Interoffice Correspondence 3M COMPANY

Subject: Fluorocarbons in Human Blood Plasma

August 20, 1975

CONFIDENTIAL

TO: L. C. KROGH - COMMERICAL CHEMICAL DIVISION - 223-6SE
J. D. LAZERTE - COMMERCIAL CHEMICAL DIVISION - 236-1
R. A. NEWMARK - CENTRAL RESEARCH - 201-2W
J. A. PENDERGRASS - MEDICAL DEPARTMENT - 220-2E

FROM: G. H. CRAWFORD - PHOTOGRAPHIC PRODUCTS - 209-1S

Record of a Telephone Conversation - August 14, 1975

Person calling - Dr. William Guy
College of Medicine
University of Florida
Gainesville, Florida

Dr. Guy called again, following up on the subject (vide my earlier memo) to see if we had any further ideas as to possible sources of the fluorocarbon carboxylic acids found in human blood samples from Texas and New York. I got John Pendergrass on the line and Guy brought in a Dr. Tays (who apparently was involved in the original observation).

The original sampling involved plasma specimens from Albany, New York, Rochester, New York (low natural fluoride in the water) Hillsborough, Texas, Andrews, Texas, and Corpus Christi, Texas (high natural fluoride). There was no measurable difference by region (10^{-6} molar F^-). F^{19} NMR studies run by Prof. Wallace Brey (Dept. of Chem., U. of F.) indicate that the fluorine is organic and the suspected species is fluorocarbon carboxylic acid with a C_6 or C_7 fluoroalkyl group. Dr. Brey suspects a branched end on the chain, e.g. perfluoro t-butyl.

The discussion involved Dr. Guy's speculative questions as to where such a "universal" presence of such compounds in human blood could come from. (The compounds are not present in laboratory animals.) These included:

1. Biosynthesis from inorganic F^-.

2. Biosynthesis from aerosol freons (but they don't find chlorine).

Exhibit
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State of Minnesota v. 3M Co.,
Court File No. 27-CV-10-28862

3. Teflon cookware.

4. "Scotchgarded" fabrics.

Somewhere he got the information that 3M's fluorocarbon carboxylic acids are used as surfactants and wanted to know if they were present in "Scotchgard" or other items in general use by the public. We plead ignorance but advised him that "Scotchgard" was a polymeric material not a F.C. acid.

Apparently an earlier ('59-'60) study turned up similar quantities of F^- in human plasma (not necessarily FC derived); this would presumably antedate the increased use of either "Scotchgard" or "Teflon" cookware.

They have done experiments involving water boiled in Teflon cookware with negative results.

We suggested obtaining plasma specimens from uncivilized areas, e.g. New Guinea where they don't use too much "Teflon" cookware or "Scotchgard".

Of all the unlikely explanations above, the <u>least</u> unlikely is residual FC 143 (or whatever) we sell to DuPont to polymerize TFE in Teflon cookware. This is still pretty far-fetched. This was <u>not</u> (I hasten to say) suggested to Dr. Guy.

We adopted a position of scientific curiosity and desire to assist in any way possible and suggested that our own analytical people might be able to clarify Dr. Brey's NMR findings (I know Wallace Brey from way back. He is highly respected, conservative and not given to frivolous speculations).

After we hung up I called CRL Analytical, talked to John McBrady and Richard Newmark. It turns out that Newmark is acquainted with Brey and has, in fact, published in a NMR journal edited by Brey.

My recommendation (with J.P.'s concurrence) is to get Richard in touch with Brey, obtain spectra for his own interpretation perhaps samples to run on our equipment, etc. in other words, keep scientists talking to scientists in the spirit of cooperative scientific inquiry.

On the <u>positive</u> side - if it is confirmed to our satisfaction that everybody is going around with fluorocarbon surfactants in their bloodstreams with no apparent ill-effects, are there some medical possibilities that would bear looking into? We

know that fluorocarbons are good oxygen carriers (but this is straight FC-75, not dissolved FC 143). Can fluorocarbon surfactants improve the hemodynamics, wetting and capillary permeation of blood in cases of atherosclerosis, kidney blockage, senility and the like? Can hemolysis, platelet destruction and other blood damage during hemodialysis and cardiovascular surgical procedures be reduced by fluorocarbon surfactants? This is speculation (but not completely wild). I would like to suggest that we consider some animal experiments to see just how much of these materials can, in fact, be tolerated in the bloodstream - both from a defensive point of view and for the above (to me) intriguing reasons. What do you think, John?

GHC/lr

Interoffice Correspondence 3M

CONFIDENTIAL

Subject: Meeting Minutes - Review of Animal Studies

dc: R.J. Davis - 220-12E
J.D. LaZerte - 236-1
L.J. Magill - 223-6SE
A.L. Rosenthal - 230-3
T.J. Scheuerman - 220-12E
F.A. Ubel - 220-2E

May 17, 1978

THOSE PRESENT:

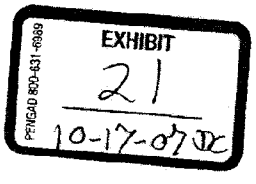
M. T. CASE	218-2
J. E. LONG	220-2E
R. A. NELSON	218-3
R. E. OBER	218-2
R. A. PROKOP	236-3B

Those present met on April 28, 1978 to discuss results of the 90 day animal studies carried out at I.R.D.C. The dosing phase of studies on rats using FC-95, FM-3422 and FC-143 have been completed. Dosing of the monkeys on FC-143 is complete while the FM-3422 and FC-95 monkey dosing will be completed in May. An up-to-date status summary of all studies was supplied by J. E. Long and is attached to these minutes. A complete report from I.R.D.C., including histopathological data is due in June or July for the rat studies and later in the fall for the monkey experiments.

After a very brief discussion of the most recent results from the animal studies, M. T. Case, J. E. Long, R. A. Nelson and R. E. Ober agreed that FC-95, FM-3422 and FC-143 should be regarded as toxic although the degree of toxicity was left undefined.

R. E. Ober inquired as to the types and amounts of impurities present in FC-95, FM-3422 and FC-143. Some impurities, if sufficiently toxic, could cause erroneous conclusion from the animal studies. During the discussion, it was pointed out that FC-95 has been identified in the blood of rats which were fed FM-3422. The question arose as to whether FC-95 might be an impurity in FM-3422. The answer was not known. R. A. Prokop agreed to supply the committee with all available information on impurities present in FC-95, FM-3422 and FC-143.

RECEIVED
MAY 22 1978
R. J. DAVIS



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R. E. Ober raised the question as to which compounds related to FM-3422 and FC-95 would cause greatest worker exposure. Because of the many products related to these compounds, no definite answer could be given. However, because of the large volumes involved, it is believed that FM-3422 itself and FC-807 would contribute most to exposure. It was agreed that Industrial Hygiene should spend more time in identifying the intensity of employee exposure related to FM-3422 and FC-95.

A discussion then took place on analytical methods for determining FC-95 in serum and tissue. Due to the (high?) toxicity of FC-95, it would be advisable to sample the blood of workers exposed to FC-95 or related compounds in order to determine $C_8F_{17}SO_3^-$ levels. The analytical method which has been developed for FC-95 is claimed to be sensitive down to a level of 0.5 ppm, however supporting data is lacking. Also, if the method is satisfactory down to a level of 0.5 ppm, it should be adequately sensitive for determining the amount of FC-95 in serum and liver from I.R.D.C. rat studies. However, supporting data are not available. It was concluded that R.E. Ober and R.A. Prokop should meet with Central Research Analytical personnel and analyze the data on determination of FC-95 in serum and tissue in order to assess the reliability of the method.

A discussion then took place on having the capability of analyzing for FC-95 in serum and liver of animals before starting the two year animal studies. R. A. Nelson and J. E. Long felt that FC-95 should be identified as being present before proceeding with the studies since it is possible that a metabolite of FC-95 might be responsible for toxic effects rather than FC-95 itself. R. E. Ober regarded such studies as supplemental. It was agreed that analytical work on FC-95 in the serum and liver of rats should be completed as rapidly as possible.

It was questioned why FC-95, FM-3422 and FC-143 were chosen for the animal studies. FC-143 and FC-95 have been found in the employees. FM-3422 is an intermediate which goes into a variety of products. R. E. Ober suggested that a two year study on FM-3422 would give information on the effects of FM-3422, and possible metabolites. It was agreed that this suggestion should be given further consideration.

R.A. Nelson stated that I.R.D.C. is now saving monkeys for the two year animal studies. If we are to use these animals, we must purchase them now at a cost of $61,280 and pay $7800 per month to maintain them. I.R.D.C. wants an answer by May 1, 1978. If we do not purchase and maintain these monkeys, none may be available later for the animal studies. However, since it has not yet been decided with certainty that monkeys will be used in the two year studies, those present recommended not purchasing the monkeys at this time.

Those present again considered the available toxicity data on FC-95, FM-3422 and FC-143. It was pointed out that male rats fed FC-143 at the 1000 ppm level had about 50 ppm FC-143 in their blood and that one Chemolite worker had a level of 53 ppm in his blood. At the 1000 ppm feeding level, male rats had liver discoloration (females had none). It was concluded that the liver discoloration in rats associated with a blood level of 50 ppm suggests a possible human health problem for individuals who have this level (or above) in their blood for long periods of time. Those present also concluded the following:

As concluded previously by the full committee, available data in man indicates that no substantial risk exists under the Toxic Substances Control Act. However, those present urgently recommended that all reasonable steps be taken immediately to reduce exposure of employees to these compounds.

It was also agreed that:

1. R. E. Ober will make proposals on metabolic studies and make a presentation to the committee on such studies.

2. R. A. Prokop and J. E. Long will make certain that Riker has all previous analytical and toxicological data involving fluorochemicals in blood.

3. A protocol should be written for sampling of employees blood.

4. It will be necessary to have a method for analyzing FC-95, FM-3422 and FC-143 in the food used in animal studies.

Submitted by: R. A. Prokop

R. A. Prokop

RAP:df

attachment

3MA10067061

Phil Raths

THIRD DRAFT
FC-143 Decatur
Standby Press Statement
April 15, 1981
Lowell Ludford (3-6154)

CONFIDENTIAL

APR 27 1981

HOLD FOR RELEASE

As a precautionary measure, approximately 25 women of childbearing potential have received job reassignments at the 3M Decatur plant this week so they will not be exposed to a type of fluorochemical that can cause birth defects in rats.

Preliminary results of a recent 3M toxicology study showed that three related fluorochemicals affected eye development in the fetuses of rats, according to Phil Raths, manager of the Chemical Resources Division plant.

The study currently is being repeated on rats and other species to clarify the initial finding, Raths explained. Until these results are known and evaluated, he said the 3M Medical Department felt it was prudent to recommend this action.

The women are being reassigned to jobs in the adjacent 3M Film and Allied Products Division plant. This is being done in an equitable way that will protect their present seniority status, benefits and pay, Raths pointed out.

-more-

"While we are not aware of any adverse health effects on our men and women employees," Rathe said, "we are transferring these women as a precautionary step pending further tests."

The three fluorochemicals are used in the manufacture of specialty chemicals by 3M and various other industrial firms.

-30-

For further information contact:

Lowell Ludford
3M Public Relations Department
3M Company, 3M Center
St. Paul, Minnesota 55133
Telephone: (612) 733-6154

Internal Correspondence

CONFIDENTIAL

F. D. GRIFFITH - MEDICAL - 220-2E-02
L. F. LUDFORD - CORPORATE INFORMATION - 225-5N-04
W. C. MC CORMICK - MEDICAL - 220-2E-02
To: D. E. ROACH, M.D. - MEDICAL - 220-2E-02

From: F. A. UBEL, M.D. - (3-5181) -MEDICAL - 220-2E-02

Subject: Phone conversation from Dr. McKusick - DuPont - 12/14/81

Date: December 14, 1981

3M

"This is what we are going to tell our employees and we are going to start telling them at 1:00 o'clock on Wednesday, December 16."

"On April 1 we advised you that 3M in a preliminary study had observed birth defects in the eyes of unborn rats when C-8, also known as FC-143 or ammonium perfluoro-octanoate, was fed to pregnant female rats. Based upon those findings, we decided it was necessary to exclude female employees of childbearing capability from areas where there is potential for exposure to C-8. We indicated further studies by DuPont and 3M would be undertaken promptly to determine the significance, if any, of the findings as they might relate to employee exposure. We would like to share with you the results of these studies that we have today."

"Thus far, based on our review of the results of the further studies, it does not seem that the observed effects on the eyes of the unborn rats were due to C-8. Also, in the new studies, rat pups delivered by C-8 exposed females showed no eye defects. Rather, it is believed that in the original studies, 3M's technique for the very difficult job of preparing the fetal eye tissue for microscopic examination resulted in the alterations noted".

"3M has another toxicological test underway that will be completed in the first quarter of 1982. At that time we expect to have all the data available and will assess if it is necessary to continue excluding female employees of childbearing capability from areas of potential exposure. Until final determination is made, we continue to advise that employees defer giving blood until the blood level of C-8 returns to background levels. We also advise that females who have an organic flouride level above background should consult with their personal physician prior to contemplating pregnancy. We will provide all information we have on C-8 to employees' personal physicians".

FAU:mam

Exhibit 1266
State of Minnesota v. 3M Co., Court File No. 27-CV-10-28862

UNIVERSITY OF MINNESOTA
TWIN CITIES

Division of Environmental
and Occupational Health
School of Public Health
Box 197 Mayo
420 Delaware Street S.E.
Minneapolis, Minnesota 55455

April 6, 1989

Larry R. Zobel, M.D.
Staff Physician
3M Center
Medical Department
220-2E-02
St. Paul, MN 55144-1000

Dear Larry;

Enclosed please find the tables containing the results of the comparison with the Minnesota population. As I mentioned on the telephone, these must be interpreted cautiously because of the uncertainty regarding the Minnesota rates prior to 1959. Deaths among the study cohort occurred in 41 states; therefore, the U.S. rates may be more appropriate.

As you will see from the tables, the results are similar to those presented previously which used the U.S. rates. The only consistent finding between the two comparisons is for prostatic cancer which we addressed in our initial report. For cancer of the digestive organs and peritoneum there was a statistically significant excess (SMR=176, 95% C.L.=1.09, 2.69) for the entire cohort. However, this was not found among the Clinical Division employees. Furthermore, no single site within the gastrointestinal tract was elevated suggesting that this was probably due to chance. Also worth noting is the fact that these are not sites typically associated with chemical exposures.

If you have any questions please feel free to call me at 626-4810.

Yours sincerely,

Jack

Jack S. Mandel, Ph.D.
Associate Professor

TABLE 5. OBSERVED AND EXPECTED DEATHS BY CAUSE, STANDARDIZED MORTALITY RATIO (SMR), 95 PERCENT CONFIDENCE LIMITS AND CHI SQUARE VALUES, MALES, CHEMICAL DIVISION (COMPARED TO MINNESOTA DEATH RATES)

	OBSERVED	EXPECTED	OBS/EXP	LL	UL	CHISQ
0ALL CAUSES OF DEATH	100	89.79	1.11	0.91	1.35	1.05
1ALL MALIGNANT NEOPLASMS	23	16.39	1.40	0.89	2.11	2.27
2ALL INFECTIVE AND PARASITIC DISEASE	0	0.68	0.00	0.00	5.41	0.05
9ALL TUBERCULOSIS	0	0.20	0.00	0.00	18.48	0.46
140CANCER OF BUCCAL CAVITY AND PHARYNX	0	0.52	0.00	0.00	7.05	0.00
149CANCER OF DIGESTIVE ORGANS AND PERITONEUM (1925-APPROXIMATE)	6	4.23	1.42	0.52	3.09	0.38
150CANCER OF ESOPHAGUS (1925-APPROXIMATE)	1	0.40	2.49	0.03	13.83	0.02
151CANCER OF STOMACH	0	0.71	0.00	0.00	5.16	0.06
153CANCER OF LARGE INTESTINE (1925-APPROXIMATE)	3	1.40	2.14	0.43	6.25	0.86
154CANCER OF RECTUM (1925-APPROXIMATE)	0	0.45	0.00	0.00	8.23	0.01
155ALL CANCER OF LIVER (1925-APPROXIMATE) 1970 PLUS-PRIMARY ONLY	0	0.27	0.00	0.00	13.68	0.20
157CANCER OF PANCREAS (1925-APPROXIMATE)	2	0.88	2.28	0.26	8.23	0.44
160CANCER OF RESPIRATORY SYSTEM (1925-APPROXIMATE)	5	4.66	1.07	0.35	2.50	0.01
161CANCER OF LARYNX (1925-,1930- APPROXIMATE)	1	0.19	5.16	0.07	28.73	0.48
162ALL CANCER OF LUNG-PRIMARY AND SECONDARY (1925-,1930-APPROXIMATE)	4	4.41	0.91	0.24	2.32	0.00
170CANCER OF BONE (1925-,1930,1945-APPROXIMATE)	0	0.12	0.00	0.00	29.60	1.14
172CANCER OF SKIN	1	0.40	2.49	0.03	13.85	0.02
185CANCER OF PROSTATE (1925-APPROXIMATE)	4	0.51	7.80	2.10	19.96	17.39
186CANCER OF TESTIS (OTHER GENITAL ORGANS-1925-49) (1925-,1930-APPROXIMA	1	0.38	2.61	0.03	14.55	0.04
188CANCER OF BLADDER (1925-APPROXIMATE)	0	0.26	0.00	0.00	14.08	0.22
189CANCER OF KIDNEY (1925-APPROXIMATE)	0	0.53	0.00	0.00	6.91	0.00
190CANCER OF EYE (1950-1969 ONLY)	0	0.02	0.00	0.00	181.46	11.39
191ANCER OF BRAIN AND OTHER CENTRAL NERVOUS SYSTEM (1925-APPROXIMATE)	1	0.87	1.15	0.02	6.42	0.15
193CANCER OF THYROID (1950-1969 ONLY)	0	0.04	0.00	0.00	93.44	5.41
200LYMPHOSARCOMA AND RETICULOSARCOMA (1950-1969 ONLY)	0	0.52	0.00	0.00	7.04	0.00
201HODGKIN'S DISEASE (1940-,1945-APPROXIMATE)	0	0.55	0.00	0.00	6.62	0.01

204LEUKEMIA AND ALEUKEMIA	0	2.63	0.00	0.00	1.40	1.72
208CANCER OF OTHER LYMPHATIC TISSUE (1950-1969 ONLY)	3	1.42	2.11	0.42	6.15	0.81
209ALL LYMPHOPOIETIC CANCER	6	6.69	0.90	0.33	1.95	0.01
210BENIGN NEOPLASMS (PLUS UNSPECIFIED)	0	0.49	0.00	0.00	7.50	0.00
240ALLERGIC,ENDOCRINE,METABOLIC,NUTRITIONAL DISEASES (1950-1969 ONLY)	4	4.74	0.84	0.23	2.16	0.01
250DIABETES MELLITUS	3	3.71	0.81	0.16	2.36	0.01
280ALL DISEASES OF BLOOD AND BLOOD-FORMING ORGANS (1925-,1930-APPROXIMAT	1	0.44	2.28	0.03	12.68	0.01
319MENTAL,PSYCHONEUROTIC, AND PERSONALITY DISORDERS (1950-1969 ONLY)	1	1.86	0.54	0.01	3.00	0.07
320ALL DISEASES OF NERVOUS SYSTEM AND SENSE ORGANS	3	3.21	0.94	0.19	2.73	0.03
390ALL DISEASES OF CIRCULATORY SYSTEM	104	96.73	1.08	0.88	1.30	0.47
393CHRONIC RHEUMATIC HEART DISEASE (1925-APPROXIMATE)	3	2.89	1.04	0.21	3.03	0.05
410ARTERIOSCLEROTIC HEART DISEASE, INCLUDING CHD (1925-APPROXIMATE)	84	72.88	1.15	0.92	1.43	1.55
430ALL VASCULAR LESIONS OF CNS	7	10.58	0.66	0.26	1.36	0.90
460ALL RESPIRATORY DISEASES (1925-,1930-APPROXIMATE)	7	8.68	0.81	0.32	1.66	0.16
480ALL PNEUMONIA (1925-,1930-APPROXIMATE)	3	3.09	0.97	0.20	2.84	0.05
492EMPHYSEMA (1950-,1955 APPROXIMATE)	1	1.95	0.51	0.01	2.86	0.10
493ASTHMA (1925-,1930-APPROXIMATE)	1	0.43	2.34	0.03	13.02	0.01
520ALL DISEASES OF DIGESTIVE SYSTEM	12	11.45	1.05	0.54	1.83	0.00
531ALL GASTRIC AND DUODENAL ULCER	2	1.12	1.78	0.20	6.44	0.13
571CIRRHOSIS OF LIVER	3	6.88	0.44	0.09	1.27	1.66
580ALL DISEASES OF GENITO-URINARY SYSTEM	0	2.16	0.00	0.00	1.70	1.27
582CHRONIC NEPHRITIS	0	0.91	0.00	0.00	4.04	0.18
709ALL DISEASES OF THE SKIN AND CELLULAR TISSUE	0	0.14	0.00	0.00	26.14	0.92
739ALL DISEASES OF THE BONES AND ORGANS OF MOVEMENT	1	0.52	1.94	0.03	10.78	0.00
799SYMPTOMS,SENILITY,AND ILL DEFINED CONDITIONS	1	1.66	0.60	0.01	3.36	0.01
800ALL EXTERNAL CAUSES OF DEATH	46	52.17	0.88	0.65	1.18	0.62
801ALL ACCIDENTS	34	38.27	0.89	0.62	1.24	0.37
810MOTOR VEHICLE ACCIDENTS	27	21.42	1.26	0.83	1.83	1.20
959SUICIDE	9	10.86	0.83	0.38	1.57	0.17
TOTAL RESIDUAL	1	-65.08	-0.02			

CANCER RESIDUAL

13	3.00	4.33

28 March 1999

To: 3M

I resign my position as Environmental Specialist effective 6 April 1999. My resignation is prompted by my profound disappointment in 3M's handling of the environmental risks associated with the manufacture and use of perfluorinated sulfonates (PFOS)(CAS# 29081-56-9) and its precursors, such as ethyl FOSE alcohol (CAS #1691-99-2) and methyl FOSE alcohol (CAS #24448-09-7).

Perfluorooctansefulfonate is the most insidious pollutant since PCB. It is probably more damaging than PCB because it does not degrade, whereas PCB does; it is more toxic to wildlife; and its sink in the environment appears to be biota and not soil and sediment, as is the case with PCB.

I have worked within the system to learn more about this chemical and to make the company aware of the dangers associated with its continued use. But I have continually met roadblocks, delays, and indecision. For weeks on end I have received assurances that my samples would be analyzed soon--never to see results. There are always excuses and little is accomplished. I can illustrate with several examples.

- For more than twenty years 3M's ecotoxicologists have urged the company to allow testing to perform an ecological risk assessment on PFOS and similar chemicals. Since I have been assigned to the problem a year ago, the company has continued its hesitancy.
- Over a period of seven months I made frequent requests that ecological risk consultants be hired to help me plan toxicity testing, environmental sampling, chemical fate studies, and ecological risk procedure. I still have not received authorization even to bring people in to interview.
- I requested, very frequently, over a nine-month period, a sample of chemical to send out for fate property and ecotoxicity testing. Finally I was provided with one that apparently the division had had all along.
- I put together a pioneer risk assessment on PFOS that indicated a greater than 100% probability of harm to sea mammals, based on preliminary data on the concentration of PFOS in menhaden fish meal. The 8e committee told me that they would like to reconsider the assessment after we had a validated value for fishmeal. That analysis was given high priority by the committee. After three months the analysis is still not done--not because there were technical problems, but because management did not actually give the analysis high priority.
- 3M submitted a TSCA 8e last May. There is tremendous concern within EPA, the country, and the world about persistent bioaccumulative chemicals such as PFOS. Just before that submission we found PFOS in the blood of eaglets--eaglets still young enough that their only food consisted of fish caught in remote lakes by their parents. This finding indicates a widespread environmental contamination and food chain transfer and probable bioaccumulation and bio-magnification. This is a very significant finding that the 8e reporting rule was created to collect. 3M chose to

report simply that PFOS had been found in the blood of animals, which is true but omits the most significant information.

- One of our customers, Griffin, has data on some of our chemicals. They developed this data for pesticide registration purposes. I started regularly asking for permission to visit Griffin and view the data last May. Their data can help us plan our studies of similar chemicals. It can also indicate if there is an unforeseen risk to certain biota or via certain exposure pathways. It was ten months before I was allowed to visit Griffin, at which time I did not get to see the data. I have to return another time to see it.
- 3M waited too long to tell customers about the widespread dispersal of PFOS in people and the environment. We knew before May of 1998, yet 3M did not start telling customers until January of 1999. I felt guilty about this and told customers I personally knew earlier. Still, it was not as early as it should have been. I kept waiting for 3M to do its duty, as I was continually assured that it would. Some of the customers have done risk assessments on the PFOS precursor they use. They assume there is not a background in the environment and in wildlife. Since there is a background, their risk assessments are inaccurate. Thus they can make inappropriate business decisions and not realize that their use of PFOS precursors contributes to an aggregate risk.
- 3M continues to make and sell these chemicals, though the company knows of an ecological risk assessment I did that indicates there is a better than 100% probability that perfluorooctansulfonate is biomagnifying in the food chain and harming sea mammals. This chemical is more stable than many rocks. And the chemicals the company is considering for replacement are just as stable and biologically available. The risk assessment I performed was simple, and not worst case. If worst case is used, the probability of harm exceeds 100,000%.

- 3M told those of us working on the fluorochemical project not to write down our thoughts or have email discussions on issues because of how our speculations could be viewed in a legal discovery process. This has stymied intellectual development on the issue, and stifled discussion on the serious ethical implications of decisions.

I have worked to the best of my ability within the system to see that the right actions are taken on behalf of the environment. At almost every step, I have been assured that action will be taken—yet I see slow or no results. I am told the company is concerned, but their actions speak to different concerns than mine. I can no longer participate in the process that 3M has established for the management of PFOS and precursors. For me it is unethical to be concerned with markets, legal defensibility and image over environmental safety.

