00

* Adjusted for age, gender, body mass index, water district, and estimated glomerular filtration.

dominated by reabsorption kinetics in the healthy kidney (Fenton et al., 2020). It is also consistent with recent findings that inherited allelic OAT variants do not materially affect PFAS serum concentrations (Ingelido et al., 2018). It is further consistent with recent findings that moderate to severe renal failure is associated with increased serum PFAS excretion (Jain and Ducatman, 2019c), especially in the presence of albuminuria (Jain and Ducatman, 2019b). If reabsorption dominates human renal PFAS kinetics as hypothesized, then albuminuria and advancing renal failure are expected to lead to lower serum PFAS whereas inhibition of OAT might have little effect on serum PFAS.

In contrast to the nil or equivocal findings with the OAT inhibitor probenecid, participants taking the anion exchange resin cholestyramine experienced definitively reduced PFAS serum concentrations in a community setting. Our findings provide practical confirmation at a population level for previous case reports that this fecal excretory route for PFAS is greatly enhanced by taking cholestyramine (Genuis et al., 2010, 2013) especially for PFOS. The effect in our data is that the relative potency for decrease in PFAS in cholestyramine takers is PFOS>>PFNA > PFHxS > PFOA. This relative degree of implied effectiveness might be different in populations with different initial and ongoing PFAS exposures. The cholestyramine data are also consistent with the observation that gastrointestinal kinetics contribute to inter-individual uncertainties for predicting measures of serum PFAS (Fabrega et al., 2014).

PFAS exposures have been previously shown to be independently associated with significant increases in serum uric acid in this community population (Steenland et al., 2010) and in other populations where serum PFAS has been measured (Geiger et al., 2013; Gleason et al., 2015; Kataria et al., 2015; Scinicariello et al., 2020; Shankar et al., 2011; Zeng et al., 2019) and in a PFOA-exposed workforce (Costa et al., 2009). "Confounding by indication" therefore deserves discussion because gout is the common indication for probenecid. However, to the degree that such indication bias may exist for probenecid per se, it is *a priori* more likely to lead to higher serum PFAS, given that higher uric acid is associated with higher serum PFAS, whereas our analysis found no association or an equivocal association with probenecid. A secondary mechanistic consideration for such a bias is via the effect of gout upon kidney function. However, our data show that adjustment for eGFR does not affect the findings, and it has been independently shown that the PFAS-uric acid association is not due to confounding by renal disease (Jain and Ducatman, 2019a). If these potential sources of bias are relevant to our findings, they would tend to overstate an association that is already equivocal or nil in our data, reinforcing the inference that pharmacologic inhibition of OAT1 and 3 has small or no effect on serum PFAS.

Sources of bias related to cholestyramine can also be considered in a community setting. Cholestyramine is generally mixed into and taken with a liquid, often water. Although we have no data to address water intake, this medication requirement could have increased PFOA intake by inhabitants of the contaminated water districts who participated in our study, especially in the case of cholestyramine users. If this theoretical source of bias is important, the data may understate the excretory effect of cholestyramine, especially upon PFOA, which was in the drinking water. Further, an historic indication for cholestyramine is elevated total and LDL cholesterol, which are also associated with higher PFOA and PFOS serum concentrations in multiple studies including in this population (Steenland et al., 2009a, 2009b). If confounding by indication does influence our cholestyramine findings, then the excretory effect of cholestyramine may be slightly stronger than the data indicate, especially for PFOA. Finally, it is worth considering if the strong negative impact of cholestyramine on serum PFAS shown in these data, notably on PFOS, could be a source of bias if not adjusted for in other previously reported associations such as to lipid outcomes. However, the prevalence of use is very low (36/56175) in this population, and some studies of PFAS associations with lipids have excluded subjects taking lipid lowering agents, including in this population (Fitz-Simon et al., 2013; Steenland et al., 2009b).

An obvious study weakness is its prevalence survey design. Prevalence studies have important limitations as regards causation. However, the findings are highly consistent with two short-term demonstrations of cholestyramine used as an excretory agent of PFAS in an individual or small group (Genuis et al., 2010, 2013) as well as with toxicologic data concerning the effects of cholestyramine on PFAS kinetics (Jandacek and Tso, 2007; Johnson et al., 1984; Lau et al., 2007) and with historic human data concerning cholestyramine treatment of other anionic contaminants (Cohn et al., 1978). The data in this cross sectional study cannot address the time course required for cholestyramine to reduce PFAS. The data also do not address permanence of cholestyramine effects on serum PFAS following cessation of medication. Because PFAS are known to be stored in a variety of tissues other than serum, there is possibility that some "bounce-back" could occur following the cessation of treatment due to tissue compartment re-equilibration.

Our data provide evidence that cholestyramine enhances PFAS excretion in a community setting, especially for PFOS. This information is potentially interesting to exposed workers and inhabitants of communities with known PFAS water contamination, as there are a growing list of human health effects associated with PFAS exposure and parallel toxicology findings of concern (Fenton et al., 2020; Hagstrom et al., 2021). Importantly, it is possible but not proved by these data that addressing the levels of a circulating toxicant risk factor with a pharmaceutical agent will necessarily also decrease the health risk of exposure. We do not know if and to what degree alterations in liver metabolism and other changes following from PFAS exposure are reversible, although modeled exposure studies in this same population do suggest that the lipid associations may respond to declining serum PFAS (Fitz-Simon et al., 2013). Access to available means such as pharmaceutical agents to decrease serum PFAS concentrations is a repeated inquiry posed to care givers by members of exposure populations. We are providing data that excretion can be enhanced by cholestyramine, but the cross-sectional data do not directly address the impact on the burden in other tissues. More fundamentally, the data cannot provide evidence that increasing the speed of excretion

otherwise improves the health of those exposed to PFAS. That conversation initiated by individuals known or inferred to have heavy internal PFAS contamination can be guided by evidence that excretion can be enhanced, especially for PFOS, but is not yet informed by clinical trial evidence of the balance of risks and benefits for medications such as cholestyramine that appear to decrease human biological PFAS burdens.

5. Conclusion

We conclude that bile acid sequestrant cholestyramine prescribed for reasons that did not consider PFAS can nevertheless assist in the more rapid excretion of PFAS in community settings, especially PFOS and, to a lesser degree, other PFAS including PFNA, PFHxS, and PFOA. Using the same method, we sought but did not find clear-cut evidence that probenecid, an inhibitor of organic anion transporters (OAT), affects PFAS clearance. The cholestyramine results provide confirmation that gastrointestinal kinetics can be important to inter-individual differences in serum PFAS concentrations, especially for long-chain sulfonate compounds. The probenecid results support the understanding that reabsorption plays a dominant kinetic role for PFAS in the healthy kidney.

Author contributions

Alan Ducatman proposed the study and the original study design, and was the principal author. Tony Fletcher modified the study design including the statistical approach and performed the statistical comparisons. Michael Luster assisted with the interpretation of the physiology and the preparation of the paper. All authors have reviewed the submitted manuscript.

Declaration of Competing Interest

Dr. Fletcher has served as paid consultant to plaintiff and defendant law firms conducting litigation involving PFAS. Dr. Ducatman has provided testimony assisting attorneys representing community populations to obtain settlement funding for population PFAS and related medical monitoring testing. Dr. Luster served as a contractor for the C8 Science Panel providing scientific expertise. The work and conclusions are independent of any party to a lawsuit.

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The authors of this manuscript declare that their ability to design, conduct, interpret, or publish research was unimpeded by and fully independent of the court and/or settling parties.

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