Sincerely,

Rich Purdy

8/4/24 DRAFT

TO: Carly Griffith, Water Program Director, MCEA

Cc: Akilah Sanders-Reed; Heidi Guenther, MCEA

FROM: Gary L Krueger, PSS

RE: Timeline of 3M actions regarding PFAS releases – Superfund enforcement

Carly, the following is a rough timeline of the actions taken by 3M in response to requests made by MPCA to investigate and undertake cleanup actions regarding releases of PFAS at the Cottage Grove (and Oakdale/Woodbury disposal sites)

- 2002 - 3M informed MPCA of PFOS and PFOA detected in water supply wells at the Cottage Grove facility. MPCA requested 3M collect groundwater samples at Oakdale and Woodbury sites. MPCA also requested 3M conduct facility wide assessment at Cottage Grove to identify potential PFAS disposal sites on facility and any other off-site disposal locations.
- 2003 – PFAS identified at the Oakdale and Woodbury disposal sites in groundwater monitoring wells (also found at Washington County Landfill by MPCA)
- 2004 – 3M completed Cottage Grove facility assessment which identified most likely PFAS disposal areas at facility and other potential off-site disposal locations. MPCA requested 3M conduct preliminary remedial investigation at Cottage Grove. Highest concentrations found in D1, D2 and D9 disposal locations and sediment in East Cove (point at which wastewater from facility flows before discharge to Mississippi River. Additional investigation (2006) conducted to determine extent and magnitude of PFAS releases, Included surface water, sediment and pore water in Mississippi River.
- MPCA proposed Request for Response Action (RFRA)(i.e. Superfund enforcement order) and requested MPCA Board approve RFRA at April 2007 Board Meeting. Main dispute between 3M and MPCA was determination PFOS and PFOA were “hazardous substances” under MERLA (Minn. Stat 115B) MPCA Board deferred issuance and directed MPCA staff and 3M negotiate Consent Order.
- MPCA Board approved Consent Order on May 22, 2007. (2007 SACO) Included language regarding hazardous substance determine that was “agree to disagree” between MPCA and 3M, and was in best interest to move forward. Consent Order also included language regarding cleanup required and drinking water response that was more restrictive than RFRA, and provided funding towards MPCA PFAS research activities and cleanup actions at the Washington County Landfill.
- 3M completed Feasibility Studies at each of the 3M PFAS disposal sites in compliance with Consent Order. 3M subsequently completed soil/sediment excavation activities at each of the 3 sites, per Consent Order, between 2008 and 2011.
- 3M also completed upgrade/expansion of groundwater control systems at each site by 2012. Building 92 (GAC treatment) treats all of the ground water from the on-site production wells and pump-out wells at Cottage Grove and pump-out water from Woodbury. This pretreatment of all groundwater pumped at both sites before use at plant was requirement of consent order.
- By 2012/2013, 3M completed all response actions as required under the consent order and outlined in the Minnesota Decision Document (MDD)(MPCA version of ROD)

- 3M reimbursed in full, all appropriate MPCA costs incurred as part of oversight activities. These included all costs for residential well sampling, home GAC installations/maintenance, MPCA/MDH staff costs and MPCA contractor costs. Only time when 3M delayed reimbursement was during 2017/18, when NRDA lawsuit/negotiations were very active. MPCA costs for 2017 were covered as part of the $850 million settlement agreement.
- Through 2010's, 3M conducted on-going GW/SW monitoring at each of the sites, as appropriate. At MPCA's request, 3M needed to add/adjust ground water pump-out wells at Cottage Grove and Oakdale. In 2020, at MPCA request, 3M began additional investigations at Oakdale regarding surface water releases via Raleigh Creek. 3M has proposed additional response actions at Oakdale to address these releases.
- 3M has also installed or is in the process of installing additional pump-out wells at the Cottage Grove site to control migration of PFAS contaminated ground water to the Mississippi River. These actions were at request of MPCA based on gw monitoring activities.
- FYI – the consent order included a 45-day review/respond time limit for both the MPCA and 3M. In general, 3M complied with that time deadline, and informed the MPCA if it could not comply with that deadline for submittals. In reality, the MPCA needed more extensions of time in order to fully review 3M submittals.
- Overall, 3M complied with the terms of the 2007 Consent Order regarding PFAS releases from the Cottage Grove, Oakdale and Woodbury sites.
- While not under the Consent Order, the MPCA requested and 3M is conducting additional ground water investigations to determine if there have been impacts to Hastings drinking water supply from PFAS releases at the Cottage Grove site. (October 2023). This may lead to 3M requirements to address Hastings drinking water PFAS issues under the Consent Order.

<u>2018 NRDA Settlement</u>

- February 2018, MPCA and 3M reach settlement agreement regarding NRDA lawsuit. 2018 agreement outlined requirements for funds to address drinking water impacts from PFAS in the East Metro. Terms of settlement left 2007 Consent Order in place. 3M must continue to monitor and conduct any actions necessary to address PFAS releases at each site, and reimburse MPCA for its costs. Once settlement funds are exhausted, 3M must comply with terms of consent order regarding drinking water impacts from PFAS releases from 3M disposal sites. MPCA must address drinking water PFAS impacts from the Washington County Landfill.
- Settlement agreement was settlement to lawsuit filed in 2010. The only superfund "compliance" portion was 3M requirement to fund up to $40 million of "temporary" treatment drinking water systems in East Metro, over the first 5 years of agreement. These included the temporary municipal treatment systems in Oakdale, Woodbury and Cottage Grove, necessary until long term treatment systems are in place. Dispute did arise between 3M and MPCA on what was a temporary system, especially with regards to home treatment systems. Ultimately, through negotiations with Special Master, 3M proposed a 50/50 split on disputed items. MPCA agreed with 3M's proposal for this split. In end, 3M probably paid more than it would have normally owed without 50/50 proposal. 3M paid approximately $30 million dollars under the 5 Year Temporary provision in settlement, which was in addition to the $850 million settlement amount.

Wastewater enforcement

Until this current draft NPDES/stormwater permit, PFAS limits have not been included as part of wastewater discharge limits at the Cottage Grove facility. Until the present, PFAS compounds were not regulated under the CWA. There was a proposed limit for PFOS in the 2011 draft permit, however, that permit was placed on hold due to the NRDA lawsuit. There were however, monitoring requirements for PFAS. These monitoring requirements, including for stormwater, lead to a comprehensive facility wide assessment of surface water impacts at/around the Cottage Grove facility, and thus lead to the development of the PFAS surface water criteria for Pool 2. In the end, the monitoring requirements placed on 3M by the MPCA lead to the current permit requirements.

In reviewing information on MPCA's web page, (What's in my neighborhood), there have been a couple NOVs issued to 3M under the current wastewater permit. These were in 6/2018 and 1/2016, with no penalty listed. I do recall a couple instances of "inadvertent" Fire Foam/retardant discharges, with 3M reporting the instance and collecting the material for disposal.

Hazardous waste enforcement

The one main area of "non-compliance" at the 3M Cottage Grove facility has been in hazardous waste management. As noted on the NPDES permit reissuance web page there have a couple of "smaller" non-compliance issues (APO's), one in 2021($80K) and one in 2024($5K). However, there was a $2.8M stipulation agreement, which outlined some significant hazardous waste management issues (storage, labeling, sampling, etc). These were some major issues, some of which went back to 1996.

According to the MPCA web page, these storage and container management violations, did not result in any air permit violations at 3M's hazardous waste incinerator, located at the Cottage Grove facility. However, 3M has discontinued use of the incinerator, and is in the process of decommissioning the incinerator. Additional site investigations are underway under 3M's RCRA permit closure requirements. It is likely additional remediation corrective measures will be conducted under those same permit closure requirements.

Conclusion

Overall, 3M complied with terms of the 2007 Consent Order in regards to implementing MPCA determined response actions at each of the 3M PFAS disposal sites. 3M continues to conduct monitoring activities at each site and is implementing additional investigation/response actions when directed by the MPCA. 3M has reimbursed MPCA for agency costs under the 2007 SACO. 3M also provide the full $850 million to the MPCA under terms of the 2018 Settlement Agreement. Once the settlement dollars are exhausted, 3M must comply with terms of the 2007 SACO in regards to drinking water impacts from the 3M PFAS disposal sites. This also means the MPCA must comply with those same terms for PFAS releases from the Washington Co. Landfill.

While there were no PFAS limits in the current expired NPDES/stormwater permits, PFAS monitoring requirements under those permits lead to development of water quality criteria for Pool 2 and the proposed PFAS limits in the current draft permit. Also, according to MPCA staff, PFAS discharge levels have decreased since monitoring began in early 2000's.

There have been significant hazardous waste management violations, with the 2021 Stipulation Agreement. It does appear that 3M has completed the terms/requirements of that agreement.

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Accumulation of perfluoroalkyl substances in human tissues

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ABSTRACT

Perfluoroalkyl substances (PFASs) are environmental pollutants with an important bioaccumulation potential. However, their metabolism and distribution in humans are not well studied. In this study, the concentrations of 21 PFASs were analyzed in 99 samples of autopsy tissues (brain, liver, lung, bone, and kidney) from subjects who had been living in Tarragona (Catalonia, Spain). The samples were analyzed by solvent extraction and online purification by turbulent flow and liquid chromatography coupled to tandem mass spectrometry. The occurrence of PFASs was confirmed in all human tissues. Although PFASs accumulation followed particular trends depending on the specific tissue, some similarities were found. In kidney and lung, perfluorobutanoic acid was the most frequent compound, and at highest concentrations (median values: 263 and 807 ng/g in kidney and lung, respectively). In liver and brain, perfluorohexanoic acid showed the maximum levels (median: 68.3 and 141 ng/g, respectively), while perfluorooctanoic acid was the most contributively in bone (median: 20.9 ng/g). Lung tissues accumulated the highest concentration of PFASs. However, perfluorooctane sulfonic acid and perfluorooctanoic acid were more prevalent in liver and bone, respectively. To the best of our knowledge, the accumulation of different PFASs in samples of various human tissues from the same subjects is here reported for the very first time. The current results may be of high importance for the validation of physiologically based pharmacokinetic models, which are being developed for humans. However, further studies on the distribution of the same compounds in the human body are still required.

1. Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are a large group of surface-active organic compounds. Because of their chemical and thermal stability, as well as their hydrophobic and lipophobic nature, they have been used for over 50 years in a number of industrial and commercial applications (Zhao et al., 2012). PFASs are highly resistant to breakdown. Therefore, they are persistent in the environment, being able to accumulate in living organisms and biomagnified through the trophic web (Loi et al., 2011; Powley et al., 2008). Moreover, there is a growing concern related to their potentially harmful effects on human health (Vieira et al., 2013). Due to these reasons, the U.S. industry undertook voluntary actions to phase out production of perfluorooctane sulfonic acid (PFOS) between 2000 and 2002, and in 2007 the United States Environmental Protection Agency (US EPA) published the Significant New Use Rules (SNURs) to restrict the production of PFOS and related substances (Lindstrom et al., 2011). Moreover, in 2006, the major PFAS producers committed the Stewardship Program to phase out the global emissions and products containing perfluorooctanoic acid (PFOA) for 2015. Despite these measures, hundreds of other different PFASs are currently being produced and used. Thus, although the production of PFOA is being phased out by the companies participating in the Voluntary Stewardship Program, environmental contamination and human exposure from PFOA and higher homologue chemicals (e.g. PFNA, PFDA, etc.) are anticipated to continue for the foreseeable future due to a number of reasons: its persistence, their formation from precursor compounds, and the potential for continued production by other manufacturers in the U.S. and/or overseas (Lindstrom et al., 2011).

In 2008, the European Food Safety Authority (EFSA, 2008) established a series of Tolerable Daily Intakes (TDIs) values for PFOS and PFOA at 150 and 1500 ng/kg/day, respectively. PFOS was subsequently included as a persistent organic pollutant (POP) under the Stockholm Convention (UNEP 2010). In 2009, the US EPA Office of Water established the provisional health advisory values for PFOS and PFOA at 200 and 400 ng/L, respectively. It must be highlighted that, although TDIs and the water provisional health advisory were calculated in different basis, in both cases short-term exposure was considered as the relevant period of exposure. This was consistent with PFOA and PFOS toxicity data, which in turn rely upon subchronic exposure experimental values. However, long-term exposures must be considered for the accurate assessment of their potential risk on human health, taking into account that their

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presence has been reported in drinking water, ambient air, and food (Domingo et al., 2012a,b; Ericson Jogsten et al., 2012; Ericson et al., 2008, 2009; Post et al., 2009, 2012).

PFASs have been related to different toxicological effects on mammals. In mice, the neonatal exposure to PFOS and PFOA has been linked up to changes in proteins of importance for the neuronal growth and synaptogenesis in the brain developing (Johansson et al., 2009), as well as with neurobehavioral defects and changes in the cholinergic system (Johansson et al., 2008). In addition, perfluorohexanesulphonate (PFHxS) has been related to irreversible neurotoxic effects in neonatal mice, showing a similar behavior to that of other POPs, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and bisphenol A (Viberg et al., 2013). A recent study in human suggested that higher PFOA serum levels might be associated with testicular, kidney, prostate, and ovarian cancers, and non-Hodgkin lymphoma, according to the concentrations of residents in 6 areas with contaminated drinking water supplies (Vieira et al., 2013).

In the human body, the polar hydrophobic nature of fluorine-containing compounds can lead to increased affinity for proteins (Jones et al., 2003; Luebker et al., 2002; Vanden Heuvel et al., 1992; Weiss et al., 2009). A number of PFASs have been detected in human serum, cord blood and breast milk (Domingo et al., 2012a; Ericson et al., 2007; Fromme et al., 2010; Haug et al., 2009a,b; Llorca et al., 2010). As other bioacumulative halogenated contaminants (e.g., polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and PCBs), PFASs can have long persistence in the body. However, they do not tend to accumulate in fat tissue. According to outcomes of animal studies, PFOA and PFOS are mostly excreted through the urine (Cui et al., 2010), but limited observations in humans suggest that only one-fifth of the total body clearance is renal (Harada et al., 2005). The elimination half-life of PFOA in humans was roughly estimated to be 3.5 years, while that of PFOS was approximately 4.8 years (Olsen et al., 2007), according to data from retired workers. Post et al. (2012) recently reviewed studies reporting the elimination half-life values between 2.3 and 3.3 years, following an exposure to contaminated drinking water (Post et al., 2012). Information about sources, environmental fate and toxicokinetics of PFOS and PFOA is largely available, while estimation values in the half-lives of PFBS, PFHxS and PFBA (Chang et al., 2008; Lau et al., 2007). In contrast, data on most of the PFASs currently in use, continues to be very limited. It has been hypothesized that the possible harmful effects associated to PFASs accumulation are of special concern during early stages of life (Maisonet et al., 2012; Post et al., 2012; Schecter et al., 2012). However, their accumulation and distribution in the different human tissues are still poorly understood. The potential accumulation of PFASs with different chain lengths is an issue of great importance for exposure assessment and risk characterization studies. Most current investigations on human accumulation have focused on the occurrence in blood and breast milk, while very few studies have reported levels in other tissues. Kärrman et al. (2009) determined the concentrations of six PFASs in liver samples collected post-mortem in Spain. Mean concentrations of 27 and 1 ng/g of PFOS and PFOA, respectively, were found. In turn, Maestri et al. (2006) found levels of 14 ng/g of PFOS and 3 ng/g of PFOA in a pooled liver samples corresponding to seven subjects from northern Italy, while Olsen et al. (2003) reported mean PFOS and PFOA concentrations of 19 and 47 ng/g, respectively, in 30 subjects from USA. Finally, Pirali et al. (2009) detected PFOA and PFOS in thyroid tissue (median levels: 2 and 5.3 ng/g, respectively), concluding that those compounds are not actively concentrated in the thyroid.

The main objectives of the present study were the following: 1) to optimize and validate an on-line analytical approach based on turbulent flow chromatography coupled to tandem mass spectrometry (TFC-LC-MS/MS) for determining PFASs in various human tissues; 2) to measure the levels of 21 PFASs in these human tissues in order to elucidate their distribution and accumulation in the human body. The method optimized for the tissue analysis was carefully selected to accomplish the minimum sample size requirements and to reduce sample manipulation. The analytical procedure was validated for different kinds of tissues, and applied for the determination of selected compounds in liver, lung, brain, bone, and kidney samples collected post-mortem from 20 subjects. PFASs values were correlated with the concentrations of some heavy metals (unpublished results) in the same tissue samples, as well as with the levels of PCDD/Fs in adipose tissue from 15 of the same individuals (Nadal et al., 2009). To the best of our knowledge, these are the first data reporting the accumulation of a notable number of PFASs in human tissues, as well as comparing the body burden of these pollutants with that of other environmental contaminants (metals and PCDD/Fs).

2. Materials and methods

2.1. Chemicals and standards

Standard solutions were purchased from Wellington Laboratories Inc. (Guelph, ON, Canada). The standard analytes used in this study were: i) PFAC-MXB [98% purity in methanol] containing perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUdA), perfluorododecanoic acid (PFDoA), perfluorotridecanoic acid (PFTrA), perfluorotetradecanoic acid (PFTeA, perfluorohexadecanoic acid (PFHxDA), perfluorooctadecanoic acid (PFODA), perfluorobutanesulphonate (PFBS), perfluorohexanesulphonate (PFHxS), perfluorooctanesulphonate (PFOS) and perflurodecanesulphonate (PFDS); ii) FTA [98% purity in isopropanol] including perfluorohexyl ethanoic acid (FHEA), perfluorooctyl ethanoic acid FOEA, and perfluorodecyl ethanoic acid FDEA; iii) perfluorooctane sulfonamide (PFOSA) [98% pure in methanol]. Identification and quantification were performed using the following internal standards: i) MPFAC-MXA [>98%] containing [$^{13}C_4$]-perfluorobutanoic acid (MPFBA ($^{13}C_4$)), ion [$^{18}O_2$]-perfluorohexanesulfonate (MPFHxS ($^{18}O_2$)), [$^{13}C_2$]-perfluorohexanoic acid (MPFHxA ($^{13}C_2$)), ion [$^{13}C_4$]-perfluorooctanesulfonate (MPFOS ($^{13}C_4$)), [$^{13}C_4$]-perfluorooctanoic acid (MPFOA ($^{13}C_4$)), [$^{13}C_5$]-perfluorononanoic acid (MPFNA ($^{13}C_5$)), [$^{13}C_2$]-perfluorododecanoic acid (MPFDoA ($^{13}C_2$)), [$^{13}C_2$]-perfluorodecanoic acid (MPFDA ($^{13}C_2$)), [$^{13}C_2$]-perfluoroundecanoic acid (MPFUdA ($^{13}C_2$)); ii) MFTA-MXA [>98%] [$^{13}C_2$]-perfluorohexylethanoic acid (MFHEA($^{13}C_2$)), [$^{13}C_2$]-perfluorooctylethanoic acid (MFOEA($^{13}C_2$)), [$^{13}C_2$]-perfluorodecylethanoic acid (MFDEA ($^{13}C_2$)) and iii) [$^{13}C_8$]-perfluorooctanesulfonamide (MPFOSA ($^{13}C_8$)).

Water, methanol, acetonitrile, CHROMASOLV®Plus for HPLC grade, ammonium acetate salt ($AcNH_4$: MW, 77.08; 98%), and formic acid (HFo) were obtained from Sigma-Aldrich (Steinheim, Germany). To remove possible cross contamination, polypropylene (PP) insert vials and inert taps were used.

2.2. Sampling and pre-treatment

Samples from liver, kidney, brain, lung, and bone (rib) were collected in 2008 from 20 subjects who had been living in different areas of Tarragona County (Catalonia, Spain) at least for the last 10 years. Causes of death were varied, including multiple trauma, subdural hematoma, ischemic heart disease, accident or self-injury. Autopsies and extraction of samples were carried out during the first 24 h after the time of death. Additional data from the subjects, such as age (mean: 56; range: 28–83) and smoking habits information, were collected (Table S1; Supporting Information). Tissue samples were stored at −20 °C before analysis. The study protocol was reviewed and approved by the Ethical Committee for Human Studies of the School of Medicine, Universitat Rovira i Virgili, Reus/Tarragona, Spain.

Sample pre-treatment was based on a previously published protocol (Llorca et al., 2010). Briefly, 1 g of each sample was weighed and transferred into a 15 mL PP tube. Then, 2 mL of water were added, and the mixture was shaken. Homogenates were fortified with surrogate

internal standards (to obtain a concentration of each internal standard of 10 μg/L), being digested with 5 mL of sodium hydroxide (20 mM in methanol) during 4 h at 125 rpm on an orbital shaker table at room temperature. After digestion, samples were centrifuged at 4000 rpm, and 20 μL of supernatant were directly injected into the turbulent flow chromatography system.

2.3. Analysis

A turbulent flow chromatograph Aria TLX-1 system (Thermo Fisher Scientific, Franklin, MA, USA) comprised of a PAL auto sampler (CTC Analytics, Zwingen, Switzerland), two mixing binary pumps (eluting pump and loading pump), and a three-valve switching device unit with six-port valve. The entire system was controlled via Aria software, version 1.6. The on-line enrichment was achieved using a Hypersil GOLD aQ column (2.1 × 20 mm, 12 μm particle size from Thermo Fisher Scientific, Franklin, MA, USA). The analytical column used for the chromatographic separation was a Hypersil GOLD PFP (50 × 3) (Thermo Fisher Scientific, Franklin, MA). The sample was loaded into enrichment columns using ultrapure water acidified at pH 4.5 with formic acid. After the enrichment step, the analytes were transferred to the analytical column for their chromatographic separation. The gradient used is shown in Table S2 (Supporting Information).

After separation, the detection of the selected analytes was accomplished by using a triple quadrupole mass spectrometer Thermo Scientific TSQ Vantage (Thermo Fisher Scientific, San Jose, CA), equipped with a Turbo Ion Spray source. All the analyses were performed operating in the negative electrospray ionization (ESI (−)) mode. Acquisition was performed in selected reaction monitoring mode (SRM) to obtain enough identification points (IP) for confirmation of each analyte (European Commission Decision 2002/657/EC). The main m/z transitions are summarized in Table S3 (Supporting Information). For analyte identification, the following conditions had to be met: i) analyte retention time in the sample must be in agreement with analyte retention time in the calibration curve; ii) two m/z transition were confirmed for every analyte; iii) ratio between the two transitions in the sample compared to ratio in the calibration curve should be in agreement to [calibration curve average ± SD (calibration curve)]. Table S4 (Supporting Information) provides the method limit of detection (MLOD) and the method limit of quantification (MLOQ) of the selected compounds in the five analyzed human tissues.

2.4. Quality assurance and quality control

To eliminate sources of contamination from the analytical system, all the polytetrafluoroethylene (PTFE) tubing was replaced by polyether ether ketone (PEEK) connections. In addition, an extra analytical column (C8 50 × 3 Thermo Scientific) was directly placed upstream of the injector to trap the instrumental sources of analytes, and therefore, to minimize the background signal and inter-run variability of all analytes. Blanks, consisting on initial conditions of mobile phase, were analyzed every 5 sample injections. For assessment of matrix interference in the analysis, matrix-matched calibration curves, and blank samples, were introduced in each run of analysis.

Spiking experiments were performed with blank animal (pig) matrices of brain, lung, liver, bone and kidney fortified at three different concentration levels (6, 12 and 24 ng/g of tissue). To assess the initial concentrations of PFASs, these samples were analyzed prior to fortification, being in all cases below the MLOD. The method was validated according to the criteria described by the EC Decision 2002/657/EC. The following parameters were established: instrumental selectivity and methodology limits of detection and quantification (ILOD, MLOD, ILOQ and MLOQ, respectively), linearity, recoveries, and precision expressed as intraday and inter-day repeatability.

2.5. Multivariate analysis

Before executing the multivariate data analysis, non-detected values were assumed to be equal to one-half of the method limit of detection (ND = 1/2 MLOD). The whole data set from the 5 human tissues was analyzed both individually and by using a column-wise 99 × 20 matrix augmentation strategy (Navarro et al., 2006). Auto scaling was chosen as pre-treatment method. With this procedure, the mean of the column elements was subtracted from individual elements and divided by their column standard deviation. Consequently, each column has zero mean and unit variance (Brodnjak-Vončina et al., 2002; Massart et al., 1998). Auto scaling can be applied either to the individual matrices corresponding to each tissue before matrix augmentation, or once they have been arranged in the column-wise augmented data matrix. The former system identifies differences in the tissues, while the latter detects differences among individual samples.

Data were also subjected to Principal Component Analysis (PCA). This is a data reduction technique aimed at explaining most of the variance in the data by transforming a set of correlated measured variables into a new set of uncorrelated Principal Components (PCs), which preserve the relationships present in the original data (Rovira et al., 2011a). The main goal of this multivariate statistical technique is to extract useful information and provide an easier visualization of the existent relationships between objects and variables determined in large or complex data set (Rovira et al., 2011b). PCA can be easily extended to the simultaneous analysis of multiple correlated data sets. In the present study, PCA was conducted to assess the possible distribution of the different compounds in the tissues studied, as well as to assess any possible correlation between age and smoking habits of the subjects and their PFASs accumulations. PCA modelling was conducted using the PLS Toolbox (Eigenvector Research, Manson WA, USA) appropriate functions under the MATLAB computer and visualization environment (The Mathworks, Natick, MA, USA). Finally, a hierarchical cluster analysis was conducted to confirm some of the conclusions obtained by the PCA. Data were also treated by normalization. The dissimilitude matrix was conducted by the Euclidean distance, while the Ward method was chosen for the aggrupation approach. This part of the multivariate analysis was conducted using the XLSTAT module version 2012.042.

3. Results and discussion

The concentrations of detected PFASs in human samples of brain, liver, lung, bone and kidney are depicted in Fig. 1. The complete set of results of each one of the 99 analyzed samples is given in Table S5 (Supporting Information), while a summary of median and range values is presented in Table 1. All samples showed detectable values of at least two of the investigated compounds. Although PFASs accumulation followed different trends depending on the specific tissue, some similarities were observed between liver and brain, on one hand, and between kidney and lung, on the other hand. In liver, PFHxA, PFOS and FHEA were the most prevalent compounds, with median concentrations of 68.3, 41.9 and 16.7 ng/g, respectively. PFOS, one of the most toxic PFASs, was present in 90% of the samples, while PFOA could be quantified in 45% of the samples (median: 4.0 ng/g). In brain, PFHxA was the main compound, being detected in all the samples at concentrations ranging from 10.1 to 486 ng/g. The contributions of PFNA (median: 13.5 ng/g) and PFDA (median: 12.4 ng/g) were also relatively important in brain samples. In contrast, PFOS was only quantified in 20% of the samples (median: 1.9 ng/g), whereas PFOA was not detected in any of them. In general terms, lung was the tissue showing the highest accumulation of PFASs. PFBA and PHFxA were the compounds presenting the highest median concentrations (807 and 207 ng/g, respectively). Only two lung samples showed PFOS levels under the limit of detection, with a median value of 28.4 ng/g. Although the percentage of samples with detected values of PFOA fell down to 45%, the contribution of PFOA to the total PFASs in lung was quite important, in comparison to

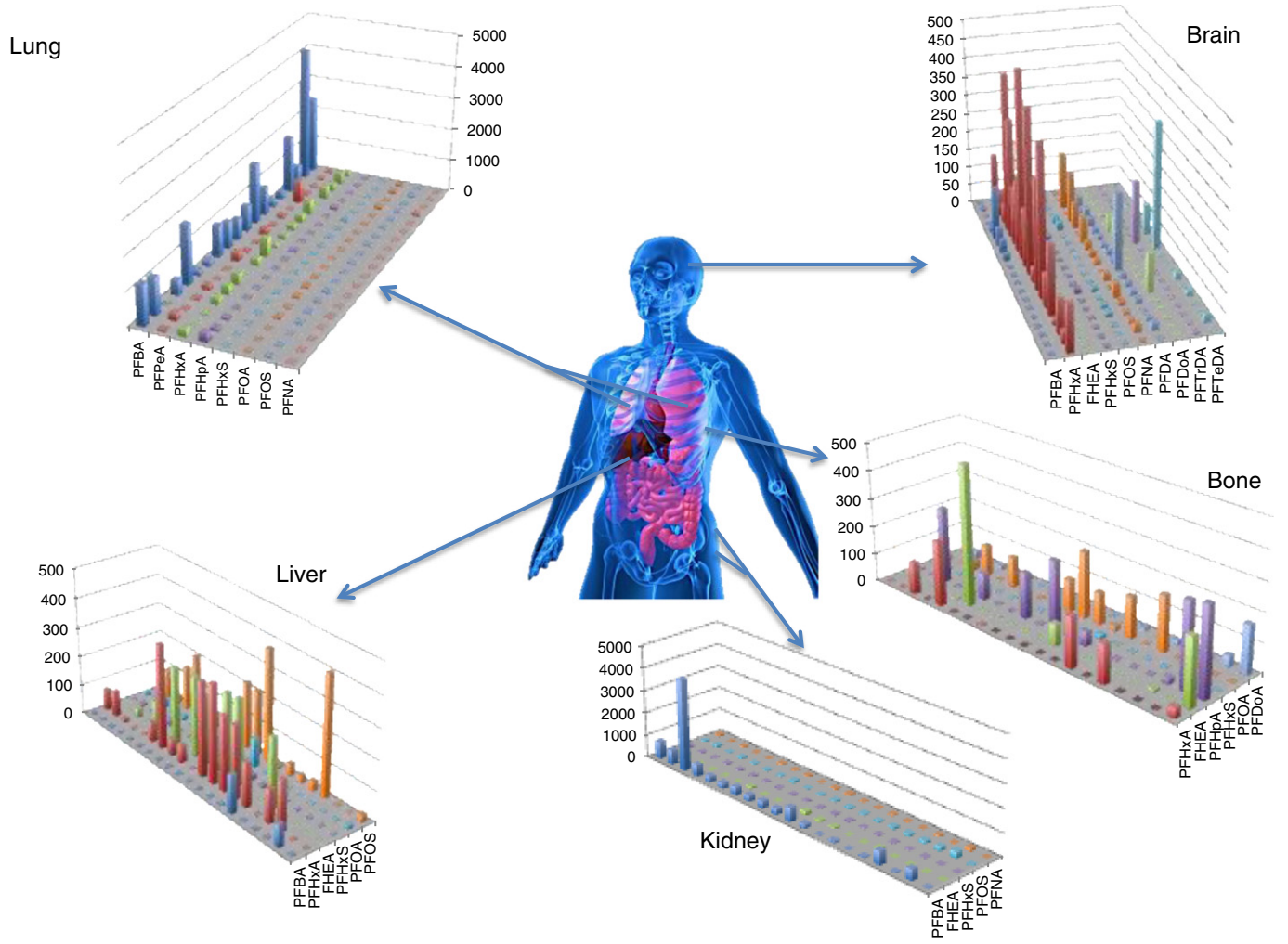

Fig. 1. Concentrations of various PFASs (in ng/g) in 5 human tissues from 20 residents of Tarragona (Catalonia, Spain).

other tissues and analytes. PFBA was also the predominant compound in all kidney samples, whose median concentration was 263 ng/g. PFDoDA and PFDA were also detected in kidney samples, but at much lower concentrations (median: 91.4 and 90.2 ng/g, respectively). High concentrations of PFOS were also found in kidney (median: 55.0 ng/g), while the presence of PFOA was minor. In contrast to lung, bone was identified as the tissue with the lowest burdens of PFASs. Furthermore, the PFAS profile was substantially different from those of the remaining tissues, as PFOA was, far the major contributor to the total concentration of PFASs (median: 20.9 ng/g). In turn, PFOS was not detected in any of the bone samples (Table 1). In summary, the profiles of PFASs accumulation in the different tissues reflected some common trends. Thus, PFHxA showed the highest concentrations in brain and liver, while PFBA presented the maximum median levels in kidney and lung, with PFOA as the predominant compound in bone. PFOS accumulated basically in lung, liver and kidney, while the levels of PFOS in bone and brain were very low. We hypothesized that since PFBA is a short chain compound, its predominance in lung could reflect the inhalation of contaminated dust and the industrial replacement of the eight carbons chain compounds by shorter ones. In addition, the human half-life of this compound is much shorter (3 days) (Chang et al., 2008) compared to the half-life to other longer chain compounds as those with 8 carbon-chain thereby accounting for its detection in other tissues as kidney. As aforementioned, there is an important lack of studies reporting PFASs levels in human tissues, excepting plasma. In comparison to previous results (Kärrman et al., 2009; Maestri et al., 2006; Olsen et al., 2003), the current concentrations of PFOS in liver from residents in Tarragona fall in the higher part of the range. However, this comparison can be only taken into account as a first indication.

The physical–chemical properties of each chemical are responsible for their tissue-specific accumulation profiles. However, the overall body burden can be similar although the chemicals accumulate in different tissues. In order to determine whether exposure to PFASs is related to exposures to other contaminants, the levels of PFASs in each sample were evaluated to determine whether they correlate with the concentrations of some metals and PCDD/Fs. The content of arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), tin (Sn), and thallium (Tl) had been previously determined in the same human tissue samples (unpublished results). With a few exceptions, the levels of PFASs were not associated with those of most trace elements. However, a significant Pearson correlation was noted between PFOA and As ($p < 0.001$), as well as between PFOA and Pb ($p < 0.001$). Manganese was the element presenting a significant correlation with a higher number of PFASs: PFDS, PFUDA, and PFTeDA ($p < 0.001$ in all cases). Finally, Ni correlated with PFHxDA. However, PFOS did not correlate with any of the above elements (Table S6; Supporting Information). The concentrations of PCDD/Fs had been also analyzed in adipose tissues from 15 of the same 20 individuals (Nadal et al., 2009). The mean PCDD/F concentration in adipose tissue was 14.6 pg WHO-TEQ/g of fat (range: 3.3–55.4 pg WHO-TEQ/g of fat). The total levels of PCDD/Fs, as well as those of the 17 2,3,7,8-chlorinated congeners, were compared with the concentrations of PFASs accumulated in the 5 human tissues here analyzed. Although not statistically significant, a negative correlation was

Table 1
Summary of PFAS concentrations (in ng/g wet weight) in 5 autopsy tissues from 20 individuals of Tarragona (Catalonia, Spain).

	Liver				Bone				Brain				Lung				Kidney			
	Mean	Median	Range	MLOD	% of detection	Mean	Median	Range	MLOD	% of detection	Mean	Median	Range	MLOD	% of detection	Mean	Median	Range	MLOD	% of detection
PFBA	12.9	3.0	128-Bdl.	6.00	10	Bdl.	–	–	0.03	0	13.5	1.4	137-Bdl.	2.71	25	304.2	807	4138-Bdl.	0.01	95	464	263	4026-Bdl.	0.01	95
PFPeA	1.4	Bdl.	27.1-Bdl.	0.001	5	0.8	0.8	0.8-Bdl.	1.51	0	Bdl.	–	–	0.59	0	44.5	40.8	695-Bdl.	6.006	74	Bdl.	–	–	0.006	5
PFBS	0.9	0.7	1.5-Bdl.	1.39	0	3.2	2.4	17.8-Bdl.	14.41	5	Bdl.	–	–	0.96	0	17.8	1.1	9.7-Bdl.	2.10	47	8.0	1.7	80.4-Bdl.	3.40	5
PFHxA	115	68.3	353-Bdl.	2.73	70	35.6	1.5	230-Bdl.	0.001	30	180	141	486-10.1	0.72	100	50.1	207	569-Bdl.	9.42	89	5.6	2.7	57.1-Bdl.	5.37	25
FHEA	92.6	16.7	289-Bdl.	4.40	45	42.5	2.0	494-Bdl.	3.52	25	18.6	2.0	93.1-Bdl.	4.00	25	2.4	3.9	3.9-Bdl.	5.54	0	23.7	1.4	153-Bdl.	2.85	5
PFHpA	33.3	1.5	638-Bdl.	3.00	5	77.1	2.4	309-Bdl.	2.89	45	Bdl.	–	–	2.70	0	17.4	1.5	245-Bdl.	3.00	37	7.8	2.6	94.3-Bdl.	5.26	95
PFHxS	4.6	1.8	20.6-Bdl.	3.00	10	1.8	1.2	13.8-Bdl.	2.40	5	3.2	2.3	14.4-Bdl.	4.54	5	8.1	5.7	47.6-Bdl.	3.30	32	20.8	18	37-Bdl.	4.20	5
PFOA	13.6	4.0	98.9-Bdl.	3.00	45	60.2	20.9	234-Bdl.	3.00	55	Bdl.	–	–	2.40	0	29.2	12.1	87.9-Bdl.	6.00	42	2.0	1.5	11.9-Bdl.	3.00	95
PFOS	102	41.9	405-Bdl.	3.00	90	Bdl.	–	–	3.00	0	4.9	1.9	22.5-Bdl.	3.00	20	29.1	28.4	61.8-Bdl.	3.00	89	75.6	55	269-Bdl.	6.00	45
PFNA	1.3	1.0	6.6-Bdl.	1.99	0	Bdl.	–	–	4.18	0	29.7	13.5	150-Bdl.	3.27	55	15.3	3.5	126-Bdl.	7.13	11	28.3	10.9	128-Bdl.	4.04	0
FOEA	2.8	2.8	2.8-Bdl.	5.67	0	3.6	1.6	35.7-Bdl.	3.20	5	Bdl.	–	–	8.80	0	13.2	4.9	87-Bdl.	5.60	21	Bdl.	–	–	3.55	0
PFODA	2.5	1.5	6.5-Bdl.	3.00	0	Bdl.	–	–	6.00	0	Bdl.	–	–	2.91	0	Bdl.	–	–	2.91	0	Bdl.	–	–	0.01	0
PFDA	Bdl.	–	–	0.001	0	Bdl.	–	–	0.30	0	23.4	12.4	204-Bdl.	2.94	70	17.1	1.5	108-Bdl.	2.973	32	6.2	Bdl.	90.2-Bdl.	0.001	10
PFOSA	Bdl.	–	–	2.60	0	Bdl.	–	–	2.04	0	Bdl.	–	–	2.04	0	Bdl.	–	–	10.16	0	Bdl.	–	–	3.78	0
PFDS	Bdl.	–	–	0.001	5	1.7	1.5	5.7-Bdl.	2.97	0	0.3	Bdl.	1.4-Bdl.	0.00	25	3.1	0.6	9-Bdl.	1.200	37	4.9	Bdl.	21-Bdl.	0.001	25
PFUdA	Bdl.	–	–	0.003	0	Bdl.	–	–	0.30	0	Bdl.	–	–	18.00	0	2.8	1.4	20.4-Bdl.	2.700	11	7.1	1.5	55.4-Bdl.	3.00	10
FDEA	3.7	0.7	59.3-Bdl.	3.00	5	Bdl.	–	–	0.30	0	Bdl.	–	–	2.91	0	Bdl.	–	–	0.01	0	Bdl.	–	–	9.01	0
PFDoA	2.4	1.5	20.2-Bdl.	1.45	5	16.6	5.1	169-Bdl.	0.98	70	13.2	1.5	102-Bdl.	1.32	25	20.7	Bdl.	253-Bdl.	4.76	11	14.7	4.5	91.4-Bdl.	2.30	15
PFTrDA	2.1	Bdl.	32-Bdl.	0.001	10	15.8	0.3	311-Bdl.	0.60	5	9.9	1.4	167-Bdl.	2.88	10	138.6	6.9	1582-Bdl.	2.970	42	Bdl.	–	–	6.00	0
PFTeDA	Bdl.	–	–	0.001	0	Bdl.	–	–	0.001	5	24.8	1.4	335.7-Bdl.	2.85	30	9.8	1.5	82.8-Bdl.	2.910	16	6.2	30.8	Bdl	0.002	25
PFHxDA	Bdl.	–	–	3.00	0	16.6	2.9	171.8-2.9	5.85	10	Bdl.	–	–	2.91	0	8.5	1.5	80.2-Bdl.	2.95	16	Bdl.	–	–	0.01	0

MLOD: Method Limit of Detection. Bdl.: Below limit of detection.

observed between the total sum of PCDD/Fs and the total amount of PFASs (Table S7; Supporting Information). It is well known that the toxicity of dioxins is mediated through the activation of the Aryl hydrocarbon Receptor (AhR) (White and Birnbaum, 2009). In contrast, the mode-of-action (MoA) for PFOA as well as other PFASs, is not so well understood (Post et al., 2012). Notwithstanding, it must be noted that data on PCDD/Fs were only available for adipose tissue, while PFASs levels refer to another 5 different tissues (liver, brain, kidney, bone, and lung). Therefore, these data are not entirely comparable, and consequently, this indication cannot be confirmed.

The pharmacokinetic properties of PFOA and PFOS are well studied (Loccisano et al., 2012). These parameters have been used in the development of pharmacokinetic models, aimed at describing the human distribution of PFOA and PFOS (Loccisano et al., 2011; Thompson et al., 2010), among other PFASs. Physiologically based pharmacokinetic (PBPK) models are mathematical representations of the human body, where organs are considered as compartments (Fàbrega et al., 2011). The overall goal of developing these PBPK models is to extrapolate to humans the distribution of chemicals in the body, in order to enhance the scientific basis for human health risk assessment of PFASs (Loccisano et al., 2012). According to the results of studies with experimental animals, these compounds are well absorbed orally (Loccisano et al., 2012). Therefore, ingestion should be considered a key pathway. A clear relationship between the intake of PFOA, basically through drinking water consumption, and serum concentrations in humans, has been found (Emmett et al., 2006), with a with a serum:drinking water ratio of about 100:1 (Post et al., 2012). Although a number of PBPK models have been described, most of them have been based only on animal data, while human data are still very scarce. To the best of our knowledge, we here report, for the very first time, the simultaneous accumulation of PFASs in various human tissues. This information should be beneficial for the development of theoretical PBPK models, whose validation is still incomplete. Consequently, forensic analyses offer a practical way to explore the real accumulation of those pollutants in the human body.

In the current study, PCA analysis was used to determine the variation of PFASs accumulation between tissues, as well as to extract possible relations between the individual concentrations and other factors, such as age and smoking. The PCA results are summarized in Table 2. The first PC explained a variance ranging between 12% and 29% of the total variance, for all the different tissues analyzed, while PC2 and PC3 variances ranged 19–20% and 8–15% respectively. The percentage of explained variance for those PCAs performed in the individual tissues was always higher than that in the augmented matrices. The explained variances differed in the two groups of PCAs. In the augmented matrices, they increased very slowly, not reaching 50% of the total variance until PC6. This indicates the presence of multiple independent distribution processes of PFASs in the considered tissues. On the other hand, in the individual PCAs of each tissue, the variance increased faster, reaching 50% of the total variance in the PC3 in most of the cases, indicating similar distribution processes when the same tissue is considered. Fig. 2 depicts the loadings plot for the first two PCs of the augmented and auto scaled data matrices. The first PC had positive loadings for all acidic compounds, from low to high contributions depending on the compound, except for PFHxA, PFHpA, with moderate negative loadings, and PFDOA, with a high negative loading. In this first PC, perfluoroalkyl sulphonates presented positive loadings, with higher contributions of PFHxS and PFDS. Regarding telomer acids (FHEA, FDEA and FOEA), the three compounds showed moderate loadings, negatively for FHEA and FDEA, and positively for FOEA. The second PC showed positive loadings for most acidic compounds except for PFNA, PFDA, PFUdA and PFTeDA, with especially high contributions of PFBA, PFOA, PFDA and PFTrDA. Perfluoroalkyl sulphonates presented moderate contributions to the second PC, being positive for PFBS and PFDS, and negative for the remaining two. The telomer acids presented positive loadings for FOEA and FDEA, and negative for FHEA. When plotting

Table 2
Percentages of explained variances obtained by PCAs applied to $All_{aug\text{-}auto}$, $All_{auto\text{-}aug}$ and the individual matrices of the 5 tissues.

Matrix	$All_{aug\text{-}auto}$	$All_{auto\text{-}aug}$	Liver	Brain	Bone	Lung	Kidney
PC1	11.98	12.49	28.68	22.69	25.80	18.65	20.20
PC2	9.26	11.23	19.91	17.10	16.18	16.28	14.75
	(21.25)	(23.72)	(48.59)	(39.79)	(41.98)	(34.93)	(34.96)
PC3	7.65	9.82	15.50	13.68	13.64	12.55	14.56
	(28.90)	(33.53)	(64.09)	(53.47)	(55.63)	(47.48)	(49.51)
PC4	7.49	8.28	9.78	10.96	12.52	9.66	12.17
	(36.39)	(41.81)	(73.87)	(64.43)	(68.15)	(57.14)	(61.69)
PC5	6.75	7.53	8.53	9.30	8.20	8.02	8.75
	(43.14)	(49.35)	(82.40)	(73.73)	(76.35)	(65.16)	(70.43)
PC6	6.47	6.22	5.47	7.55	6.95	7.74	8.26
	(49.61)	(55.57)	(87.87)	(81.28)	(83.30)	(72.90)	(78.70)
PC10	4.60	4.55	1.07	2.07	1.79	3.58	2.82
	(70.28)	(76.06)	(99.74)	(98.01)	(100)	(91.42)	(95.92)

In parenthesis, percentage of accumulated variance for that particular component.
$All_{aug\text{-}auto}$: augmented matrix of the 5 individual tissues and then autoscaled.
$All_{auto\text{-}aug}$: individually autoscaled matrixes of the 5 tissues and then augmented.

the scores using these two PCs, the samples can be grouped into each one of the 5 tissues analyzed (Fig. 3). This means that the profile of PFASs found in each tissue is different from the others. Thus, PC1 allowed the separation between lungs, kidney and brain, with positive contribution, while bone and liver showed a negative contribution. In turn, PC2 reflected a separation between lung and bone, with positive loadings, and the remaining three tissues, with negative loadings. When considering the remaining PCs, the behavior was similar.

Fig. S1 (Supporting Information) depicts the loadings plot for the first PC of each PCA performed in the individual matrices of each tissue. In liver, the first PC showed high positive loadings for acidic compounds with an odd number of carbons (PFPeA, PFHpA, PFNA and PFTrDA). In kidney, a similar profile was obtained, with acidic PFASs with an odd number of carbon chain (PFPeA, PFNA, PFUdA and PFTrDA) acting as prevalent compounds. In brain, acidic compounds with a pair number of carbon chain (PFBA and PFHxA) and sulphonates (PFBS, PFOS and PFDS) were the predominant compounds. Unlike other PFASs, sulphonates also showed a high contribution in bone. Lung samples also presented positive loadings for most acidic compounds with a pair number of carbon chain (PFBA, PFHxA, PFOA, PFDoA), as well as some of the sulphonates (PFOS, PFDS) and FOEA. This different profile of PC1 confirms the different distribution pattern of PFASs according to each specific tissue. The influence of smoking in the accumulation of PFAs in the lungs was also studied. As shown in Fig. 4, smoker subjects presented lower contributions of PC1 and PC2 than non-smokers. It means less accumulation of the PFASs, which contribute to these PCs. When considering the rest of PCs, a similar behavior is observed. Considering the samples included in this study, a negative correlation between smoking habits and accumulations of PFAs in lung is observed. Further investigation with a higher number of subjects should be performed to check this relationship.

The accumulation of PFASs with age was studied in the analyzed tissues. In general terms, older people (more than 60 years) showed higher concentrations of PFASs, which is a clear indication that these compounds accumulate after a long-term exposure. All middle-age (40–60 years) individuals presented fairly similar levels of PFASs in the different tissues. However, some young subjects (18–39 years) also showed relatively high levels of PFASs. These values could be due to differential accumulation factors, such as dietary intake, living habits, and/or early exposure. This was also confirmed after performing a hierarchical classification (Fig. S2). Finally, a special correlation between smoking habits and PFAS accumulation in lung was performed. Although PCs did not show a positive relation between both

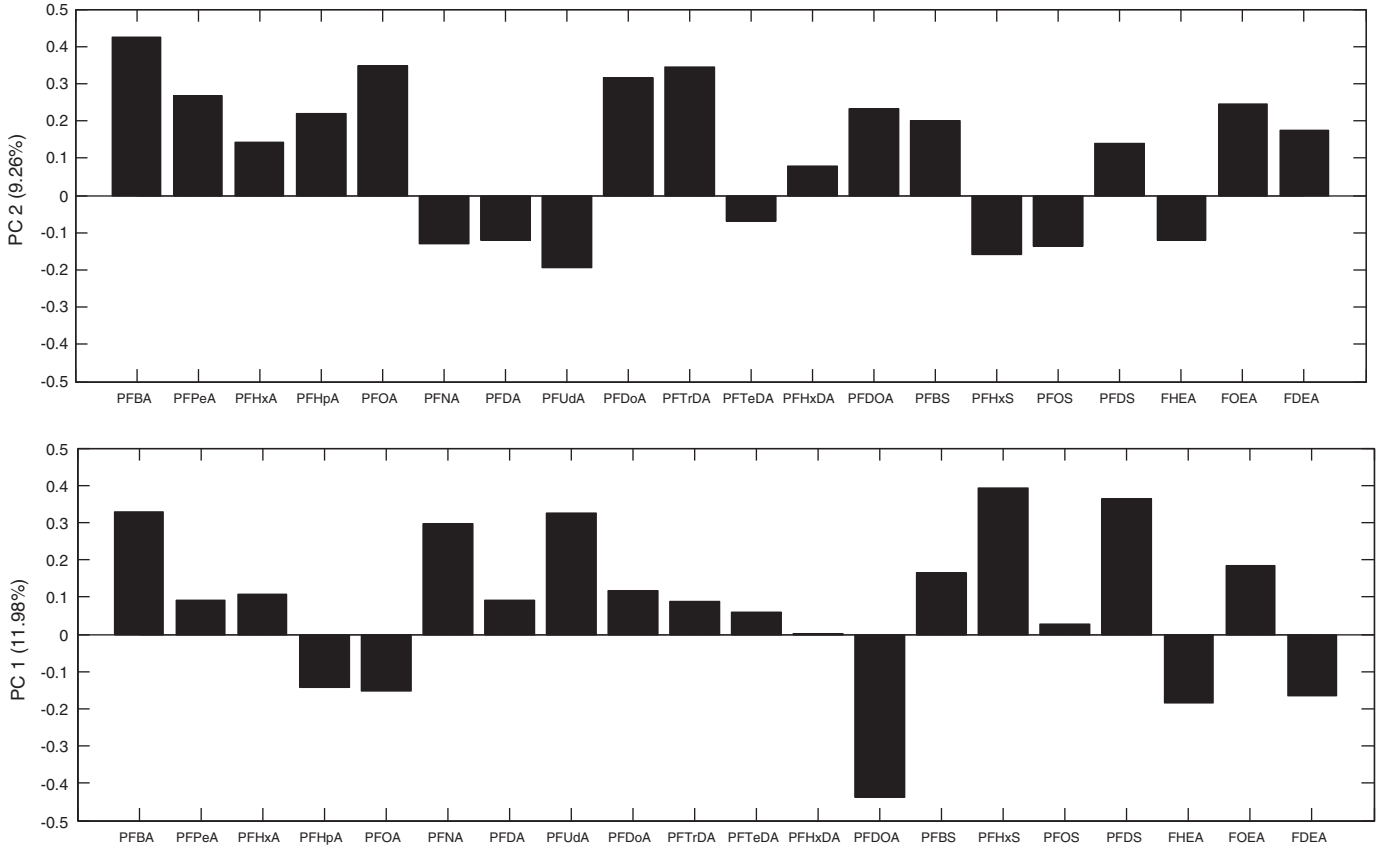

Fig. 2. Loadings of the first two principal components (PCs) for the augmented and auto scaled matrices.

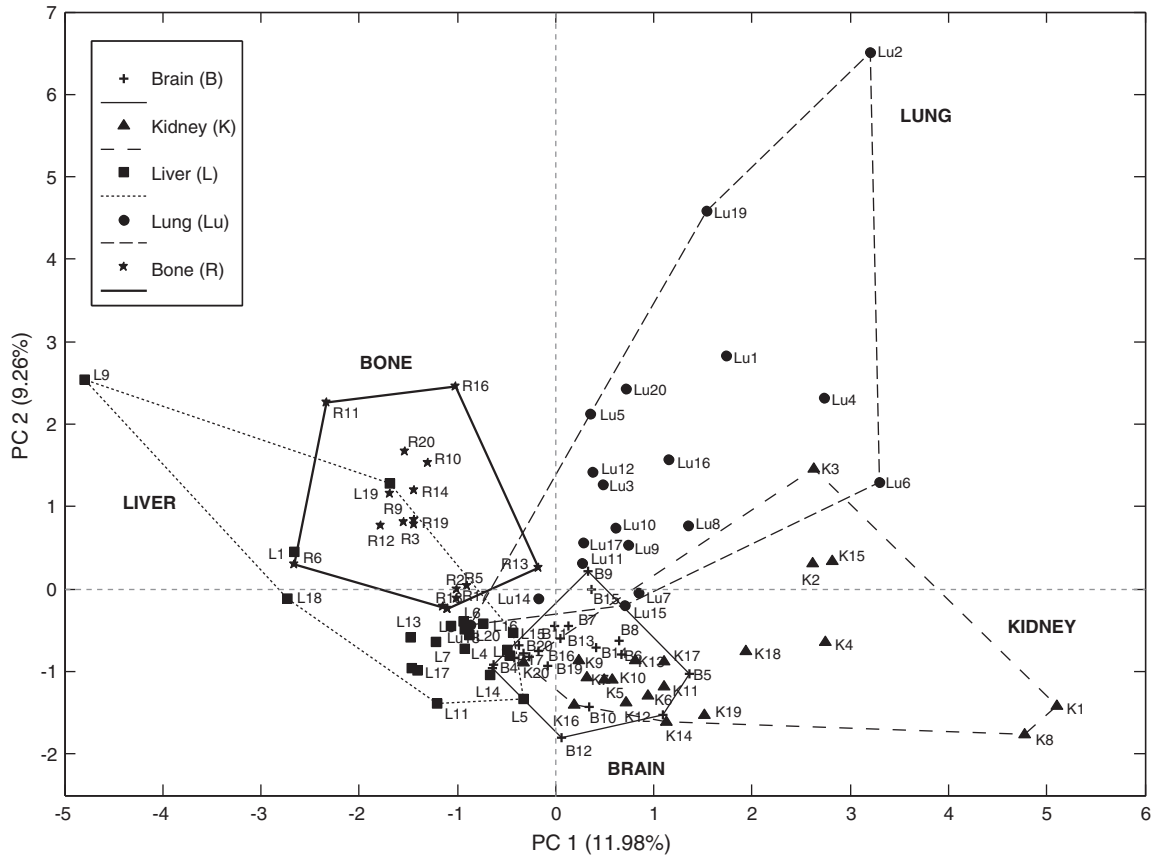

Fig. 3. Scores plots for the first two principal components (PCs) for the augmented and autoscaled matrix.

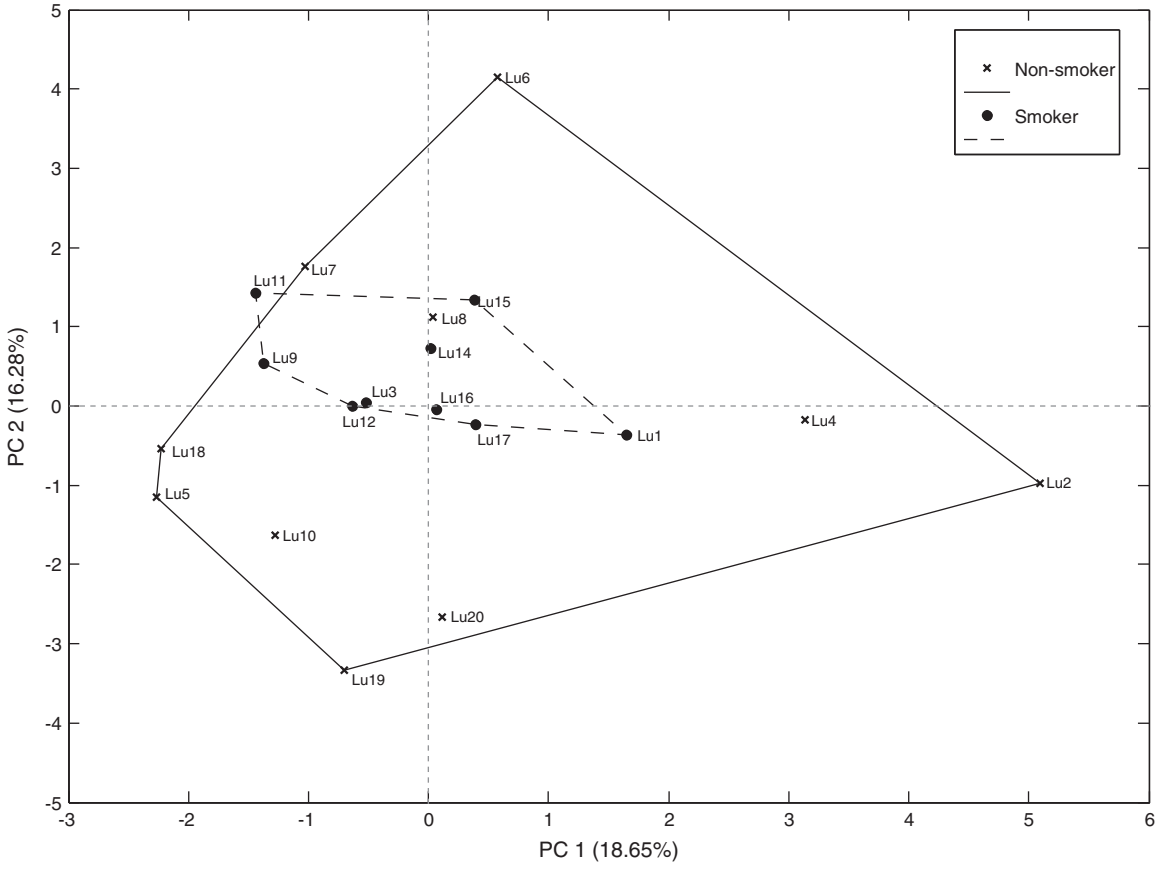

Fig. 4. Principal Component Analysis (PCA) of PFASs in lung samples.

parameters, the current number of samples was not sufficient to establish conclusions on this issue. Further investigations involving a higher number of subjects are necessary.

4. Conclusions

In this study, an effective analytical method optimized for the ultra-trace analysis of 21 PFASs in human tissues, using both small sample sizes (amount: 1 g) and a reduced sample manipulation, was addressed. The application of this approach to the analysis of 99 samples of five different tissues from 20 subjects demonstrated, for the very first time, the accumulation of certain short chain compounds, such as PFBA and PFHxA, in human tissues. Moreover, the results from the chemical analysis, together with the application of multivariate statistical techniques, showed a different accumulation pattern of the analyzed compounds in human tissues. Only few correlations were noted in the concentrations of metals and those of PFASs. However, interestingly, certain negative association between the contents of PFASs in those 5 autopsy tissues, and the levels of PCDD/Fs in adipose tissue, was observed. This finding suggests the need to fully characterize the toxicity mechanisms of PFASs, which are not currently so well understood as those of PCDD/Fs. Notwithstanding, as data refer to different biological compartments, values are not entirely comparable. In any case, the current results should be of importance for the validation of PBPK models, which are being developed for humans.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2013.06.004.

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RESEARCH ARTICLE

Severity of COVID-19 at elevated exposure to perfluorinated alkylates

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Abstract

Background

The course of coronavirus disease 2019 (COVID-19) seems to be aggravated by air pollution, and some industrial chemicals, such as the perfluorinated alkylate substances (PFASs), are immunotoxic and may contribute to an association with disease severity.

Methods

From Danish biobanks, we obtained plasma samples from 323 subjects aged 30–70 years with known SARS-CoV-2 infection. The PFAS concentrations measured at the background exposures included five PFASs known to be immunotoxic. Register data was obtained to classify disease status, other health information, and demographic variables. We used ordered logistic regression analyses to determine associations between PFAS concentrations and disease outcome.

Results

Plasma-PFAS concentrations were higher in males, in subjects with Western European background, and tended to increase with age, but were not associated with the presence of chronic disease. Of the study population, 108 (33%) had not been hospitalized, and of those hospitalized, 53 (16%) had been in intensive care or were deceased. Among the five PFASs considered, perfluorobutanoic acid (PFBA) showed an unadjusted odds ratio (OR) of 2.19 (95% confidence interval, CI, 1.39–3.46) for increasing severities of the disease. Among those hospitalized, the fully adjusted OR for getting into intensive care or expiring was 5.18 (1.29, 20.72) when based on plasma samples obtained at the time of diagnosis or up to one week before.

the secure server at the Danish Health Data Authority, pending necessary approvals from the Authority (instructions at www.sundhedsdata.dk) and the Regional Committee on Health Research Ethics for researchers who meet the criteria for access to confidential data. Aggregated data underlying the results presented in the study are available from the corresponding author, provided that no data are based on less than 5 individuals.

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Conclusions

Measures of individual exposures to immunotoxic PFASs included short-chain PFBA known to accumulate in the lungs. Elevated plasma-PFBA concentrations were associated with an increased risk of a more severe course of COVID-19. Given the low background exposure levels in this study, the role of exposure to PFASs in COVID-19 needs to be ascertained in populations with elevated exposures.

Introduction

Elevated exposure to community pollution is associated with a worsened outcome of coronavirus disease 2019 (COVID-19) [1–4]. While replicated in different populations, this evidence relies solely on ecological study designs of air pollution without measures of individual exposures. Several environmental chemicals are known to suppress immune functions [5, 6] and worsen the course of infections [7]. Of particular relevance, the perfluorinated alkylate substances (PFASs) are persistent, globally disseminated chemicals known to be immunotoxic [8]. Thus, elevated blood-PFAS concentrations are associated with lower antibody responses to vaccinations in children [9] and in adults [10]. Also, infectious disease occurs more frequently in children with elevated exposure [11–13]. In support of the potential impact of these substances, a modeling study suggested that endocrine disruptors, including major PFASs, may interfere with proteins involved in critical pathways, such as IL-17, associated with severe clinical outcomes of the COVID-19 infection [14].

Substantial differences occur in the clinical course of the disease, and the reasons for this variability are only partially known [15, 16]. As a possible contributor, a deficient antibody response may be an important contributor to a more severe clinical course of the infection [17], as also suggested by the poorer prognosis in patients with bacterial co-infection [18]. The most serious clinical consequences are associated with male sex, older age, and the presence of co-morbidities, including obesity and diabetes [19–23]. In parallel, serum-PFAS concentrations are higher in men than in women and also tend to increase with age [8, 24]. Because elevated PFAS exposure has been linked to both obesity and diabetes [25, 26], these substances may potentially affect the progression of COVID-19 directly as well as indirectly.

Several PFASs can be reliably determined in human blood samples, where most of them show long biological half-lives of 2–3 years or more [27], thereby providing a measure of cumulated exposure. Still, blood concentrations may not accurately reflect the retention in specific organs, e.g., the short-chain perfluorobutanoic acid (PFBA), which accumulates in the lungs [28].

To assess if elevated background exposures to immunotoxic PFASs are associated with the clinical course of the infection, a study was undertaken in Denmark to determine individual plasma-PFAS concentrations in adults confirmed to be infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and examine the association with the severity of COVID-19 development.

Methods

Population

Plasma samples for PFAS analysis were obtained from medical biobanks that store excess material from diagnostic tests, viz., the Danish National Biobank at the Statens Serum Institut

(SSI) and Odense University Hospital (OUH). Eligible subjects were identified from the Danish cohort of COVID-19 patients [29]. All cases were tested by quantitative polymerase-chain-reaction (PCR) and had a positive response for SARS-CoV-2 infection, as recorded in the Danish Microbiology Database (MiBa), a national database that contains both positive and negative results of the majority of microbiology testing done in Denmark [30].

The study included non-pregnant subjects aged 30–70 years at the time of the positive test by early March 2020 through early May 2020, provided that the biobanks could provide a plasma sample of 0.15 mL. Although most blood samples were obtained soon after SARS-CoV-2 infection was identified, we also included subjects, mainly those not hospitalized, whose plasma in the SSI biobank had been obtained up to 28 months earlier, i.e., less than a half-life for major PFASs [27]. We calculated the time interval from blood sampling to the time of diagnosis, of relevance mainly for non-hospitalized subjects. In those hospitalized, we computed the interval from admission to the time of sampling the plasma used for PFAS analysis.

All samples were coded, and the Personal Identification Number for each subject was separately transferred to the Danish Health Data Authority (FSEID-00005000) to allow linkage to demographic and medical information from the Danish Civil Registration System (CRS) [31], the Danish National Register of Patients (DNRP) [32], and the National Health Insurance Service Register [33]. We used the following classification of disease status: no hospital admission and completed infection within 14 days of testing positive, hospitalization with COVID-19 up to, or above, 14 days, admission to intensive care unit, or death. Presence of chronic disease was based on the following diagnoses in the register data: diabetes type I and II (ICD10 codes E10-E11), malignant cancers (C00-C99), cerebrovascular and coronary disease (I00-I99), pulmonary disease (J00-J99), and obesity (E66-E68). Renal disease (N0-N2) was treated as a separate covariate due to the possible impact of kidney function on plasma-PFAS concentrations [34]. The linked data set was analyzed via secure server without access to information on the Personal Identification Numbers of the subjects involved. For confidentiality reasons, all tabular information had to be based on at least five subjects.

The protocol was approved by the Regional Committee on Health Research Ethics (S-20200064), which also allowed the project to proceed without seeking informed consent from the subjects identified for study participation. Additional approvals were obtained from the Danish Data Protection Agency as well as institutional and regional authorities for the transfer blood samples and linkage of subject information to the PFAS analyses, while protecting confidentiality.

Chemical analysis

The plasma samples were analyzed in successive series for PFAS concentrations, including PFBA, perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA), which are known from previous studies to be associated with immunotoxicity in humans [8, 35, 36]. We also determined plasma concentrations of PFASs so far not linked to immunotoxicity, i.e., short-chain perfluorobutanesulfonate (PFBS), perfluoroheptanesulfonate (PFHpS), perfluorodecanoate (PFDA), and perfluoroundecanoate (PFUdA) (results shown in the Supporting information). We used online solid-phase extraction followed by liquid chromatography and triple quadropole mass spectrometry (LC–MS/MS) at the University of Southern Denmark [37]. Accuracy of the analysis was ensured by inclusion of quality control (QC) samples comprising proficiency test specimens from the HBM4EU program organized by Interlaboratory Comparison Investigations (ICI) and External Quality Assurance Schemes (EQUAS). All results of the QC samples were within the

acceptance range. The between-batch CVs for the actual series ranged between 3% and 14% for all compounds. Both PFOS and PFOA were quantified in all blood samples, and all PFASs were detectable in at least 30% of the samples. Results below the limit of detection (LOD, 0.03 ng/ml) were replaced by LOD/2 before uploading to the secure server at the Danish Health Data Authority, where linkage to other information took place.

Statistical analysis

Correlations between PFASs were examined using Spearman's correlation coefficient. The PFAS concentrations were compared between demographic groups (age in years, sex, national origin, place of inclusion), presence of comorbidities, and number of days between blood sampling and diagnosis, and differences were tested using Kruskal-Wallis and Wilcoxon rank-sum test. Furthermore, associations of COVID-19 severity with age were tested using Kruskal-Wallis test, and relations with each of the variables sex, national origin, presence of comorbidities, and number of days between blood sampling and diagnosis were tested using χ^2 test. Associations between place of inclusion and COVID-19 severity could not be displayed and tested, as some cells contained less than five individuals.

Because COVID-19 severity was categorized, the association between the continuous plasma-PFAS concentrations and COVID-19 severity was tested in ordered logistic regression models. More than half the short-chain PFAS concentrations were below the LOD, and they were therefore treated as binary variables (below/above LOD). Potential confounding variables were identified based on a priori knowledge as summarized above and included age (continuous, years) sex, and national origin (Western European yes/no). Among those of Western European national origin, 94% were Danish, while most of the participants of non-Western European national origin were born in or of parents from Somalia (20% of the sample), Pakistan (13%), Iraq (12%), Morocco (11%), Eastern Europe (9%), and Turkey (9%). Kidney disease may affect PFAS elimination, and PFAS exposure could potentially increase the risk of certain other chronic diseases that may affect COVID-19 severity [8]. Kidney disease (yes/no) and other chronic disease (yes/no) were thus considered potential confounders to allow estimation of the direct, rather than the total effect of plasma-PFAS concentrations. Due to changes in PFAS exposures over time, the timing of blood sampling was included as covariate. Further, due to the short elimination half-life for short-chain PFASs [8], we carried out sensitivity analyses excluding plasma samples obtained more than one week before or after diagnosis. We also adjusted for the place of inclusion (OUH/SSI) but, under the circumstances of this study, detailed data on socioeconomic status (e.g., income, education or labor market affiliation) were unavailable for this study. Dichotomous analyses comparing severities of the disease were performed in logistic regression models.

The default assumption of dose-response linearity was tested by including PFAS squared along with PFAS in the regression models. No significant ($p<0.05$) deviation from linearity was found. The proportional odds assumption in the ordered logistic regression was tested by a likelihood-ratio test using the Stata *omodel* package. In a model adjusting for age, place of inclusion, and timing of blood sampling, the hypothesis of proportional odds was accepted ($p>0.05$) in all analyses. Odds ratios (ORs) between groups of COVID-19 severity were therefore calculated using logistic regression models.

Results

The predominant PFAS in plasma was PFOS, with an average concentration of 6.1 ng/mL (median, 4.7 ng/L), approximately equally distributed between the normal and branched isomers. Other PFASs quantified showed averages below 1 ng/mL. In a sensitivity analysis, one

Table 1. Spearman's correlation coefficients for pairwise comparisons of detectable PFASs in plasma from 323 subjects included in the study.

	PFBA	PFHxS	PFOA	PFOS
PFHxS	0.0520			
PFOA	0.0617	0.7072		
PFOS	0.0591	0.8406	0.7248	
PFNA	0.0127	0.7133	0.7759	0.8406

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extreme PFHxS outlier at 12.9 ng/mL was omitted. The PFAS concentrations correlated well, with Spearman correlation coefficients generally above 0.5 (Table 1 and S1 Table), except for short-chained PFAS. PFOS on average contributed 69% of the total PFAS concentrations by weight and correlated particularly well with most other PFASs quantified.

In general, serum-PFAS concentrations were higher at older ages, in men, and among those of Western European origin. Although the presence of chronic disease did not seem to be associated with PFAS, the plasma concentrations appeared to be higher in the presence of kidney disease (Table 2 and S2 Table).

In the study population, males, older subjects, and those with chronic disease, were more frequently represented among subjects with severe COVID-19, while there was no difference in regard to national origin for disease severity (Table 3). The PFAS-associations with disease severity were similar in Western Europeans and subjects with other backgrounds ($P > 0.2$ for population differences).

A more severe disease outcome was associated with higher plasma-PFBA concentrations, also after adjustment for all covariates (Table 4 and S4 Table). None of the other PFASs showed a similar tendency. If leaving out presence of chronic disease as a non-significant predictor, the adjusted OR for PFBA was 1.77 (95% CI, 1.09, 2.87). More importantly, when excluding samples collected earlier than one week before the time of diagnosis (148 samples), or more than one week later (5 samples), stronger ORs emerged for PFBA (Table 4). Counter to the *a priori* hypothesis, some PFASs, including PFHxS, seemed associated with a lower risk, but this tendency was weakened when relying on plasma samples collected in close connection to the diagnosis of corona infection (Table 4 and S3 Table).

In dichotomous analyses comparing severities of the disease (S4 Table), detectable PFBA in plasma also showed a clear association with a more severe clinical course of the disease, most pronounced for odds between hospitalization and admission to intensive care unit/death, especially when based on plasma samples obtained at the time of diagnosis or up to one week before where the adjusted OR was 5.18 (1.29, 20.72). No such tendency was seen for the other PFASs detected (S4 Table). The association between PFBA and disease severity was similar for men and women (Fig 1).

Discussion

The present study aimed at determining the potential aggravation of COVID-19 associated with elevated exposures to PFASs. Several of these substances are known immunotoxicants in laboratory animals [35] and in humans [8, 9]. In addition to immunotoxicity, major PFASs can potentially interfere with major pathways that are predictive of a serious clinical outcome of the infection [14]. An association of PFAS exposure with disease severity therefore appears biologically plausible.

Among the PFASs, presence of detectable PFBA in plasma showed the strongest positive association with the severity of the disease. This finding may at first seem surprising, as this

Table 2. Median plasma-PFAS concentrations (25th, 75th percentiles) in ng/mL by population characteristics.

			PFAS (ng/mL) median (25th,75th percentile)			
Population characteristics	**n (%)**	**PFBA**	**PFHXS**	**PFOA**	**PFOS**	**PFNA**
Total	323 (100)	<LOD (<LOD, 0.04)	0.48 (0.28, 0.71)	0.77 (0.43, 1.18)	4.86 (2.85, 8.29)	0.38 (0.23, 0.59)
Age (years)						
30–39	37 (11)	<LOD (<LOD, 0.03)	0.32 (0.19, 0.46)	0.59 (0.43, 0.86)	3.30 (1.89, 5.27)	0.29 (0.21, 0.43)
40–49	64 (20)	<LOD (<LOD, 0.03)	0.35 (0.15, 0.57)	0.58 (0.35, 0.89)	3.11 (2.24, 5.06)	0.27 (0.19, 0.39)
50–59	106 (33)	<LOD (<LOD, <LOD)	0.50 (0.31, 0.75)	0.83 (0.43, 1.18)	5.41 (2.79, 8.84)	0.40 (0.24, 0.61)
60–70	116 (36)	<LOD (<LOD, 0.05)	0.56 (0.39, 0.89)	0.97 (0.56, 1.51)	6.11 (3.83, 9.60)	0.48 (0.30, 0.70)
p-value [a]		0.008	<0.001	<0.001	<0.001	<0.001
Sex						
Male	174 (54)	<LOD (<LOD, 0.04)	0.59 (0.40, 0.87)	0.81 (0.51, 1.26)	5.96 (3.65, 10.17)	0.40 (0.25, 0.61)
Female	149 (46)	<LOD (<LOD, 0.04)	0.35 (0.17, 0.52)	0.70 (0.40, 1.04)	3.43 (2.06, 5.66)	0.36 (0.22, 0.56)
p-value [b]		0.713	<0.001	0.011	<0.001	0.131
Kidney disease						
yes	34 (11)	<LOD (<LOD, 0.06)	0.55 (0.34, 0.77)	0.91 (0.54, 1.46)	5.60 (3.08, 8.38)	0.50 (0.24, 0.67)
no	289 (89)	<LOD (<LOD, 0.03)	0.47 (0.28, 0.71)	0.76 (0.43, 1.15)	4.76 (2.82, 8.10)	0.36 (0.23, 0.57)
p-value [b]		0.040	0.466	0.065	0.489	0.141
Other chronic disease						
Yes	220(68)	<LOD (<LOD, 0.04)	0.47 (0.28, 0.68)	0.71 (0.42, 1.15)	4.70 (2.87, 7.99)	0.38 (0.23, 0.57)
No	103 (32)	<LOD (<LOD, 0.03)	0.51 (0.28, 0.76)	0.87 (0.47, 1.23)	5.35 (2.72, 8.41)	0.41 (0.23, 0.65)
p-value [b]		0.075	0.314	0.124	0.850	0.407
National origin						
Western Europe	224 (69)	<LOD (<LOD, 0.04)	0.52 (0.35, 0.76)	0.91 (0.60, 1.29)	5.61 (3.40, 9.18)	0.43 (0.29, 0.64)
Other	99 (31)	<LOD (<LOD, 0.04)	0.34 (0.16, 0.57)	0.44 (0.31, 0.80)	2.86 (1.61, 5.13)	0.23 (0.16, 0.36)
p-value [b]		0.552	<0.001	<0.001	<0.001	<0.001
Place of inclusion						
Odense	48 (15)	<LOD (<LOD, 0.06)	0.45 (0.32, 0.69)	0.67 (0.42, 0.95)	4.67 (3.29, 8.09)	0.36 (0.24, 0.45)
Copenhagen	275 (85)	<LOD (<LOD, 0.03)	0.48 (0.28, 0.72)	0.79 (0.44, 1.20)	4.89 (2.72, 8.31)	0.39 (0.23, 0.62)
p-value [b]		0.003	0.967	0.203	0.697	0.299
Timing of blood sampling						
After diagnosis—1 week before	193 (60)	<LOD (<LOD, 0.04)	0.48 (0.30, 0.71)	0.70 (0.40, 1.11)	4.63 (2.83, 7.65)	0.34 (0.23, 0.56)
>1 week—1 year before	46 (14)	<LOD (<LOD, 0.03)	0.45 (0.21, 0.66)	0.82 (0.38, 1.35)	4.81 (2.36, 8.62)	0.38 (0.20, 0.65)
> 1year before diagnosis	84 (26)	<LOD (<LOD, 0.03)	0.50 (0.30, 0.72)	0.87 (0.57, 1.22)	5.48 (3.10, 10.28)	0.45 (0.28, 0.65)
p-value [a]		0.185	0.756	0.085	0.209	0.053

[a] Variables with more than two categories tested using Kruskal-Wallis rank test.

[b] Binary variables tested using Wilcoxon rank-sum test.

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PFAS has a short elimination half-life in the blood and is often considered of less importance to health [27]. However, in tissue samples from autopsies, PFBA is the only PFAS that is substantially accumulated in the lungs [28]. Given the persistence of the PFASs in general, the unique retention of PFBA in lung tissue may offer a clue to interpreting the findings in this study.

Some odds ratios for PFBA were weakened after adjustment for covariates. However, adjustment for all covariates may result in over-adjustment bias. Thus, older age and male sex are known to be strong predictors of higher blood-PFAS concentrations, and simple adjustment for these factors could potentially result in a bias toward the null. As PFAS exposure has been linked to important comorbidities, such as diabetes and obesity [25, 26], both of which

Table 3. COVID-19 severity by population characteristics.

	COVID-19 severity			
Population characteristics	**No. of subjects**	**No hospitalization**	**Hospitalization**	**Intensive care unit and/or deceased**
Total No. of subjects (%)	323 (100)	108 (33)	162 (50)	53 (16)
Age (years) median (25th,75th percentile)	55 (46, 62)	49 (41, 57)	57 (51, 63)	62 (53, 67)
P value [a]	<0.001			
Sex				
Male, n (%)	174 (100)	44 (25)	94 (54)	36 (21)
Female, n (%)	149 (100)	64 (43)	68 (46)	17 (11)
P value [b]	0.002			
Kidney disease				
Yes, n (%)	34 (11)	7 (21)	13 (38)	14 (41)
No, n (%)	289 (89)	101 (35)	149 (52)	39 (13)
P value [b]	<0.001			
Other chronic disease				
Yes, n (%)	220 (100)	54 (25)	119 (54)	47 (21)
No, n (%)	103 (100)	54 (52)	43 (42)	6 (6)
P value [b]	<0.001			
National origin				
Western Europe, n (%)	224 (100)	76 (34)	113 (50)	35 (16)
Other, n (%)	99 (100)	32 (32)	49 (49)	18 (18)
P value [b]	0.844			
Days between blood sampling and diagnosis				
median (25th,75th percentile)	0 (-1, 393)	335 (22.5, 639.5)	0 (-1, 0)	0 (-2, 1)
P value [a]	<0.001			

[a] Associations tested using Kruskal-Wallis rank test.

[b] Associations tested using Pearson's chi-squared test.

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may exacerbate the virus infection, adjustment for chronic disease may also not be justified. Leaving it out slightly strengthened the PFBA association with the disease severity. The strongest associations for PFBA, but not for other PFASs, appeared when focusing on the most representative blood samples obtained close to the time of diagnosis.

Table 4. Ordered logistic regression OR of increased Covid-19 severity for an increase by 1 ng/mL in plasma-PFAS concentrations.

PFAS	**No. of subjects**	**OR (95% CI)**		**No. of subjects**	**OR (95% CI)**
		Crude	**Adjusted for main covariates[a]**		**Exposure at time of diagnosis[a,b]**
PFBA (>LOD/<LOD)	104/219	2.19 (1.39, 3.46)	1.57 (0.96, 2.58)	61/109	2.10 (1.02, 4.33)
PFHxS (ng/mL)	323	0.85 (0.63, 1.15)	0.52 (0.29, 0.91)	170	0.52 (0.24, 1.14)
PFHxS [c] (ng/mL)	322	1.00 (0.62, 1.61)	0.52 (0.29, 0.93)	169	0.53 (0.22, 1.27)
PFOA (ng/mL)	323	0.99 (0.72, 1.36)	0.83 (0.57, 1.20)	170	0.62 (0.36, 1.08)
PFOS (ng/mL)	323	1.00 (0.96, 1.04)	0.97 (0.92, 1.02)	170	0.98 (0.89, 1.07)
PFNA (ng/mL)	323	1.18 (0.67, 2.09)	1.04 (0.54, 2.02)	170	0.73 (0.25, 2.11)

[a] Adjusted for age, sex, kidney disease, other chronic disease, national origin, place of testing, and days between blood sampling and diagnosis.

[b] Excluding individuals who had blood sampled more than one week before or after diagnosis.

[c] PFHxS >10 ng/mL excluded.

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Fig 1. Proportion of plasma samples with detectable PFBA concentrations at different disease severities. Results are shown for 44 men and 64 women with up to two weeks of hospitalization, 94 men and 68 women with longer hospitalization, and 36 men and 17 women admitted to the intensive care unit (ICU) or deceased ($P = 0.003$).

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An additional consideration is that the present study relates to low background exposure levels, in comparison with PFAS concentrations to findings in, e.g., U.S. adults [38]. Given the wide occurrence of highly contaminated drinking water in other countries [39], the present study results should not be interpreted as evidence that most PFASs do not contribute to a worsened clinical course of COVID-19.

The results for PFBA in this study appear to parallel the findings in regard to other environmental toxicants, viz., air pollutants [1–4] and suggest a need to ascertain the impact of relevant occupational or environmental exposures on COVID-19 severity. Of note, the evidence on air pollution relies solely on ecological study designs without measures of individual levels of exposure, while the present study benefitted from measurements of plasma-PFAS concentrations of all study subjects.

In regard to limitations, the study population may not be representative of corona-positive subjects, as inclusion in the study depended solely on the existence of plasma from diagnostic blood samples at the participating hospitals. Thus, subjects with chronic disease or more severe COVID-19 likely had more frequent hospital visits or longer admissions and thereby a greater chance of having plasma available for inclusion in this study. With a corona-related fatality rate of Danish blood donors below 70 years of age at 89 per 100,000 infections [40], the

presence of 17 deaths in the present material (i.e., against 0.3 deaths expected) confirms that the blood samples represent a highly selected population. Still, a total of 108 subjects were known to have been infected, though not hospitalized. In many cases, their plasma had been stored on previous occasions, and the PFAS concentrations may reflect slightly higher exposures in the recent past [8], which could possibly explain the apparent protective effects of some PFASs, although adjustment for the time interval since sample collection was included in the analyses. However, the strongest associations for PFBA, but not for other PFASs, were seen when excluding samples not obtained in close temporal connection with the infection.

The study population included mostly older subjects who were more frequently male, and a large proportion of foreign-born subjects and second-generation immigrants (Table 3), thereby possibly deviating from the background population of corona-infected patients in Denmark. Still, the results do not suggest major biases affecting PFAS exposure and its association with COVID-19 outcomes.

Among immigrants, adverse associations appeared slightly stronger, also after adjustments, in accordance with national origin, perhaps as related to demographic or social factors, resulting in a greater likelihood also to PFAS-associated aggravation of the infection. Difference in age, sex, or comorbidities did not explain this tendency, but is in agreement with previous findings of ethnic differences in vulnerability [41]. However, national origin may be a surrogate marker for other factors, such as exposure at work or exposure within crowded households, as immigrant origin tends to be associated with certain occupations including front-line workers and living in areas with higher population density [42]. Still, in agreement with higher PFAS exposure being associated with higher socioeconomic position [43], we found that the association between PFBA exposure and disease severity was independent of national origin.

Conclusions

Increased plasma-PFBA concentrations were associated with a greater severity of COVID-19 prognosis, and this tendency remained after adjustment for sex, age, comorbidities, national origin, sampling location and time. Although occurring in fairly low concentrations in plasma, PFBA is known to accumulate in the lungs. Thus, as immunotoxic substances, the PFASs may well contribute to the severity of COVID-19. The present findings on a short-chain PFAS at background exposures suggest a need to ascertain if elevated exposures to environmental immunotoxicants may worsen the outcome of the SARS-CoV-2 infection.

Supporting information

S1 Table. Spearman's correlation coefficients for pairwise comparisons of detectable PFASs in plasma from 323 subjects included in the study.
(DOCX)

S2 Table. Median plasma concentrations of additional PFASs (25^{th}-75^{th} percentiles) in ng/mL by population characteristics.
(DOCX)

S3 Table. Ordered logistic regression odds ratios (ORs) of increased COVID-19 severity at an increase by 1 ng/mL in plasma concentrations of additional PFASs.
(DOCX)

S4 Table. Logistic regression odds ratios (ORs) of increased COVID-19 severity at an increase by 1 ng/mL in plasma concentrations of all PFASs detected.
(DOCX)

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Short-chain per- and polyfluoralkyl substances (PFAS) effects on oxidative stress biomarkers in human liver, kidney, muscle, and microglia cell lines

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ABSTRACT

Long-chain per- and polyfluoralkyl substances (PFAS) are ubiquitous contaminants implicated in the induction of intracellular reactive oxygen species (ROS), compromising antioxidant defense mechanisms *in vitro* and *in vivo*. While a handful of studies have assessed oxidative stress effects by PFAS, few specifically address short-chain PFAS. We conducted an evaluation of oxidative stress biomarkers *in vitro* following exposures to low (1 nM) and high (1 μM) concentrations of five short-chain PFAS compounds: perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), [undecafluoro-2-methyl-3-oxahexanoic acid (HFPO-DA)], 6:2 fluorotelomer alcohol (6:2 FTOH) and perfluorohexanesulfonic acid (PFHxS). We conducted experiments in human kidney (HEK293-hTLR2), liver (HepaRG), microglia (HMC-3), and muscle (RMS-13) cell lines. Fluorescence microscopy measurements in HepaRG cells indicated ROS generation in cells exposed to PFBS and PFHxA for 24 h. Antioxidant enzyme activities were determined following 24 h short-chain PFAS exposures in HepaRG, HEK293-hTLR2, HMC-3, and RMS-13. Notably, exposure to PFBS for 24 h increased the activity of GPX in all four cell types at 1 μM and 1 nM in HepaRG and RMS-13 cells. Every short-chain PFAS evaluated, except for PFHxS, increased the activity of at least one antioxidant enzyme. To our knowledge, this is the first study of its kind to explore antioxidant defense alterations to microglia and muscle cell lines by PFAS. The findings of this study hold great potential to contribute to the limited understanding of short-chain PFAS mechanisms of toxicity and provide data necessary to inform the human health risk assessment process.

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large group of industrial compounds distinguished by the presence of highly stable carbon-fluorine chains that have made them suitable for use in a broad range of consumer products. These compounds' structural characteristics and widespread use have resulted in ubiquitous and persistent environmental contamination rendering them a scientific, regulatory, and public concern (Sunderland et al., 2019). Human exposure to PFAS is documented to be primarily through drinking water sources near fluorochemical manufacturing locations and contaminated food (Daly et al., 2018; DeLuca et al., 2022; Domingo and Nadal, 2019). In addition, PFAS-containing aqueous film-forming foam (AFFF) used in training exercises on military bases and airport facilities is a significant source of groundwater and soil contamination (Garrett et al., 2022). Inhalation of dust particles and dermal contact with household cleaning products have also been reported as routes of exposure (East et al., 2021). Recently, a study from Muensterman et al. (2022) found that PFAS-treated facemasks worn for long periods while combatting the spread of COVID-19 may have contributed to human exposure from dermal absorption, inhalation of gas-phase PFAS and ingestion of particulate-phase PFAS.

Perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are long-chain ($C \geq 8$) perfluorinated compounds that are often considered to be the most problematic PFAS. However, the import and manufacturing of long-chain PFAS has halted in the United States (Teaf et al., 2019; USEPA, 2016). Short-chain PFAS have since emerged as alternatives to long-chain PFAS. The decrease in the carbon-fluorine chain was designed to be less persistent in tissues and, by extension, less toxic (Jensen and Warming, 2015). However, long-chain and short-chain PFAS are known to be readily absorbed and strongly bind human serum albumin following exposure, leading to enhanced distribution throughout the body (Moro et al., 2022). Human PFAS exposure has been liked to an increased risk of thyroid disease, increased blood cholesterol, immune suppression, cancers of the kidney and testes, cardiovascular disease, kidney disease, liver damage, neurological

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disruptions, type II diabetes, osteoarthritis, and respiratory illnesses (ASTR, 2018; DeWitt, 2015; Li et al., 2021). Sheng et al. (2018) used the human liver HL-7702 cell line to determine the cell viability impacts of PFOA, PFOS, 6:2 fluorotelomer carboxylic acid (6:2 FTCA), 6:2 fluorotelomer sulfonic acid (6:2 FTSA), 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA), and hexafluoropropylene oxide (HFPO). Compared with PFOA and PFOS, 6:2 Cl-PFESA, HFPO trimer acid (HFPO-TA), HFPO tetramer acid (HFPO-TeA), and 6:2 FTSA showed greater toxic effects on cell viabilities. Therefore, short-chain alternatives may have more significant hepatotoxicity than the long-chain PFAS – highlighting the need to further explore the risks of short-chain PFAS to human health.

There is also a need to understand PFAS effects in a broader range of cell types representing various human tissues. For example, few studies evaluate the impact of PFAS on skeletal muscle despite evidence that accumulation in these tissues occurs with the bodily distribution of PFAS in humans (Cao and Ng, 2021). PPARα expression levels are high in skeletal muscle, and disruptions to signaling in these tissues represent a potential target of PFAS-induced metabolic effects (Jiang et al., 2015). Moreover, disruptions to mitochondrial function are often cited as a significant pathway in xenobiotic-induced organ toxicities, necessitating the advancement of *in vitro* models suitable for mitochondrial toxicity assays that can be employed in mechanistic investigations (Meyer et al., 2013; Rana et al., 2019). In this study, we included RMS-13, a human skeletal muscle cell line used to study cellular oxygen consumption rates and the regulation of mitochondrial biogenesis (Barretina et al., 2012; Hinson et al., 2013; Ohnstad et al., 2011; Roberts et al., 1989).

Our choice to use HEK293-hTLR2 was based on understanding the mechanisms of PFAS-induced kidney injury. HEK293 is a commonly used cell line; however, the original lineage lacks a toll-like receptor 2 (TLR2) involved with inflammatory immune disorders. Therefore, HEK293-hTLR2 cells were designed to study the stimulation of human TLR2, which may have implications for understanding the role of PFAS-induced kidney injury (InvivoGen, n.d.). This study also includes HMC-3, a human fetal brain-derived microglial cell line selected based on studies that have implicated oxidative and inflammatory responses associated with PFAS-induced tissue damage in the brain (Wang et al., 2021).

Although there are many *in vitro* studies using liver cell lines, many of these have been conducted in the HepG2 cell line (Behr et al., 2018, 2020; Dale et al., 2022; Eriksen et al., 2010; Ojo et al., 2020, 2021; Wen et al., 2020; Wielsøe et al., 2015). HepG2 cells express conjugating enzymes and demonstrate many liver-specific functions but lack a functional expression of almost all the relevant human liver cytochrome P450s (Donato et al., 2015; Skolik et al., 2021). On the other hand, the HepaRG human liver cell maintains essential hepatic functions after differentiation, including high expression of xenobiotic-metabolizing enzymes and drug transporters, providing metabolic competence comparable to primary human hepatocyte cultures (Franzosa et al., 2021; Kamalian et al., 2018; Ott et al., 2017; Solan et al., 2022; Tascher et al., 2019).

In human health risk assessment, cancer mechanisms are essential for hazard identification, and mechanistic evidence supports hazard classifications. Smith et al. (2016) conducted a scoping literature review to identify 10 key characteristics exhibited by established human carcinogens, including the ability to (1) form DNA and protein adducts as a direct-acting or metabolically activated electrophile; (2) act as a genotoxicant; (3) cause genomic instability or alter DNA repair mechanisms; (4) induce epigenetic alterations; (5) induce oxidative stress; (6) cause chronic inflammation; (7) have immunosuppressive effects; (8) modulate receptor activity; (9) facilitate aberrant replication in cells (immortalization); and (10) alter cell proliferation, death, or nutrient supply. Agonism of peroxisome proliferating (PPAR) receptors in mammalian models have been proposed as a significant driver of the adverse health effects associated with PFAS exposure (Corton et al., 2018; DeWitt et al., 2009; Pawlak et al., 2015). However, the carcinogenic potential of PFAS has been proposed to be through the generation of cellular oxidative stress (Wielsøe et al., 2015).

Additionally, previous studies have pointed to increased ROS generation as plausible mechanisms of PFOA- and PFOS- induced toxicity *in vitro* and *in vivo* (Jiao et al., 2021; Liao et al., 2012; Qian et al., 2010; Shi and Zhou, 2010; Souders II et al., 2021; Wielsøe et al., 2015). The cytotoxicity estimates provided in our previous study (Solan et al., 2022) demonstrated marginal differences between short- and long-chain PFAS for some of the cell types evaluated, therefore, we hypothesized that short-chain PFAS would increase antioxidant enzymes, indicating similar mechanisms of toxicity. In the present study, we evaluated biomarkers of oxidative stress effects *in vitro* following exposure to five short-chain PFAS compounds: perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), [undecafluoro-2-methyl-3-oxa hexanoic acid (HFPO-DA)], 6:2 fluorotelomer alcohol (6:2 FTOH) and perfluorohexanesulfonic acid (PFHxS). This study aimed (1) to determine if overall ROS production would occur following exposure to short-chain PFAS in HepaRG and (2) to determine antioxidant enzyme activities as biomarkers of specific ROS production. Exposures were conducted using the previously described human cell lines that are representative of four different tissue types, including muscle (RMS-13), kidney (HEK293-hTLR2), brain microglia (HMC-3), and liver (HepaRG).

2. Materials and methods

2.1. Chemicals

Perfluorobutanesulfonic acid (PFBS), perfluorohexanoic acid (PFHxA), [undecafluoro-2-methyl-3-oxahexanoic acid (HFPO-DA)], 6:2 fluorotelomer alcohol (6:2 FTOH), perfluorohexanesulfonic acid (PFHxS), ethanol (EtOH), and Dimethyl Sulfoxide (DMSO) were obtained from Sigma-Aldrich (St. Louis, MO). All PFAS chemical stock solutions were prepared in analytical grade DMSO, except for HFPO-DA, which was prepared in EtOH. This solvent was selected to prevent the formation of HFPO-DA's degradation product, heptafluoropropyl-1,2,2,2-tetrafluoroethyl ether (fluoroether E−1), as recommended by Liberatore et al. (2020). More detailed information for each compound tested is presented in Table 1.

Eagle's Minimum Essential Medium and RPMI-1640 media were obtained from American Type Culture Collection (ATCC, Manassas, VA). Williams E Medium, trypsin-EDTA, Penicillin/streptomycin, and phosphate-buffered saline (PBS) were obtained from Life Technologies (ThermoFisher Scientific, Waltham, MA). Fetal bovine serum was obtained from Atlas Biologicals (Fort Collins, CO).

2.2. Cell culture and exposures

The cell lines and the culture conditions used in this study are shown in Table 2. All growth media was supplemented with 2 mM L-glutamine, 50 U/mL penicillin G, and 50 μg/mL streptomycin. Experiments were carried out on confluent cell monolayers. Serum-supplemented media during *in vitro* exposures to PFAS have been reported to mitigate toxicity by reducing cellular uptake (Bangma et al., 2020; Solan and Lavado, 2021; Zhang et al., 2020). Therefore, the growth medium was changed and replaced by FBS-free media before dosing with the selected PFAS chemicals or solvent control medium. We have previously demonstrated no significant differences in the expansion and survival of these cell lines in FBS-free media for up to 72 h compared to FBS media controls (Solan et al., 2022).

Solvent sensitivity determinations for the cell lines used were also reported by Solan et al. (2022). Notably, DMSO and ethanol showed no significant mortality up to 1% v/v during 48 h exposures. Based on those results, DMSO (0.1% v/v in 6-well plates, 1% v/v in 96-well plates) was the solvent vehicle for 6:2 FTOH, PFBS, PFHxA, and PFHxS. EtOH was used as the solvent vehicle (0.1% v/v in 6-well plates, 1% v/v in 96-well plates) for HFPO-DA due to the rapid degradation of this compound in

Table 1
Short-chain PFAS chemicals used in this study. All chemicals were of analytical grade (≥97%) (CAS: Chemical Abstracts Service. IUPAC: International Union of Pure and Applied Chemistry. MW: Molecular Weight (g/mol). PFCAs: Perfluoroalkyl carboxylic acids. PFSAs: Perfluoroalkyl sulfonic acids).

Preferred name	Abbreviation	Classification	CAS	IUPAC	Formula	MW	Provider
Undecafluoro-2-methyl-3-oxahexanoic acid	HFPO-DA	PFCAs	13,252-13-6	2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy) propanoic acid	$C_6HF_{11}O_3$	330.05	Synquest Labratories (2121-3-13)
Perfluorohexanoic acid	PFHxA	PFCAs	307-24-4	Undecafluorohexanoic acid	$C_6HF_{11}O_2$	314.05	Sigma-Aldrich (43,809)
6:2 Fluorotelomer alcohol	6:2 FTOH	Other	647-42-7	Potassium 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctan-1-ol	$C_8H_5F_{13}O$	364.10	Sigma Aldrich (370,533)
Perfluorobutanesulfonic acid	PFBS	PFSAs	375-73-5	Nonafluorobutane-1-sulfonic acid	$C_4HF_9O_3S$	300.09	Sigma-Aldrich (562,629)
Perfluorohexanesulfonic acid potassium salt	PFHxS	PFSAs	3871-99-6	1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluorohexane-1-sulfonate	$C_6HF_{13}KO_3S$	438.20	Sigma-Aldrich (50,929)

Table 2
Human cell lines used in this study (ATCC: American Type Culture Collection. EMEM: Eagle's minimum essential medium. FBS: fetal bovine serum).

Cell line	Human Tissue Type	Provider	Culture Media	Culture Conditions	Reference
HEK293-hTLR2	Kidney	InvivoGen (hkb-htlr2)	EMEM + 10% FBS	Humidified incubator with 5% CO_2 at 37 °C	Lin et al. (2014)
HepaRG	Liver	BioPredic International (HPR101)	William's E Medium + 2 mM Glutamax + 10% FBS + 5 μg/mL insulin +50 μM hydrocortisone hemisuccinate	Humidified incubator with 5% CO_2 at 37 °C	Gripon et al. (2002)
HMC-3	Brain	ATCC (CRL-3304)	EMEM + 10% FBS	Humidified incubator with 5% CO_2 at 37 °C	Janabi et al. (1995)
RMS-13	Muscle	ATCC (CRL-2061)	RPMI-1640 Medium + 10% FBS	Humidified incubator with 5% CO_2 at 37 °C	Douglass et al. (1987)

aprotic, polar solvents (Liberatore et al., 2020). The experiments were performed with biological (plates), and technical (wells) replicates. Three to four biological with four technical replicates were used.

All experiments were performed at a high PFAS concentration (1 μM) and a low PFAS concentration (1 nM). These concentrations were selected below the median effective concentrations (EC_{50}) for cytotoxicity values determined by Solan et al. (2022).

2.3. HepaRG differentiation

Undifferentiated HepaRG cells were grown using the culture conditions in Table 2, as recommended by Tascher et al. (2019). Undifferentiated HepaRG cells were maintained in growth media for 28 days before undergoing differentiation. When confluence was reached, the HepaRG medium was changed to growth medium supplemented with 1.7% DMSO for two additional weeks, leading to confluent differentiated cultures containing hepatocyte-like cells and choliangiocytes. HepaRG exposures were started following the differentiation process.

2.4. Detection of reactive oxygen species (ROS) by microscopy

Oxidative stress was assessed in glass-bottomed 96-well fluorescence plates (Costar) of differentiated HepaRG cells following 24 h exposures to PFAS. Our lab-established protocol calls for the use of 96-well plates with glass bottoms that are optimal for visualization but incompatible with the other cell lines used in this study. Therefore, the detection of ROS using microscopy was only conducted in HepaRG. 100 μM of tert-butyl hydroperoxide (tBHP) was included as a positive control in each plate. CellROX Deep Red Reagent (Life Technologies Corporation, Carlsbad, CA) is a cell-permeable fluorogenic probe designed to measure ROS in living cells.. CellROX Deep Red is non-fluorescent while in a reduced state; upon oxidation by free radical ROS, the probe exhibits bright fluorescence that can be measured using an appropriate filter. Cells were stained with 5 μM of CellROX Deep Red Reagent (Life Technologies, Inc.) following the manufacturer's instructions. Cells were subsequently fixed with 4% paraformaldehyde, followed by counter-staining with 1 μg/mL of Hoechst 33,342 (Life Technologies Corporation, Carlsbad, CA). Finally, cells were washed with PBS, and the resulting fluorescence was measured using a Lionheart FX automated microscope (BioTek, Winooski, VT). Imaging was carried out with the Texas Red and DAPI imaging filter cubes to capture the signal from the CellROX Deep Read Reagent and Hoechst 33,342, respectively, using 4x magnification. Images were analyzed using Gen 5 version 3 software (BioTek). The mean intensity of the CellROX was normalized to the cell count obtained from the Hoechst 33,342 counter-stained nuclei.

2.5. Collection of cell lysates and protein determination

Cell lysates were collected from HEK293-tTLR2, HMC-3, HepaRG, and RMS-13 following 24 h exposure to PFAS in 6-well plates. Preparations were completed following the instructions for cell lysate preparation from the manufacturer of the antioxidant enzyme bioassay kits (Cayman Chemical, Ann Arbor, MI). Briefly, cells were collected using a rubber policeman and centrifuged at 2,000*g* for 10 min at 4 °C. The cell pellet was homogenized in cold 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA and centrifuged once more at 10,000*g* for 15 min at 4 °C. Finally, the supernatant was collected and immediately stored at −80 °C. Protein concentrations were determined using a Coomassie Blue method kit (Pierce Inc., Rockford, IL) and bovine serum albumin (BSA) as a standard. The protein content of the cell lysates included in the assays was between 50 and 100 μg/mL, with the specific concentration of each sample used to determine the final activity calculations.

2.6. Measurement of antioxidant enzymes

Measurements of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) were performed in the cell lysates using 96-well plate bioassay kits (Cayman Chemical, Ann Arbor, MI). CAT was measured from its reaction with methanol in the presence of hydrogen peroxide; the formaldehyde produced was measured colorimetrically with the chromogen 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole at 540 nm. Total SOD was measured by detecting superoxide radicals generated by xanthine oxidase and hypoxanthine in the presence of a tetrazolium salt. A unit of SOD is defined as the concentration of enzyme necessary for 50% dismutation of the superoxide radical measured as the change in absorbance at 450 nm per

minute. Lastly, GPX activity was indirectly measured with a coupled reaction with glutathione reductase, where endogenous NADPH was oxidized to $NADP^+$; the decreasing rate of GPX activity was measured kinetically at 340 nm for 5 min. The manufacturer-supplied assay-positive controls were used for quality control during data analysis.

2.7. Statistics

All data were analyzed before statistical analysis to meet the homoscedasticity and normality assumptions. Statistical analyses and graphing were carried out using GraphPad Prism version 9 (GraphPad Software, San Diego, CA). All values were calculated using the average of the assessed endpoint of the three independent experimental results and their associated errors. Statistical significance was assessed using one-way ANOVA tests. A p-value of <0.05 was considered statistically significant unless otherwise indicated. Dunnett's test was used to test significant differences with the respective control if an overall significance was detected.

3. Results

3.1. ROS screening in HepaRG cells

Fluorescence microscopy measurements of CellROX Deep Red fluorescence intensity in HepaRG following 24 h exposures to low (1 nM) and high (1 μM) concentrations of short-chain PFAS are presented in Table S1 and Fig. 1. 100 μM of tertbutyl hydrogen peroxide (tBHP) was used as the positive control, the increase in intensity relative to both solvent controls (DMSO and EtOH) were significant ($p < 0.0001$).

Relevant to the solvent controls, none of the short-chain PFAS evaluated at 1 nM demonstrated a significant increase in fluorescence intensity in HepaRG after 24 h. However, 1 μM exposures of PFBS and PFHxA showed small increases that were 1.5 and 1.2 times the CellROX fluorescence intensity per cell of the DMSO control (2.01 ± 0.13) with mean values of 3.51 ± 0.54 ($p = 0.0002$) and 2.62 ± 0.35 ($p = 0.0039$), respectively.

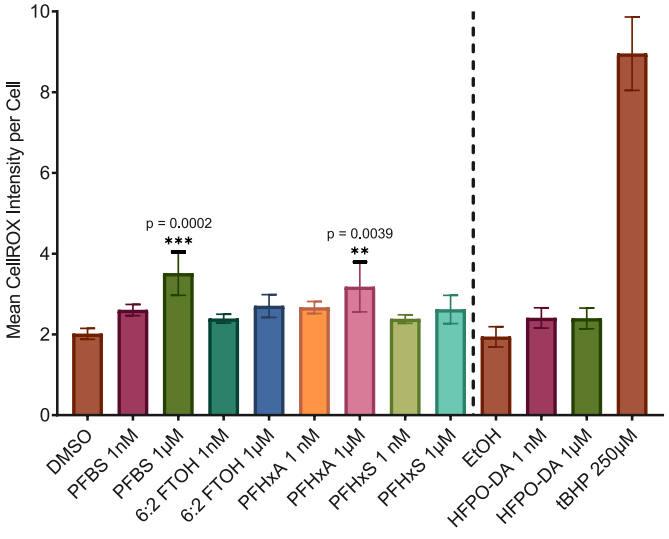

Fig. 1. Fluorescence microscopy measurements of CellROX Deep Red fluorescence intensity in HepaRG following 24 h exposure to low (1 nM) and high (1 μM) concentrations of short-chain PFAS. 100 μM of tertbutyl hydrogen peroxide (tBHP) was used as the positive control, the increase in intensity for both solvent controls (DMSO and EtOH) were significant ($p < 0.0001$). Data are presented as mean per cell count ± std. Deviation. Asterisks represent treatments with significant intensity relative to their respective solvent controls (One-way ANOVA, Dunnett's test, $p < 0.05$; n = 4).

3.2. Glutathione peroxidase (GPX) activity in cell lysates

GPX activities are presented as nmol/min/mg protein in Fig. 2. One unit of GPX activity is defined as the concentration of enzyme that will cause the oxidation of 1.0 nmol of NADPH to $NADP^+$ by GPX per minute at 25 °C (Paglia and Valentine, 1967). The activity values were corrected for the protein in the sample. Averages are shown in the Supplementary Data in Table S2.

In HEK293-hTLR2 cells, the GPX activity in the DMSO control was 22.4 ± 8.50 nmol/min/mg protein. The only notable change in activity was found in exposures to 1 μM of PFBS, with a mean activity of 165 ± 62.0 nmol/min/mg protein ($p = 0.0005$). Similarly, exposures to 1 μM of PFBS in HMC-3 cells significantly increased GPX activity relative to the DMSO control (36.2 ± 21.3 nmol/min/mg protein) with the mean activity of 183 ± 39.3 nmol/min/mg protein ($p = 0.0066$).

The DMSO control values for HepaRG and RMS-13 were 243 ± 76.8 and 180 ± 64.1 nmol/min/mg protein, respectively. In HepaRG, PFBS exposures of 1 nM and 1 μM increased GPX activity in a dose-dependent fashion from 746 ± 256 nmol/min/mg protein ($p = 0.0051$) and 839 ± 159 nmol/min/mg protein ($p = 0.0010$), respectively. For RMS-13, the 1 nM and 1 μM PFBS treatments also demonstrated dose-dependent GPX activities with corresponding values of 384 ± 103 nmol/min/mg protein ($p = 0.0479$) and 414 ± 21.1 nmol/min/mg protein ($p = 0.0198$).

3.3. Catalase (CAT) activity in cell lysates

HEK293-hTLR2 was the only cell line that had an increase in CAT activity following a 24 h exposure to PFBS at 1 μM with a mean activity of 251 ± 51.5 nmol/min/mg protein, which was significant compared to the DMSO solvent control activity value of 119 ± 16.4 nmol/min/mg protein ($p = 0.0095$) (Fig. 3). One unit of CAT activity is defined as the concentration of enzyme needed to initiate the formation of 1.0 nmol of formaldehyde produced by enzyme in methanol in an optimal concentration of H_2O_2 per minute at 25 °C (Wheeler et al., 1990).

HEK293-hTLR2 also showed an increase in CAT activity in the exposures to 1 μM of HFPO-DA with a mean of 200 ± 27.1 nmol/min/mg protein, as shown in Table S3. This was significant compared to the corresponding EtOH solvent control activity of 129 ± 25.2 nmol/min/mg protein ($p = 0.0178$). Increases in CAT activity with 1 μM of HFPO-DA were also observed in HepaRG and RMS-13 with mean activities of 314 ± 22.4 and 383 ± 130 nmol/min/mg protein, respectively. HMC-3 showed no significant catalase activity increases for any PFAS exposure conditions (Fig. 3).

3.4. Superoxide dismutase (SOD) activity in cell lysates

Similar to the observations with GPX and CAT activities in HEK293-hTLR2 cells, a notable change in activity was found in exposures to 1 μM of PFBS (Fig. 4), with a mean activity of 30.4 ± 8.84 U/mg protein ($p < 0.0001$). Significance was also observed in the mean SOD activity in HEK293-hTLR2 cells exposed to 1 μM of 6:2 FTOH (22.6 ± 5.33 U/mg protein) relative to the DMSO solvent control (7.25 ± 0.906, $p = 0.0025$). As shown in Fig. 4 and Table S4, HepaRG SOD activity was increased by PFHxA at 1 μM with a mean activity of 8.74 ± 1.68 U/mg protein compared to the DMSO solvent control activity value of 4.71 ± 0.685 U/mg protein ($p = 0.0005$).

Likewise, the HMC-3 treatments of 1 μM 6:2 FTOH ($p = 0.0421$) and PFHxA ($p = 0.0421$) showed a significant increase compared to the DMSO solvent control (1.58 ± 1.17 U/mg protein), with similar mean activities of 3.38 ± 0.283 and 3.38 ± 1.13 U/mg protein, respectively. For RMS-13, the 1 μM PFBS and HFPO-DA treatments increased activities with corresponding values of 9.86 ± 1.22 U/mg protein ($p = 0.0019$), and 6.77 ± 0.754 U/mg protein (p = 0.0247), which were significant relative to the respective DMSO (4.35 ± 1.17 U/mg protein) and EtOH (4.35 ± 1.17 U/mg protein) controls.

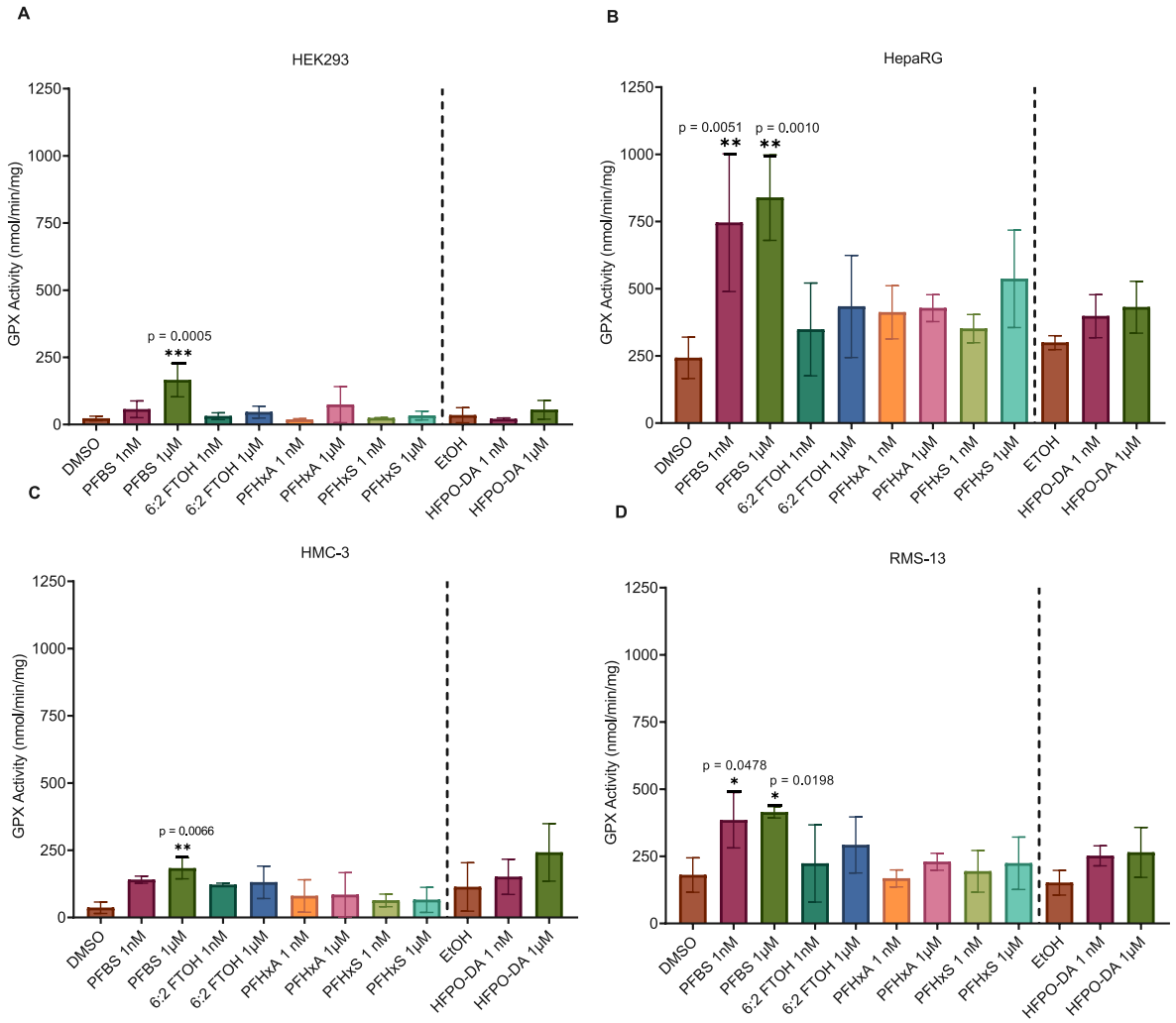

Fig. 2. Glutathione peroxidase (GPX) activity following 24 h exposure to low (1 nM) and high (1 µM) concentrations of short-chain PFAS in (**A**) HEK293-hTLR2 (denoted as HEK293 in the figure), (**B**) HepaRG, (**C**) HMC-3 (**D**) and RMS-13. Data are presented as mean GPX activity (nmol/min/mg protein) ± std. Deviation. Asterisks represent treatments with significant intensity relative to their respective solvent controls (One-way ANOVA, Dunnett's test, $p < 0.05$; n = 3).

4. Discussion

Blood PFAS levels in humans have been associated with disruptions in redox-related pathways and biomarkers, with evidence derived from proteomic, metabolomic, and lipidomic analyses (Omoike et al., 2021; Taibl et al., 2022). Studies point to PFOA and PFOS exposure resulting in increased ROS generation and altering oxidative defense mechanisms *in vitro* and *in vivo*. (Jiao et al., 2021; Liao et al., 2012; Qian et al., 2010; Shi and Zhou, 2010; Souders II et al., 2021; Wielsøe et al., 2015). Thus, the carcinogenic potential of these compounds has been proposed to be through the generation of cellular oxidative stress (Wielsøe et al., 2015). While redox imbalances are a common characteristic of human carcinogens, oxidative stress is not unique to cancer induction and may not necessarily lead to adverse outcomes (Smith et al., 2016). Nonetheless, redox imbalances resulting in cellular oxidative stress are associated with several pathological conditions and chronic diseases, including cardiovascular disease, diabetes, and Alzheimer's disease (García-Sánchez et al., 2020; Pisoschi and Pop, 2015; Smith et al., 2016) (Pisoschi et al., 2021).

Alterations to the activity levels of antioxidant enzymes are considered biomarkers of antioxidant reactions in cells due to their protective role against free radical-induced damage (Cuello et al., 2010; Murphy et al., 2022). ROS participate in many cellular processes to maintain homeostasis by activating essential cell growth and proliferation signaling pathways (Cecerska-Heryć et al., 2021). Glutathione peroxidase (GPX) is an enzyme that plays a critical role in reducing lipid and hydrogen peroxides (Espinoza et al., 2008). Superoxide dismutase (SOD) is a frontline antioxidant enzyme that catalyzes the dismutation of superoxide (O2–) to hydrogen peroxide (Trist et al., 2021). Catalase (CAT) enzymes mitigate oxidative stress by destroying cellular hydrogen peroxide to produce water and oxygen (Nandi et al., 2019). Antioxidant enzymes are said to have "paradoxical" roles in physiological processes – while poor antioxidant enzyme is frequently cited as a key driver of oxidant-induced damage, overexpression may also be problematic (Lei et al., 2016). When deviations from basal physiological activity occur, and free radical scavenging systems are overwhelmed, inflammation, hypersensitivity, and autoimmune conditions may result (García-Sánchez et al., 2020).

In our initial evaluation of ROS production in HepaRG cells, the CellROX fluorescence microscopy results indicated the generation of ROS at 1 µM of PFBS and PFHxA. Increases in GPX and SOD activities that were similarly detected following exposure to these compounds supported the microscopy observations. However, the fluorescent probe used to assess ROS, CellRox DeepRed, was not predictive of the increased CAT activity following HFPO-DA exposures. CellRox DeepRed can sensitively detect free radical ROS typically associated with the GPX and SOD activity; CAT uses nonradical ROS as its substrate.

GPX activity increases were observed in HepaRG, HMC-3, HEK293-

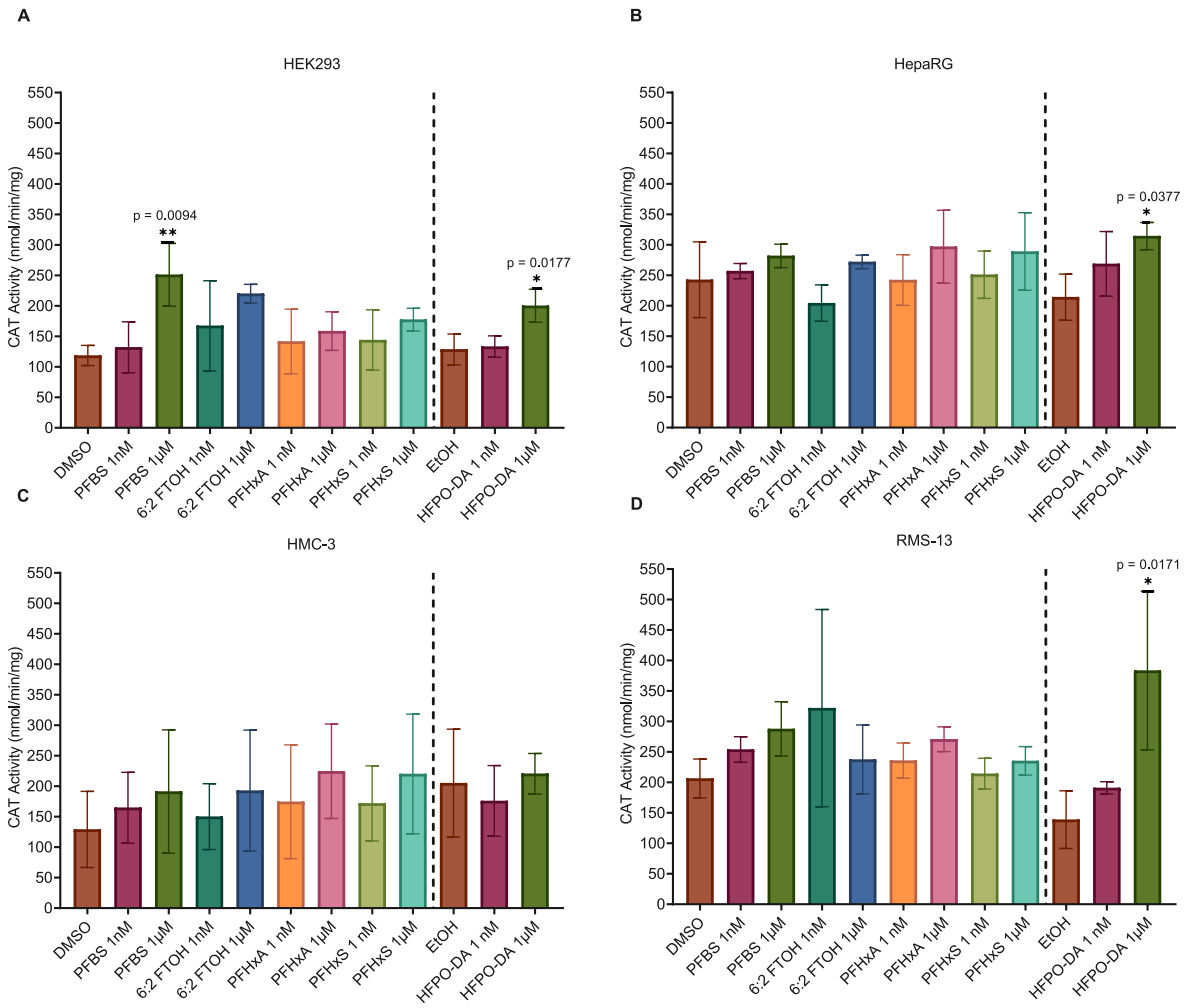

Fig. 3. Catalase (CAT) activity following 24 h exposure to low (1 nM) and high (1 μM) concentrations of short-chain PFAS in (**A**) HEK293-hTLR2 (denoted as HEK293 in the figure), (**B**) HepaRG, (**C**) HMC-3 (**D**) and RMS-13. Data are presented as mean CAT activity (nmol/min/mg protein) ± std. Deviation. Asterisks represent treatments with significant intensity relative to their respective solvent controls (One-way ANOVA, Dunnett's test, $p < 0.05$; n = 3).

hTLR2, and RMS-13. For HepaRG and RMS-13, these increases were observed at low (1 nM) and high (1 μM) concentrations. GPX breaks down hydrogen peroxides (H2O2) to water and protects cells from oxidative stress by inhibiting the lipid peroxidation process (Ighodaro and Akinloye, 2018). In addition, the mitochondrial isoform of GPX is involved in mediating the apoptotic response to oxidative stress (Liang et al., 2009). Studies evaluating GPX activity following long-chain PFAS exposure have had variable outcomes, with some reporting increased activity and others reporting decreases or no changes (Lee et al., 2020; Shi et al., 2018). Similar to our observations with PFBS, the PFOS alternative, F–53 B, was found to significantly induce GPX activity in zebrafish larvae and adults (Wu et al., 2019a, 2019b).

The enzyme with the fewest observations of increased enzyme activity following the PFAS exposures was catalase (CAT). The short-chain PFAS exposures had no significant effect on CAT activity in HMC-3. However, 1 μM HFPO-DA increased CAT activity in the other three cell lines, HepaRG, HEK293, and RMS-13. These findings align with a recent study in zebrafish embryos where observed CAT activity increases were also observed following exposures to HFPO-DA (Wang et al., 2023). The enzymatic activities of CAT and GPX often have a complementary and inversely correlated role within the antioxidant system (Bonato et al., 2020; Franchi et al., 2012). Our results were consistent with this, as 1 μM HFPO-DA treatments, generally, did not have simultaneous increases in GPX activity.

The most frequent observations of increased enzyme activity following the exposures were with SOD. SOD enzymes are often cited as the first line of defense against oxygen-derived free radicals. They can be rapidly induced to inhibit oxidative damage in mitochondria stress in response to accumulations of ROS (He et al., 2017). Bonato et al. (2020) reviewed outcomes reported in environmental models, noting that exposure to PFAS leads to a generalized increase in SOD activity.

Differences in cell line sensitivity were apparent in this study. For example, among the cell types evaluated, HMC-3 had the fewest significant activity increases following 24 h exposures to the short-chain PFAS despite viability losses demonstrated near 1 μM in 48 h cytotoxicity experiments (Solan et al., 2022). This may partly be explained by the type of tissue from which HMC-3 was derived – microglia. ROS generation is tightly regulated as a crucial part of the defense mechanisms employed in brain immune functions (Simpson and Oliver, 2020). In addition, overactivation-induced apoptosis is time-dependent in microglia (Liu et al., 2008).

In contrast, HEK293-hTLR2 frequently demonstrated activity increases despite negligible viability decreases within the concentration range used in our cytotoxicity study. In addition, it has been established that HEK293 cells have high expression of antioxidant enzymes and are not easily overwhelmed by ROS generation (Forkink et al., 2015). This may indicate that the HEK293-hTLR2 cellular redox homeostasis is being altered following exposure to these PFAS but may not be overwhelming the antioxidant capacity of the cells.

As mitochondria are a significant source of intracellular ROS, it is

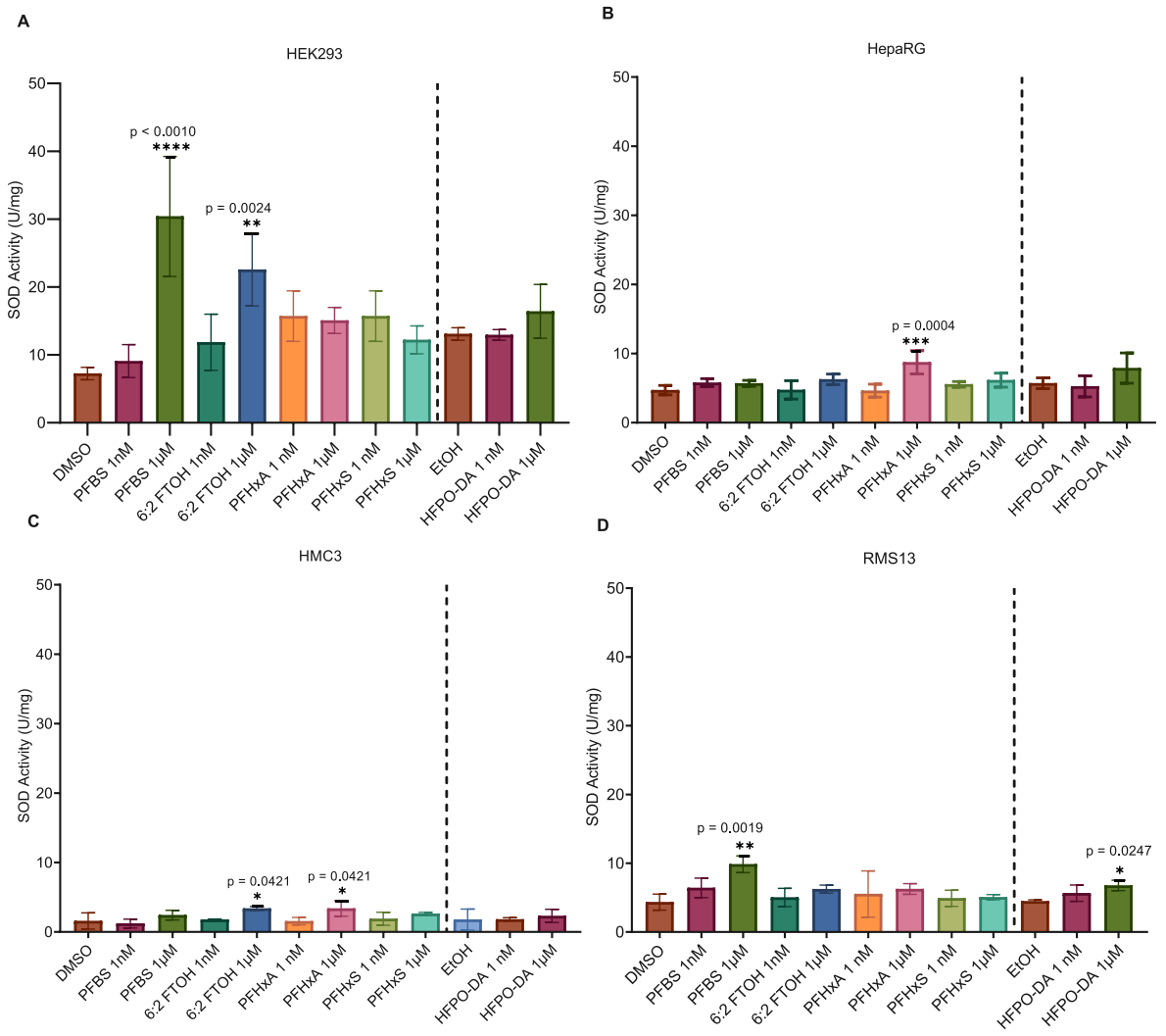

Fig. 4. Superoxide dismutase (SOD) activity following 24 h exposure to low (1 nM) and high (1 µM) concentrations of short-chain PFAS in (**A**) HEK293-hTLR2 (denoted as HEK293 in the figure), (**B**) HepaRG, (**C**) HMC-3 (**D**) and RMS-13. Data are presented as mean SOD activity (U/mg protein) ± std. Deviation. One unit (U) is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical measured in change in absorbance per minute at 25 °C and pH 8.0. Asterisks represent treatments with significant intensity relative to their respective solvent controls (One-way ANOVA, Dunnett's test, $p < 0.05$; n = 3).

crucial to consider that differences in sensitivities between each cell type may be related to differences in energy needs and endogenous substrates. Using rat tissues, Tahara et al. (2009) demonstrated ROS formation rates varied considerably in the liver, brain, kidney, and skeletal muscle due to attributing the tissue-specific differences to energy needs and the composition of endogenous substrates.

The observations here may indicate that PFAS-induced oxidative stress may still occur at concentrations the below the EC_{50}s we previously reported (Solan et al., 2022). GPX and SOD activities in all four cell types were frequently associated with PFBS, 6:2 FTOH, and PFHxA exposures. However, HFPO-DA had the strongest associations with CAT activity. This discrepancy highlights the notion that, while some of the PFAS evaluated in this study were associated with dysregulations to redox homeostasis, the mechanisms are likely distinct despite similar cytotoxicity estimates. In an exploration of PFAS/ROS-induced mechanisms of toxicity, Xu et al. (2019) used primary hepatocytes to demonstrate that PFOA and PFOS could interact with SOD via hydrophobic forces, with aggregations forming at low concentrations, resulting in activity increases. The authors found that the aggregations were destroyed as the concentrations increased over 500 µM, overwhelming the antioxidant capacity of the cells and leading to cell apoptosis and death. Molecular docking studies have demonstrated that while longer chains favor stronger hydrophobic interactions, several short-chain PFAS (including PFHxS, PFBS, PFHxA, and 6:2 FTOH) have interaction energies with some proteins that were similar to longer-chain PFAS (Dharpure et al., 2022). The molecular drivers of PFOA- and PFOS-induced apoptosis through the destabilization of SOD and the possibility of similar interactions may partially explain the observations here. However, further investigation of structural dynamics would be necessary to confirm this.

Furthermore, the carcinogenic potential of PFAS and the role of oxidative stress may be understood through the lens of Adverse Outcome Pathways (AOPs). The AOP concept was developed by Ankley et al. (2010) as conceptual that links information from molecular initiating events (MIEs) and intervening key events (KEs) to an adverse outcome at higher levels of biological organization. There are currently two Working Party on Hazard Assessment (WHPA)/Working Group of the National Coordinators of the Test Guidelines Programme (WNT) endorsed AOPs with oxidative stress as a key event (https://aopwiki.org/events/1392) including Cyp2E1 Activation Leading to Liver Cancer (https://aopwiki.org/aops/220) and Oxidative stress and Developmental impairment in learning and memory (https://aopwiki.org/aops/17). In the AOP outlining chronic activation of Cyp2E1 as an MIE, subsequent increases of reactive oxygen species (KE1), causes cytoxicity

in hepatocytes (KE2) that results in dysregulated cellular proliferation (KE3) as the liver attempts to regenerate itself following injury. The sequence of key events leads to liver tumor formation as an adverse outcome (AO).

A significant strength of our study was using the HepaRG cell line. Indeed, much of the *in vitro* research on PFAS and endpoints related to oxidative stress has focused on the impact of long-chain PFAS on liver cells, with many using HepG2 cells (Eriksen et al., 2010; Ojo et al., 2021; Wielsøe et al., 2015). In contrast to our findings, a study in HepG2 did not find ROS production following PFAS exposure (Ojo et al., 2021). Unlike HepG2 cells, the HepaRG cells used in our study have demonstrated high sensitivity in detecting oxidative stress induction and have retained distinct liver functions, drug-metabolizing enzymes, hepatobiliary transporters, and nuclear receptors that are essential for understanding the mechanism of hepatoxicity (Donato et al., 2022; Gomez-Lechon et al., 2008). The properties of the heme group in cytochrome P450 (CYP450) enzymes that are adequately expressed in HepaRG cells can facilitate ROS generation through reaction uncoupling or via reactive intermediates which modify endogenous substrates, including lipids, proteins, and nucleic acids, leading to oxidative stress (Veith and Moorthy, 2018). Further explorations into the complex dynamics between CYP450 expression, oxidative stress, and PFAS may help to elucidate the mechanistic underpinnings of exposure-related outcomes.

Some potential limitations to our study design include the culture conditions used and the use of solvents during exposures. Studies have reported that using a serum-supplemented medium during *in vitro* exposures to PFAS may mitigate toxic effects by reducing cellular uptake (Bangma et al., 2020; Solan and Lavado, 2021; Zhang et al., 2020). However, we previously tested cell growth and viability without adding FBS, and no significant differences were observed with the FBS-exposed cells up to 72 h (Solan et al., 2022). The study referenced above also evaluated the tolerance of the selected cell lines to EtOH and DMSO and showed no significant changes in growth, expansion, or survival up to 1% v/v. While the inclusion of the appropriate controls and our best efforts to ensure our test conditions would not influence the results, we acknowledge the exclusion of serum and the use of solvents has the potential to alter metabolic capacity in ways beyond those indicated by our preliminary studies and may have impacted the potency observations presented here.

5. Conclusion

Here we presented findings suggesting that short-chain PFAS can increase the activities of antioxidant enzymes following 24 h exposures. To our knowledge, this is the first study of its kind to explore biomarkers of oxidative stress by short-chain PFAS in microglia and muscle cell lines. It is apparent that there are tissue-specific differences in effects associated with short-chain PFAS exposure-related outcomes. Each cell line used here holds great potential to contribute to expanding our knowledge of PFAS health effects in humans. Moreover, while some of the PFAS evaluated in this study were associated with dysregulations to redox homeostasis, the mechanisms are likely distinct despite similar cytotoxicity estimates. The results presented here should be interpreted with caution due to the complexities of ROS and their reactions. While our results indicate increased enzyme activities as an adaptive response to oxidative stress, small amounts of ROS are crucial to cellular homeostasis, and increases in antioxidant enzymes may not necessarily indicate that oxidative damage will result from short-chain PFAS exposure.

Furthermore, the results here may support the quantitative understanding of AOPs that can be applied to PFAS hazard assessment. The utility of AOPs in risk assessment for a chemical or class of chemicals is limited by the availability of observational data and detailed mechanistic information. There is a need to continue to explore the complex relationship between PFAS mechanisms of toxicity, especially short-chain PFAS. Future studies should continue to use approaches that consider the complex physiochemical relationships between redox mechanisms and biotransformation using suitable models in a broad range of cell types to facilitate a more holistic view of human health effects using *in vitro* systems.

Credit author statement

MES and RL designed the experiments. MES, CK, and SS performed the experiments and analyzed the data. MES, CK, SS, and RL analyzed the data and wrote the paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.115424.

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Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers *in vitro*

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HIGHLIGHTS

- Effect on oxidative stress factors of seven long-chained PFAS was investigated.
- The selected PFAS were: PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnA and PFDoA.
- Four of the PFAS showed dose-dependent increase in DNA damage.
- Six PFAS increased ROS generation and the increase were dose-dependent for 2 PFAS.
- PFOA significantly decreased the total antioxidant capacity.

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ABSTRACT

Perfluoroalkylated substances (PFAS) have been widely used since 1950s and humans are exposed through food, drinking water, consumer products, dust, etc. The long-chained PFAS are persistent in the environment and accumulate in wildlife and humans. They are suspected carcinogens and a potential mode of action is through generation of oxidative stress. Seven long-chained PFAS found in human serum were investigated for the potential to generate reactive oxygen species (ROS), induce DNA damage and disturb the total antioxidant capacity (TAC). The tested PFAS were perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonic acid (PFOS), perfluoroctanoic acid (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnA), and perfluorododecanoate (PFDoA). Using the human hepatoma cell line (HepG2) and an exposure time of 24 h we found that all three endpoints were affected by one or more of the compounds. PFHxS, PFOA, PFOS and PFNA showed a dose dependent increase in DNA damage in the concentration range from 2×10^{-7} to 2×10^{-5} M determined by the comet assay. Except for PFDoA, all the other PFAS increased ROS generation significantly. For PFHxS and PFUnA the observed ROS increases were dose-dependent. Cells exposed to PFOA were found to have a significant lower TAC compared with the solvent control, whereas a non-significant trend in TAC decrease was observed for PFOS and PFDoA and an increase tendency for PFHxS, PFNA and PFUnA. Our results indicate a possible genotoxic and cytotoxic potential of the PFAS in human liver cells.

1. Introduction

Over the past 60 years perfluoroalkylated substances (PFAS) have been widely used in industrial and commercial applications. Long-chain PFAS are environmentally widespread, persistent, and accumulative in nature, animals and humans (Giesy and Kannan, 2001; Fromme et al., 2009). Humans are mainly exposed to the long-chain PFAS through food intake, house dust, and indoor air (Haug et al., 2011), and the average serum half-lives for perfluorohexane sulfonate (PFHxS), perfluorooctane sulfonate (PFOS), and perfluorooctanoate (PFOA) are 8.8, 5.4 and 2.3–3.8 years, respectively (Olsen et al., 2007; Bartell et al., 2010).

PFOA and PFOS are the most predominant PFAS and have been intensively studied although mainly in rodents. Hepatotoxicity, immunotoxicity, hormonal effects and, a possible carcinogenic potential are some of the observed effects in rodents (Lau et al., 2007). PFAS are potential endocrine disruptors and affect the

Abbreviations: DMSO, dimethyl sulfoxide; EtOH, ethanol; H_2O_2, hydrogen peroxide; LDH, lactate dehydrogenase; PBS, phosphate buffered saline; PFAS, perfluoroalkylated substances; PFCA, perfluorinated carboxylic acids; PFDA, perfluorodecanoate; PFDoA, perfluorododecanoate; PFHxS, perfluorohexane sulfonate; PFNA, perfluorononanoate; PFOA, perfluoroctanoic acid; PFOS, perfluorooctane sulfonic acid; PFOSA, perfluorooctanesulfonamide; PFSA, perfluorinated sulfonic acids; PFUnA, perfluoroundecanoate; ROS, reactive oxygen spices; TAC, total antioxidant capacity; tBuOOH, tert-butyl hydroperoxide.

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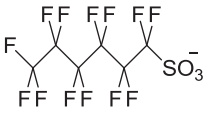
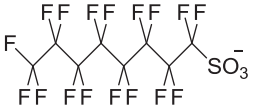
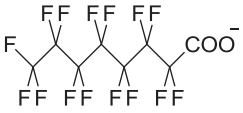
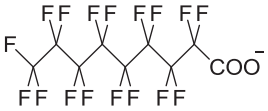
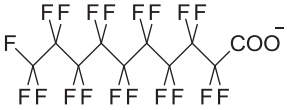
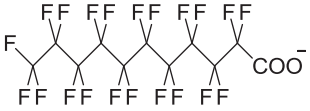
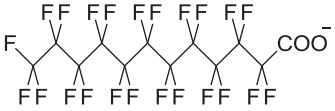
Table 1
Tested PFAS and their cytotoxicity in the HepG2 cells. As a measurement for cytotoxicity lactate dehydrogenase (LDH) leakage from the cells were measured (see Section 2.7).

Compound	Carbon atoms	Chemical structure	CAS No. [a]	Purity (%)	Cytotoxicity HepG2 (M)
Perfluorinated sulfonic acids (PFSA)					
PFHxS (perfluorohexane sulfonate)	C6		355-46-4	98	$>2 \times 10^{-4}$
PFOS (perfluorooctane sulfonate)	C8		1763-23-1	98	$>2 \times 10^{-5}$
Perfluorinated carboxylic acids (PFCA)					
PFOA (perfluorooctanoate)	C8		335-67-1	95	$>2 \times 10^{-4}$
PFNA (perfluorononanoate)	C9		375-95-1	97	$>2 \times 10^{-4}$
PFDA (perfluorodecanoate)	C10		335-76-2	98	$>2 \times 10^{-4}$
PFUnA (perfluoroundecanoate)	C11		2058-94-8	95	$>2 \times 10^{-4}$
PFDoA (perfluorododecanoate)	C12		307-55-1	96	$>2 \times 10^{-5}$

[a] The CAS No. is for the protonated acid form of the perfluoroalkylated substances.

function of thyroid hormone and functions of estrogen, androgen, and aryl hydrocarbon receptor *in vitro* (Bonefeld-Jorgensen et al., 2014). In humans, significantly higher serum levels of several PFAS including PFOS, PFOA, PFHxS, and perfluorooctanesulfonamide (PFOSA) were found in Greenlandic breast cancer patients compared with matched controls and PFOS and PFOSA were found as significant risk factors (Bonefeld-Jorgensen et al., 2011). In a prospective study of Danish women PFOSA was also found as a potential breast cancer risk factor (Bonefeld-Jorgensen et al., 2014). Another Danish prospective study did not find any association between plasma concentrations of PFOS and PFOA and the risk of prostate, bladder, pancreatic, or liver cancer, but a 30–40% increase in risk estimates for prostate cancer was observed for the three upper quartiles of PFOS compared with the lowest quartile (Eriksen et al., 2009). A significant difference in blood concentration of perfluorodecanoate (PFDA) was found between Swedish prostate cancer cases and healthy controls, with highest concentrations among the cases (Hardell et al., 2014).

The carcinogenic mechanisms of PFAS are not fully elucidated. PFAS have the ability to activate the peroxisome proliferator-activated receptor alpha and induce peroxisome proliferation in rodents, but the human relevance for this mode of action has been questioned (Klaunig et al., 2003; Andersen et al., 2008). A possible mechanism of action for PFAS in humans is generation of oxidative stress and some controversial evidence of effects in terms of oxidative stress and DNA damage exist. Several *in vitro* studies on the genotoxic and cytotoxic effects are published but the results are inconclusive (Yao and Zhong, 2005; Hu and Hu, 2009; Eriksen et al., 2010; Florentin et al., 2011; Huang et al., 2013). Some of the inconsistencies in the results may relate to differences in study and method setups.

Oxidative stress can be induced by environmental chemicals such as dioxins and heavy metals which result in increased production of reactive oxygen species (ROS) and damage of DNA (Mates et al., 2010), and similar mechanisms may be relevant for PFAS. Oxidative stress has been observed in relation to several diseases in humans, including atherosclerosis, heart attacks, chronic inflammatory diseases, central nervous system disorders, age related disorders and cancer (Aruoma, 1998; Barnham et al., 2004; Visconti and Grieco, 2009; Tsutsui et al., 2011).

The aim of this study was to assess the *in vitro* potential impact of seven long-chain PFAS on three oxidative stress endpoints: total antioxidant capacity (TAC), DNA damage, and generation of ROS. The selected endpoints were assessed using the human hepatoma cell line HepG2. The seven PFAS (PFHxS, PFOS, PFOA, perfluorononanoate (PFNA), PFDA, perfluoroundecanoate (PFUnA), and perfluorododecanoate (PFDoA)) were selected based on extent of human use and exposure, detection in human body, potential toxicity, and public concern (Posner et al., 2013).

2. Methods

2.1. Chemicals

PFHxS, PFOS, PFOA, PFNA, PFDA, and PFUnA were all purchased from ABCR (Germany). PFDoA was purchased from Sigma–Aldrich (Denmark). The purity of the test compounds was above 95% (specific purities and CAS No. are presented in Table 1). PFHxS, PFOS,

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PFOA, PFNA, PFDA, and PFUnA were dissolved in dimethyl sulfoxide (DMSO) from Thermo Scientific (Denmark). PFDoA was dissolved in ethanol (EtOH). The PFAS were diluted with culture medium immediately before use to give less than 0.04% (v/v) DMSO. For PFDoA the dilution gave a concentration of 0.4% (v/v) or less EtOH. The solvent controls did not affect the cell viability of the HepG2 cells in the used concentrations (0.04% DMSO and 0.4% EtOH). The positive controls, hydrogen peroxide (H_2O_2) and tert-butyl hydroperoxide (tBuOOH), were purchased from Sigma Aldrich (Denmark).

2.2. Cell culturing

The HepG2 cell line was obtained from American Type Culture Collection (ATCC, Rockville, Maryland, US) (HB8065), and used in all experiments. Cells were cultured in 75 cm^2 tissue culture flasks (Nunc, Denmark) and incubated at 37 °C and 5% CO_2 humidified atmosphere in Dulbecco's Modified Eagle Medium/Glutamax™ from Gibco, Invitrogen (Denmark) supplemented with 10% (v/v) fetal calf serum and 1% (v/v) penstrep (penicillin/streptomycin) also from Gibco/Invitrogen (Denmark).

For all experiments, the HepG2 cells were seeded in 24-well culture plates at cell density of 2.5×10^5 cells/well and cultured overnight to allow proper attachment. The next day the culture media was removed and the cells were incubated with culture medium containing the specified test concentrations of PFAS for 24 h.

The following seven long-chained PFAS where tested in all three assays: PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnA, PFDoA.

2.3. Comet assay

The comet assay is based on the method of Singh et al. (1988). In each experiment negative and positive test cell cultures where analyzed in parallel. Solvent control cells were treated with 0.04% DMSO (0.4% EtOH for PFDoA treated cells), and positive test cells were treated with 50 and 100 μM H_2O_2 for 15 min. After 24 h of exposure to the test compounds, the cells were trypsinized with 0.125% trypsin, centrifuged at 2000 rpm for 4 min and washed in phosphate buffered saline (PBS). The cells were embedded into 0.825% low melting agarose from Promega (USA) on gel bonds and lysed overnight in a lysis solution from Trevigen (USA). The gel bonds were then transferred to fresh made alkaline solution for 30 min (1 mM EDTA, 300 mM NaOH, pH >13.0, 4 °C) and they were subsequently subjected to electrophoresis for 30 min in the same buffer at 300 mA and ~1 V cm^{-1} at 4 °C (from anode to cathode). After electrophoresis, gel bonds were washed 3×5 min in 70% EtOH, immersed in 96% EtOH for at least 10 min and the gels were dried in an oven at 40 °C. The assay was performed in a minimum of light to prevent high background DNA damage. The gels were stained with SYBR Gold from Invitrogen (Denmark), and 100 cells/gel were scored and analyzed using Comet Assay IV software from Perceptive Instruments.

2.4. Detection of reactive oxygen species (ROS)

The generation of ROS was detected using 10 μM 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (carboxy-H_2DCFDA) from Invitrogen (Denmark). Solvent controls with 0.04% DMSO (0.4% EtOH for PFDoA treated cells) and positive test controls with 50 and 100 μM tBuOOH were included in every experiment. After PFAS exposure for 24 h the cells were washed with PBS and incubated with 10 μM carboxy-H_2DCFDA for 30 min. Subsequently, the cells were detached from the culture plates with 0.25% trypsin and 300 μL cell suspensions analyzed by flow cytometry (Cell Lab Quanta SCMPL, Bechman Coulter). A 488 nm wavelength laser was used for excitation, and fluorescence was detected in FL-1 using a 525/30 BP filter. The mean fluorescence of 2×10^4 cells was determined for each sample and analyzed using Flow Jo software ver. 7.6.5 (Tree star, INC.).

2.5. Total antioxidant assay (TAC)

After 24 h of treatment with the PFAS or solvent control (0.04% DMSO or 0.4% EtOH) the cells were washed in PBS (500 μL $well^{-1}$) and harvested in 500 μL cell dissociate buffer from Invitrogen (Denmark). The samples were centrifuged at 2000 g for 10 min at 4 °C and re-suspended in 1000 μL ice-cold buffer (5 mM potassium phosphate 7.4 pH, 0.9% NaCl, 0.1% glucose); subsequently the cells were homogenized and sonicated on ice and centrifuged at 10 000*g* for 15 min before collecting the supernatants. The samples were stored at −80 °C for maximum 1 week until the TAC measurement was carried out using the antioxidant assay kit according to the manufactory's protocol (Cayman, USA). The optical density of each sample was measured at 750 nm with a reference wavelength of 630 nm. All measurements were carried out on an EL8000 Universal Microplate Reader (BIO-TEK INSTRUMENTS, INC).

2.6. Protein measurements

To adjust for variance in cell number in the samples protein content were measured for all TAC samples using fluorescamine (Invitrogen, Denmark) dissolved in acetonitrile to a concentration of 500 mg L^{-1} as described in Kjeldsen et al. (2013). Fifty μL of each sample was added to a 96-well plate (Nunc, Roskilde, Denmark) in duplicates; as well as protein standards in the range of 0–3 μg μL^{-1} bovine serum albumin (BSA) from Promega (USA). The protein content was determined by adding fluorescamine (50 μL $well^{-1}$) to each well. The microtiter plates were covered with tin foil and placed on a shaker for 20 min. Subsequently the fluorometric measurements were carried out on a WALLAC VIVTOR 2 fluorometer (Perkin Elmer, USA) at 355/460 nm.

2.7. Cell viability (lactate dehydrogenase)

Cell viability was assessed by lactate dehydrogenase (LDH) leakage from damaged cells. The LDH leakage after PFAS exposure was measured using the Cytotoxicity Detection Kit (LDH, Roche, Denmark) as described (Ghisari and Bonefeld-Jorgensen, 2005). As a positive control, cells were lysed by Triton-X (final concentration of 1%), corresponding to a maximal release of LDH. As a negative control, culture medium from cells exposed to solvent control was used.

2.8. Statistical analysis

The PFAS exposure was tested in at least three independent experiments, each performed in duplicates with the appropriate solvent and medium controls in parallel, ensuring standardization of the assays. All experimental data from TAC and ROS assay was related to the respective solvent controls (set to 1). Statistical analyses were performed on mean values from each independent experiment. For each test compound, only results obtained at non-cytotoxic concentrations were included in the statistical analysis performed in SPSS 20.0 (SPSS Inc., Chicago, IL). The Kruskal–Wallis test was used to compare differences between concentrations and the Jonckheere–Terpstra test (two-tailed) was used to analyze for a linear trend between concentration and response. If one or both tests showed a significant difference ($p \leqslant 0.05$), the Mann–Whitney test was used to compare each concentration with the solvent control.

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3. Results

3.1. Cytotoxicity of the tested PFAS

The exposure time was 24 h for all three assays and within this timeline of exposure, in comparison with the solvent control we did not observe any significant difference in the LDH release, for PFHxS, PFOA, PFNA, PFDA and PFUnA. Cells treated with the highest tested concentration of PFOS and PFDoA (2×10^{-4} M) did, however, show a toxic reaction (Table 1). The given results refer only to the effects observed at concentrations not being toxic.

3.2. DNA damage upon exposure to PFAS (comet assay)

Comet assay trend analyses showed a significant dose-dependent increase in the level of cell DNA damage for four, PFHxS, PFOS, PFOA, and PFNA ($p \leqslant 0.001$, 0.02, 0.004, 0.01 respectively), of the seven tested PFAS (Fig. 1). For PFHxS, PFOS, PFOA, and PFNA the DNA damage reached at the highest tested concentration, 326–485% compared to the solvent set to 100% (Fig. 1). We did not observe any increase in DNA damage after exposure to PFDA, PFUnA or PFDoA (data not shown).

3.3. Intracellular ROS generation of PFAS

The generation of intracellular ROS induced by exposure to the seven selected PFAS was investigated. A significant increase of ROS was observed upon exposure to six, PFHxS, PFOS, PFOA, PFNA, PFDA and PFUnA, of the seven tested compounds (Fig. 2). Exposure to PFDoA did not increase the ROS generation. For PFHxS and PFUnA the ROS generation was dose-dependent ($p = 0.047$ and 0.045, respectively).

3.4. Total antioxidant capacity of PFAS

The effect on TAC upon exposure to the seven selected PFAS in the concentration range from 2×10^{-8} to 2×10^{-4} M was analyzed. Exposure to PFOA in the range of 2×10^{-8}–2×10^{-5} M resulted in a significant decrease in TAC (Table 2) being 0.70–0.82 fold of the values found for the solvent control (0.04% DMSO). The TAC effect was,

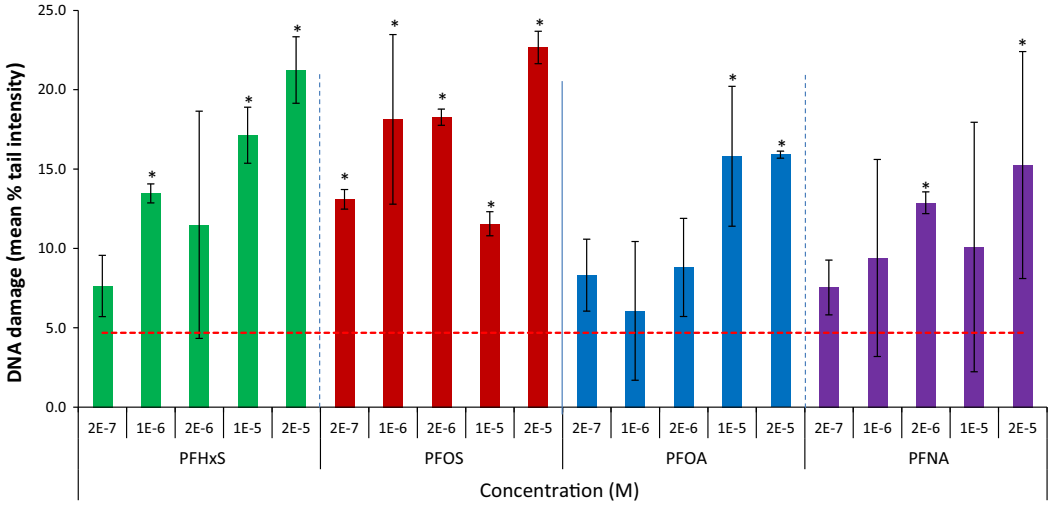

Fig. 1. The level of DNA damage after PFAS exposure for 24 h. Cells exposed to PFOS, PFHxS, PFOA and PFNA showed an increase in DNA damage in a dose depended manner. Each bar represents the mean% Tail intensity SD of three independent experiments each tested in duplicates. The symbol (*) indicates compound-induced responses significantly different ($p \leqslant 0.05$) from responses obtained with solvent control (0.04% DMSO, illustrated by a dash red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

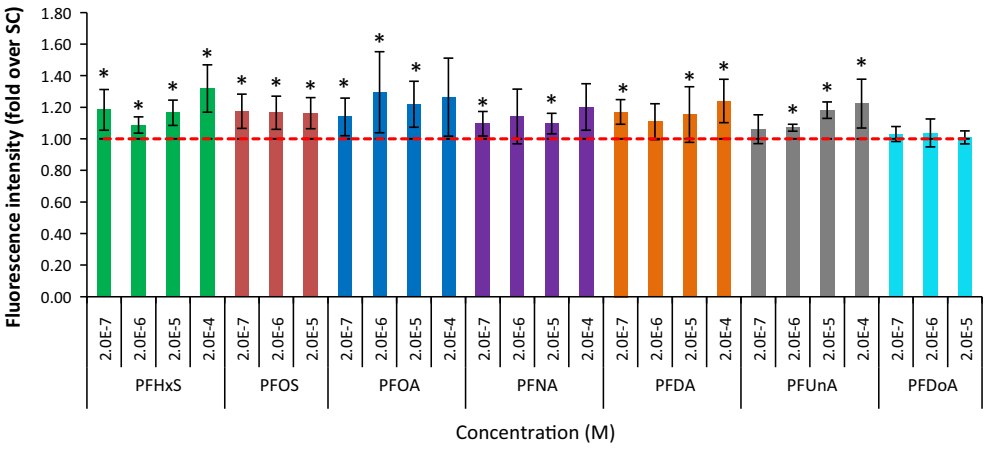

Fig. 2. Intracellular ROS generation after exposure to PFAS for 24 h. Data are reported as fold induction in fluorescence intensity relative to solvent control (0.04% DMSO or 0.4% EtOH (for PFDoA), set to 1 and indicated with a red dash line). Data are expressed as mean ± SD of three or four independent experiments, each performed in duplicates. * Statistically different at $p \leqslant 0.05$ from solvent control (SC). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 2
Total antioxidant capacities (TAC). Total antioxidant capacity after 24 h of induction with PFAS. Each value represents the mean fold induction ± SD of three independent experiments each tested in duplicates.

	PFAS		PFCA				
	PFHxS	PFOS	PFOA	PFNA	PFDA	PFUnA	PFDoA
PFAS concentration (M)							
2×10^{-8}	0.97 ± 0.11	0.85 ± 0.13	0.70 ± 0.07*	1.03 ± 0.15	0.84 ± 0.20	1.10 ± 0.11	1.43 ± 0.24
2×10^{-7}	1.10 ± 0.11	0.91 ± 0.06	0.82 ± 0.11*	1.09 ± 0.24	0.64 ± 0.38	1.44 ± 0.39	0.88 ± 0.13
2×10^{-6}	1.11 ± 0.12	0.85 ± 0.09	0.71 ± 0.14*	1.04 ± 0.13	1.15 ± 0.04	1.73 ± 0.53	0.71 ± 0.27
2×10^{-5}	1.02 ± 0.08	0.82 ± 0.13	0.82 ± 0.04*	1.00 ± 0.19	1.06 ± 0.06	1.17 ± 0.70	0.74 ± 0.18
2×10^{-4}	1.13 ± 0.10	ND	0.90 ± 0.13*	1.14 ± 0.22	1.13 ± 0.20	0.71 ± 0.08	ND

* The symbol indicates responses significantly different ($p \leqslant 0.05$) compared with solvent control (0.04% DMSO or 0.4% EtOH, set to 1). ND: Not detected.

Table 3
Summary of the results from all three assays.

	DNA damage			ROS generation			TAC		
	LOEC (M)	MOEC (M)	% of SC	LOEC (M)	MOEC (M)	% of SC	LOEC (M)	MOEC (M)	% of SC
PFSA									
PFHxS (C6)	1×10^{-6}#	2×10^{-5}*	453*	2×10^{-7}#	2×10^{-4}*	132*	NS	2×10^{-4}	113
PFOS (C8)	2×10^{-7}#	2×10^{-5}*	485*	2×10^{-7}	2×10^{-7}*	117*	NS	2×10^{-5}	82
PFCA									
PFOA (C8)	1×10^{-5}#	2×10^{-5}*	340*	2×10^{-7}	2×10^{-6}*	129*	2×10^{-8}	2×10^{-8}*	70*
PFNA (C9)	2×10^{-6}#	2×10^{-5}*	326*	2×10^{-7}	2×10^{-5}*	110*	NS	2×10^{-4}	114
PFDA (C10)	NS	2×10^{-5}	135	2×10^{-7}	2×10^{-4}*	124*	NS	2×10^{-7}[a]/2×10^{-4}[b]	64[a]/113[b]
PFUnA (C11)	NS	2×10^{-4}	101	2×10^{-6}#	2×10^{-4}*	122*	NS	2×10^{-6}[a]/2×10^{-4}[b]	173[a]/71^[b]
PFDoA (C12)	NS	2×10^{-5}	121	NS	2×10^{-5}	104	NS	2×10^{-8}[a]/2×10^{-5}[b]	143[a]/71[b]

[a] and [b]: MOEC and % of SC pairs.
* Significant data ($p \leqslant 0.05$).
# Dose-dependent results. NS, no significant effects observed. LOEC: lowest observed effect concentration, the lowest concentration at which a significant effect was detected. MOEC: maximum observed effect concentration, the lowest concentration at which the maximum effect was observed. % of SC: percent of solvent control, effect at MOEC that is given as percentage of solvent control. For PFDA, PFUnA and PFDoA two MOEC and % of SC values are given for the TAC results, as some concentrations showed a decrease whereas other resulted in an increase.
^ Data at the highest concentration might be non-detected starting cell toxicity.

however, not dose-dependent. Although not significantly, PFOS and PFDoA had a tendency to reduce the TAC, whereas PFHxS, PFNA, and PFDA non-significantly increased the TAC at the highest concentrations (Table 2). PFUnA shows a tendency to increase TAC in four of the five tested concentrations, while at the highest tested concentration (2×10^{-4} M) a decreasing TAC was observed, which might be due to non-detected cytotoxicity.

4. Discussion

We investigated the impact of seven selected PFAS (PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnA, and PFDoA) found in human blood and tissues to affect endpoints related to oxidative stress and DNA damage in the HepG2 cells. PFAS are ubiquitous pollutants and human are exposed throughout life and health risks have been reported (Giesy and Kannan, 2001; Fromme et al., 2009; Bonefeld-Jorgensen et al., 2011, 2014). Our findings suggest that PFAS can be genotoxic and have the potential to induce oxidative stress. Results from the comet assay showed that PFHxS, PFOS, PFOA, and PFNA induced DNA strand breaks dose dependently in the range of 2×10^{-7}–2×10^{-5} M. Except for PFDoA, an increased ROS generation was seen for the other PFAS, and the increase was dose dependent for PFHxS and PFUnA. Only PFOA decreased TAC significantly, and non-significant tendencies to de- or increase were seen for some of the other compounds (see Table 3 for a data overview).

Among the tested PFAS, five were perfluorinated carboxylic acids (PFCA) and two sulfonic acids (PFSA). The two investigated PFSA (PFHxS (C6) and PFOS (C8)) both increased the DNA damage dose dependently and induced intracellular ROS generation, but none of them affected the TAC level significantly. The length of the carbon chain (Table 1) seems not to affect the potential to increase oxidative stress since the two PFSA showed similar potential with respect to DNA damage and ROS generation. For the five PFCA (PFOA, PFNA, PFDA, PFUnA, and PFDoA) with carbons chains containing between 8 and 12 carbon atoms (Table 1) the length of the carbon chain seemed to some degree to be related to all the three endpoints with the highest potency of the shortest carbon length. The PFDoA (C12) elicited no significant effects in any of the three oxidative stress related assays, whereas PFOA (C8) with the shortest carbon length of the tested PFCA significantly affected all three end points: increased DNA damage, ROS generation and decreased TAC. Moreover, in the comet assay the two shorter PFCA (PFOA (C8) and PFNA (C9)) showed a significant increase in DNA damage, whereas the longer PFCA (C10, C11, C12, Table 3) did not. For the TAC assay only the shortest investigated PFCA (PFOA, C8) showed significant effect. The two compounds PFOS and PFOA have the same C8 carbon chain, but PFOS showed no significant TAC effect, although, exposure to PFOS suggested a non-significant TAC decrease. Whether the carboxylic and sulfonic groups might influence the antioxidant cell mechanisms in different ways needs further studies.

The data presented in this study were obtained at relatively high concentrations compared to levels found in humans. However, due to the persistent and the highly bioaccumulative nature of the PFAS, long human elimination half-lives as well as the lifelong human exposure we believe that knowledge gained from this study may be helpful for risk assessment of the PFAS. Our study can contribute to the elucidation of the mechanisms underlying PFAS actions e.g. as carcinogenicity.

In support to our results on DNA damage Yao and Zhong found an increased level of micronucleus and DNA damage using the

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comet assay upon PFOA exposure also using HepG2 cells (Yao and Zhong, 2005), and Eriksen et al. reported a significant increase in strand breaks upon PFNA exposure (Eriksen et al., 2010). The increased DNA damage level reported in both studies (Yao and Zhong, 2005; Eriksen et al., 2010) were, however, lower than the increase observed in our study. In contrast to our study, PFOS or PFOA did not affect the level of DNA damage in the Eriksen et al. (2010) study nor in another study also using the HepG2 cell and the comet assay (Florentin et al., 2011). The three mentioned studies used comparable exposure concentrations in the range of e.g. 100 μM and 400 μM PFOA. We used concentrations from 20 nM to 200 μM in the present study, being comparable with the above mentioned studies (Yao and Zhong, 2005; Eriksen et al., 2010; Florentin et al., 2011), but with a lower minimum and maximum concentration. Differences in exposure concentrations cannot alone explain the different results, but different method setup (buffers, media, incubation time etc.) might affect the levels of sensitivity and the observed data differences.

The DNA damage observed in our study may be an effect of PFAS-induced ROS generation. Oxidants such as ROS plays an important role as defense system against microorganisms and as cellular messengers, but a tight regulation of these highly reactive molecules are important. An uncontrolled increase may lead to damages on DNA, lipids and proteins. Our results are supported by several other studies reporting significant increases in ROS upon PFOS and PFOA exposure (Panaretakis et al., 2001; Yao and Zhong, 2005; Hu and Hu, 2009; Eriksen et al., 2010), the Eriksen et al. study did however not observe a significant increase after exposure to PFNA (Eriksen et al., 2010). However, in contrast to our results Florentin et al. reported an unchanged level of ROS upon PFOS and PFOA exposure (Florentin et al., 2011).

We also studied the effect of PFAS on the antioxidant system. The antioxidant system is complex with many different regulated pathways and consists of both specific and nonspecific antioxidants (Chaudiere and Ferrari-Iliou, 1999). The total capacity of the antioxidants (TAC) was significantly decreased by PFOA and non-significantly by PFOS and PFDoA. The TAC for other four PFAS were at the similar level of the control although with a tendency to be above 1. Whether the cells have the capacity to better overcome the oxidative stress impact upon exposure to the six other tested PFAS (PFHxS, PFOS, PFNA, PFDA, PFUnA and PFDoA) and therefore not affect the antioxidant system and thus the TAC requires further studies with more specific endpoints for factors involved in the TAC. Not many studies have investigated effect of PFAS on the cellular antioxidant level, but one study reported effects on antioxidative enzymes activity in HepG2 cells (Hu and Hu, 2009). Exposure to PFOA and PFOS resulted in an increased activity of superoxide dismutase, catalase and glutathione reductase and decreased activity of glutathione peroxidase and glutathione-*S*-transferase (Hu and Hu, 2009). The authors suggested that the observed changes in activities of antioxidative enzymes indicated that PFOA and PFOS may overwhelm the balance of the antioxidant system, boost the generation of ROS, impact the mitochondria, and result in initiation of apoptosis program. However, a direct comparison of our data and this report (Hu and Hu, 2009) are not possible as the endpoints are different. Our data shows that although the intracellular ROS generation was increased by six of the tested compounds the total levels of antioxidants were only changed by PFOA exposure. Whereas Hu and Hu (2009) were able examine at specific antioxidant levels, our TAC data only inform about the total capacity of the sum of antioxidants. Taking into consideration the limitation of the TAC method, we cannot eliminate the possibility that the six PFAS not affecting the TAC can have increased the activity on some antioxidants factors and decreased the activity on others as e.g. reported by Hu and Hu (2009). Whether PFOA affect factors in the antioxidant system or interfere with oxidative stress activation pathways require further studies. The health effects of changes in antioxidant activities are not clear, but intake of low dose supplementary antioxidants has been associated with lower total cancer incidence (Hercberg et al., 2004). Recent studies do, however, find that antioxidants (e.g. *N*-acetylcysteine and vitamin E) reduce ROS and DNA damage but increases tumor proliferation in mice, which function as lung cancer models (Sayin et al., 2014).

The levels of ROS, TAC and DNA damage are important oxidative stress factors and an imbalance in one of the factors may affect the others. The factors may interact with each other and all play a role in the oxidative state of the cells. Oxidative DNA damage is caused by reactive species, such as ROS, and more than 20 different DNA lesions are known (Halliwell and Aruoma, 1991). To prevent DNA lesions the antioxidant system neutralizes the reactive species in the cells and the DNA repair system removes oxidative DNA lesions. A high level of DNA lesions may lead to mutagenesis, cytostasis and cytotoxicity (Cooke et al., 2003). Increased oxidative DNA lesions have been shown as an effect of lowered antioxidant capacity in vivo (Fraga et al., 1991; Honda et al., 2000). We would expect, that the decrease in TAC after PFOA exposure would result in a high level of DNA damage, but the level of PFOA induced DNA lesions was not different from exposure to the other six PFAS where TAC were not affected. Thus, it might be speculated that the DNA repair system possibly is more affected by PFOA in the exposed HepG2cells. Whereas six of the seven tested compounds increased the intracellular ROS level only four of the compounds showed an enhanced level of DNA damage. These results might indicate that the mechanism behind PFAS increased DNA damage is not caused by ROS level alone, and further studies on possible factors involved is needed.

In summary, our study indicates that some PFAS have the potential to induce oxidative stress in terms of ROS production and DNA damage in the cell line representing the human liver (Table 3). Our study is supported by several other studies finding similar effects although with some degree of controversy vs other reported results. Further studies including epidemiological studies are needed to further investigate the potential health effects of PFAS exposure in humans.

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DEPARTMENT OF HEALTH

PFAS and Health

Our understanding and ability to detect PFAS in the environment has evolved since the Minnesota Pollution Control Agency (MPCA) and the Minnesota Department of Health (MDH) began investigating them in 2002. Laboratories at that time only identified two PFAS and could not detect low concentrations. We are now able to measure extremely small amounts (parts per trillion in water) of several PFAS and newer studies suggest that long-term exposure to PFAS in this range might affect the most vulnerable members of the population. MDH continues to monitor the scientific research about PFAS and we will adjust our health advice as needed.

Expand All

How people are exposed to PFAS

PFAS can be measured in the blood of most people around the world, including Minnesotans. For most people, consumer products that are grease, oil, stain and/or water resistant are a much greater source of PFAS exposure than drinking water. PFAS chemicals are commonly used in non-stick and stain-resistant consumer products, food packaging, fire-fighting foam, and industrial processes.

People can be exposed to PFAS in many ways including drinking water where the source has been impacted by PFAS contamination. For most Minnesotans, the majority of PFOS exposure comes from non-drinking water sources. These can include:

- Using consumer products treated with PFAS such as stain resistant carpeting and water-repellent clothing.
- Eating food packaged in material that contains PFAS.
- Eating fish caught from water contaminated by PFOS.
- Eating food grown or raised near places with PFAS exposure.

More information can be found at PFAS chemical exposure | ATSDR (cdc.gov). [LINK https://www.atsdr.cdc.gov/pfas/health-effects/exposure.html]

Health Risks

There are many different PFAS, and each may impact health differently. Most studies about their effects on human and animal health have been done on two PFAS chemicals, PFOA and PFOS.

The most consistently observed and strongest evidence for harmful impacts on human health is for immune suppression such as decreased vaccination response, changes in liver function such as higher cholesterol, elevated liver enzymes, and lower birth weight. In addition, lifetime exposure to PFOA has also been associated with kidney cancer. MDH develops guidance values to protect people who are most highly exposed and people who are most sensitive to the potentially harmful effects of a contaminant, including pregnant people, fetuses, infants, and children.

While we believe the immediate health risks for most people exposed to PFAS are low, the latest information indicates that fetuses and infants are more vulnerable and can be among the most highly exposed. Several PFAS are known to cross the placenta and concentrate within breastmilk. Long-term exposure to several PFAS, including PFOA, PFOS, and PFHxS, leads to a buildup of these chemicals in people of child-bearing age, which then increases exposure to fetuses and breastfed babies. Breastfeeding is a healthy activity for both baby and parent. If you have concerns about possible risks from PFOS during breastfeeding, consult with your physician. MDH recommends that women currently breastfeeding and pregnant women who plan to breastfeed continue to do so. MDH recommends that women who plan to become pregnant follow the recommendations in Reducing Exposures: Per- and Polyfluoroalkyl substances (PFAS) (PDF [LINK http://www.health.state.mn.us/communities/environment/hazardous/docs/pfas/pfasreducingexp.pdf]).

Consumption of infant formula mixed with water containing PFAS can result in higher exposure to PFAS because babies drink more water per body weight than adults. If you are concerned about exposure to PFAS by consumption of infant formula and would like to lower your baby's exposure to PFAS, consider using water that

has been filtered to remove PFAS, as your water source.

How to reduce exposures

Because PFAS are so widely found in the environment, eliminating all exposure to PFAS is unlikely; however, you can take the following steps to reduce your exposure.

- **Limit use of consumer products that contain PFAS**. PFAS is used in many consumer products. Here is a selection of items that might contain PFAS:
  - Food packaging, including grease-resistant paper, fast food containers/wrappers, microwave popcorn bags, pizza boxes, and candy wrappers.
  - Nonstick cookware.
  - Stain-resistant coatings used on carpets, upholstery, and other fabrics.
  - Water-resistant clothing.
  - Some cleaning products.
  - Some personal care products (shampoo, dental floss) and cosmetics (nail polish, eye makeup).
  - Some paints, varnishes, and sealants.
- **Follow the fish consumption guidance to choose fish low in PFAS to put on your plate** —Some PFAS, predominantly PFOS, may be present in the fish people catch and eat. Fish Consumption Guidance is available on the MDH webpage: Fish Consumption Guidance [LINK http://www.health.state.mn.us/communities/environment/fish/index.html].
- **Remove household dust.** Household dust can be a significant source of PFAS exposure, especially for infants and young children. Indoor sources (e.g., consumer products, floor waxes, stain-resistant treated upholstery and carpets) contribute most to PFAS in house dust. Keeping floors and other surfaces free of dust can limit this exposure.

People can also be exposed to PFAS from consuming water with levels of PFAS above health-based guidance. Water with PFAS levels above health-based guidance is safe for bathing, showering, swimming, washing clothes, and cleaning, but should not be used for drinking or cooking. Consider the following to understand PFAS levels in water:

- **Review PFAS Findings in Public Water Systems** by visiting MDH's Dashboard for PFAS Testing in Drinking Water and the Minnesota [LINK http://www.health.state.mn.us/communities/environment/water/pfasmap.html] Control Agency's (MPCA) Minnesota Groundwater Contamination Atlas [LINK http://www.pca.state.mn.us/about-mpca/minnesota-groundwater-contamination-atlas].
- **Test private well water to determine PFAS and other chemical contaminants.** Information about private drinking water well sampling is available on the MDH PFAS and Private Wells [LINK http://www.health.state.mn.us/communities/environment/water/wells/waterquality/pfas.html] and the MPCA Well Sampling in the East Metro Area [LINK http://www.pca.state.mn.us/air-water-land-climate/well-sampling-in-the-east-metro-area] website.
- **Consider home water treatment** if you live near a source of drinking water that is contaminated with PFAS, know there is PFAS in your drinking water, or are concerned about PFAS. Reverse osmosis and activated carbon treatment systems can reduce the levels of PFAS in drinking water. MDH provides information about inexpensive and easy-to-use systems that people can install in their home to reduce exposure to PFAS through drinking water on the following webpages:
  - Water Treatment Using Carbon Filters: GAC Filter Information [LINK http://www.health.state.mn.us/communities/environment/hazardous/topics/gac.html]
  - PFAS and Home Treatment of Water [LINK http://www.health.state.mn.us/communities/environment/hazardous/topics/pfashometreat.html]
  - Evaluation of Perfluorochemical Removal by a Small, Point-of-Use Filter (PDF) [LINK http://www.health.state.mn.us/communities/environment/hazardous/docs/pfas/poueval.pdf]
- **Prepare infant formula with filtered water or bottled water if your water source has high levels of PFAS.** People who are pregnant, fetuses, and children are sensitive to accumulating PFAS in their bodies and should reduce their exposure to PFAS. If your drinking water comes from a public water system which is treating drinking water to at or below MDH health-based guidance, tap water can be used to prepare infant formula.
- **Avoid contact with foam on water surfaces.** Several things, including PFAS, can cause foam to form on the surface of water bodies. PFAS-containing foam on water surfaces does not pose a risk to human health if

skin contact with foam is minor and infrequent. Wash skin that has come into contact with foam with soap and water.

PFAS may be present in lakes and rivers at very low levels. MDH has determined that exposure to PFAS through swimming is not a health concern. PFAS are poorly absorbed through skin and swallowing small amounts of water while swimming will not result in significant exposure. Also, because there is little evaporation of PFAS from water into the air, exposure from breathing while swimming or bathing is not a health concern.

Talking to your Health Care Provider/PFAS Blood testing

If you have been exposed to perfluoroalkyl and polyfluoroalkyl substances (PFAS) and are concerned about your health, you can talk to your Health Care Provider.

- We don't know if exposure to PFAS may cause health problems in the future. You can talk to your health care provider and ask if you need to be monitored for symptoms or conditions that may be caused by PFAS exposure in the future.
- Testing Your Blood for PFAS (PDF) [LINK http://www.health.state.mn.us/communities/environment/hazardous/docs/pfas/indbltest.pdf] - It is possible to get your blood tested for PFAS, but the results have some important limitations. This information sheet will help you understand what blood testing can tell you and whether blood testing is a good choice for you.

Health-Based Values for PFAS in Drinking Water

MDH develops guidance values to protect people who are most highly exposed and people who are most sensitive to the potentially harmful effects of a contaminant, including pregnant people, fetuses, infants, and children. A person drinking water at or below the guidance value would be at little or no risk for harmful health effects. A full list of guidance values can be found online at the Human Health-Based Water Guidance Table [LINK http://www.health.state.mn.us/communities/environment/risk/guidance/gw/table.html] .

Table of Health-based Values for PFAS

PFAS Detected in Minnesota PFAS Specific Information Sheet Available	Drinking Water Guidance Value (ppb)
perfluorobutane sulfonate (PFBS) PFBS and Drinking Water (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfbsinfo.pdf]	0.1 [same as 100 ppt]
perfluorobutanoic acid (PFBA) PFBA and Drinking Water (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfbainfo.pdf]	7 [same as 7,000 ppt]
perfluorohexane sulfonate (PFHxS) Toxicological Summary for: perfluorohexane sulfonate (PFHxS) (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfhxs.pdf]	0.047 [same as 47 ppt]
perfluorohexanoic acid (PFHxA) Toxicological Summary for: Perfluorohexanoate (PFHxA) (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfhxa.pdf]	0.2 [same as 200 ppt]

PFAS Detected in Minnesota PFAS Specific Information Sheet Available	Drinking Water Guidance Value (ppb)
perfluorooctanoic acid (PFOA) PFOA and Drinking Water (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfoainfo.pdf]	0.0000079 [same as 0.0079 ppt]
perfluorooctane sulfonate (PFOS) PFOS and Groundwater (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfosinfo.pdf]	0.0023 [same as 2.3 ppt]

How changing knowledge has impacted drinking water guidance over time

MDH has reviewed and updated guidance for PFAS in drinking water since 2002. Below is a description of how drinking water guidance has changed over time.

MDH Guidance Values (ppb) – 2002 to 2023

Year	PFOA	PFOS	PFHxS	PFHxA	PFBA	PFBS
2002	7	1	--	--	--	--
2006	1	0.6	--	--	1	--
2007	0.5	0.3	--	--	7	--
2009	0.3	0.3	--	--		7
2013			0.3	--	7	7
2016	0.07	0.07	0.07	--		
2017	0.035	0.027	0.027	--		2
2019	0.035	0.015	0.047	--		
2022				0.2		0.1
2024	0.0000079	0.0023				

How health risk is assessed when more than one chemical is present in drinking water

In some cases, water may contain multiple contaminants. Exposure to multiple contaminants may cause health effects that would not be predicted based on separate exposures to the individual concentrations of each contaminant present. When more than one PFAS is present in drinking water, MDH evaluates the "additive" risk that is created by the presence of multiple contaminants.

For more information, visit the MDH webpage: Evaluating Concurrent Exposures to Multiple Chemicals [LINK http://www.health.state.mn.us/communities/environment/risk/guidance/gw/additivity.html] .

PFAS In Air

MDH develops health-based air guidance values to evaluate potential human health risks from exposures to chemicals in ambient air. An air guidance value is a concentration of a chemical that is likely to pose little or no risk to human health. Air guidance values are developed using public health protective practices that protect

susceptible portions of the population (including but not limited to children, pregnant women and their fetuses, individuals compromised by pre-existing diseases, and elderly persons). Air guidance values apply to short time periods as well as a lifetime of exposure.

MDH has not previously derived air guidance values for PFAS. Currently, there is insufficient inhalation data available for PFAS to derive air guidance directly; however, PFAS information via the oral exposure route is more robust. Route-to-route extrapolation was implemented using MDH's health-based guidance values [LINK http://www.health.state.mn.us/communities/environment/risk/guidance/gw/table.html] information for PFAS in drinking water to derive air values.

More information can be found on the MDH Air Guidance Values [LINK http://www.health.state.mn.us/communities/environment/risk/guidance/air/table.html] webpage.

The table below shows the air guidance values (in micrograms per cubic meter, µg/m3) for five PFAS. MDH intends to derive air guidance values for additional PFAS as information becomes available.

Table of Current Air Guidance Values for PFAS

PFAS Chemical PFAS Specific Air Information Sheet	Air Guidance Value ($\mu g/m^3$)
Perfluorobutanoic acid (PFBA) Air Toxicological Summary for Perfluorobutanoic acid (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/air/pfba.pdf]	10
Perfluorohexanesulfonic acid (PFHxS) Air Toxicological Summary for Perfluorohexanesulfonic acid (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/air/pfhxs.pdf]	0.034
Perfluorohexanoic acid (PFHxA) Air Toxicological Summary for Perfluorohexanoic acid (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/air/pfhxa.pdf]	1 (short-term) 0.5 (subchronic and chronic)
Perfluorooctanoic acid (PFOA) Air Toxicological Summary for Perfluorooctanoic acid (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/air/pfoa.pdf]	0.063
Perfluorooctane sulfonic acid (PFOS) Air Toxicological Summary for Perfluorooctane sulfonic acid (PDF) [LINK http://www.health.state.mn.us/communities/environment/risk/docs/guidance/air/pfos.pdf]	0.011
Perfluorobutane sulfonic acid (PFBS)	0.3

Contact Information

For questions about health-based guidance and risk:

Contact the MDH Health Risk Assessment Unit at health.risk@state.mn.us or call 651-201-4899.

For questions about health and contaminated sites:

Contact MDH Site Assessment and Consultation Unit at health.hazard@state.mn.us or call 651-201-4897.

Information for Physicians

PFAS Resources for Health Care Providers - MN Dept. of Health (state.mn.us) [LINK http://www.health.state.mn.us/communities/environment/hazardous/topics/pfaschproviders.html]

Printable Information Sheets

- Reducing Exposures: Perfluoroalkyl Substances (PDF) [LINK http://www.health.state.mn.us/communities/environment/hazardous/docs/pfas/pfasreducingexp.pdf]
- Perfluoroalkyl Substances (PFAS) and Health (PDF) [LINK http://www.health.state.mn.us/communities/environment/hazardous/docs/pfashealth.pdf]
- Testing Your Blood for PFAS (PDF) [LINK http://www.health.state.mn.us/communities/environment/hazardous/docs/pfas/indbltest.pdf]
- Stress at Contaminated Sites: Coping with the stress that environmental contamination can cause (PDF) [LINK http://www.health.state.mn.us/communities/environment/hazardous/docs/stresscontsites.pdf]
- Perfluoroalkyl Substances (PFAS) Summary (PDF) [LINK http://www.health.state.mn.us/communities/environment/hazardous/docs/pfassummary.pdf]
- PFAS in Drinking Water (one-page summary)
  - PFAS in Drinking Water (PDF) [LINK http://www.health.state.mn.us/communities/environment/water/docs/contaminants/pfas.pdf] (English)
  - PFAS nyob hauv Cov Dej Haus (PDF) [LINK http://www.health.state.mn.us/communities/environment/water/docs/contaminants/pfashmong.pdf] (Hmong)
  - PFAS ku jirta Biyaha la Cabo (PDF) [LINK http://www.health.state.mn.us/communities/environment/water/docs/contaminants/pfassomali.pdf] (Somali)
  - PFAS en el agua potable (PDF) [LINK http://www.health.state.mn.us/communities/environment/water/docs/contaminants/pfasspanish.pdf] (Spanish)

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Multi- and *trans*-generational disturbances of perfluorobutane sulfonate and perfluorohexane sulfonate on lipid metabolism in *Caenorhabditis elegans*

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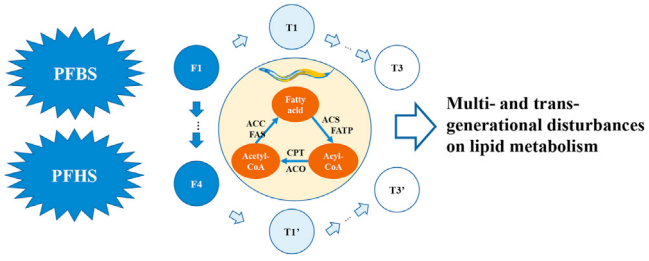
HIGHLIGHTS

- PFBS stimulated the fat content not in F1 but in F4 generation.
- PFBS disturbed lipid metabolism and IIS pathway differently from F1 to F4.
- PFHS stimulated the fat content in F1 and F4 generation.
- PFHS had similar disturbances on lipid metabolism and IIS pathway from F1 and F4.
- PFHS commonly up-regulated *daf-7* in both multi- and *trans*-generational effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Short-chained perfluorobutane sulfonate (PFBS, four-carbon) and perfluorohexane sulfonate (PFHxS, six-carbon) are widely employed to substitute long-chained per- and poly-fluoroalkyl substances (PFASs). Recent studies showed the potential persistence of PFBS and PFHxS, and also reported their correlation with obesity. However, the long-term outcome and underlying mechanisms remained poorly understood. Presently, the effects of PFBS and PFHxS were studied on *C. elegans* with multi- and *trans*-generational experiments. The multi-generational effects were measured in continuous four generational exposure (i.e., F1 to F4). Results showed that PFBS did not stimulate the fat content in F1 but in F4 with continuous but different disturbances on the lipid metabolism and the insulin and insulin-like (IIS) pathway. PFHxS stimulated the fat content in F1 and F4 with similar disturbances on the lipid metabolism and IIS pathway. The *trans*-generational results showed that the effects of PFBS and PFHxS on the lipid metabolism and IIS pathway were not totally recovered in the offspring of F1 (i.e., T1-T3) and F4 (i.e., T1′-T3′) which were not continuously exposed. PFHxS showed a common pattern to up-regulate *daf-7* in both multi- and *trans*-generational effects. The long-term consequences of the short-chained PFASs substitutes should be concerned and epigenetic regulations should be considered in future mechanism studies.

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1. Introduction

Per- and poly-fluoroalkyl substances (PFASs) are widely used in industrial production and daily life as lubricants and food packaging materials. The long-chained PFASs, e.g., perfluorooctanoic acid (PFOA), had been phased out due to their ubiquitous existence in environmental matrices and severe toxicities (Sinclair et al., 2020). Since short-chain PFASs were expected to be less toxic than the long-chained ones, perfluorobutane sulfonate (PFBS, four-carbon) and perfluorohexane sulfonate (PFHxS, six-carbon) are widely employed as substitutes. Unfortunately, PFBS and PFHxS were increasingly detected in environmental matrices and human tissues (Cui et al., 2020; Pizzurro et al., 2019). Accordingly, more comprehensive toxicological studies are still needed to fully evaluate the risk of short-chained PFASs.

Notably, the total concentration of PFBS and PFHxS in cat serum was positively correlated with its weight and obesity index (Bost et al., 2016). Such correlation demonstrated the obesogenic effects of PFBS and PFHxS. The obesogenic effects are closely related with disturbances on lipid metabolism (Yu et al., 2020), which is essential in the development, reproduction and neurodevelopment (Chaparro-Ortega et al., 2018; Fénichel and Rougier, 2019; Setayesh et al., 2018). As expected, PFBS and PFHxS showed developmental toxicity in rats (Kjølholt et al., 2015), reproductive toxicity in *C. elegans* (Chen et al., 2018a) and neurotoxicity (Mudumbi et al., 2017). Accordingly, the mechanisms underlying the influences of PFBS and PFHxS on lipid metabolism are essential to explain their toxicities.

Lipid metabolism includes complex processes involving multiple conserved molecular pathways among organisms, e.g., between *C. elegans* and human being. For example, fat accumulation is catalyzed by enzymes including glycerol-3-phosphateacyl transferases (GPAT), fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) (Takeuchi, 2009), among which ACC is the rate-limiting enzyme (Wei et al., 2018). At the same time, fat consumption is promoted by enzymes/proteins including lipase, fatty acid transport proteins (FATP), acyl-CoA synthetase (ACS), carnitine palmitoyl transferase (CPT), acyl-CoA oxidase (ACO) and fatty acid desaturase (encoded by genes including *fat-5* and *ech-1*) (Brock et al., 2007; Wang et al., 2018), among which CPT is the rate-limiting enzyme (Naher et al., 2017). It was found that PFBS or PFOS interfered with ACO and CPT levels or the expression levels of their genes in *in vitro*/*vivo* studies (Buck, 2015; Lau et al., 2007), and disturbed lipid metabolism (Muscogiuri et al., 2017).

The persistence of long-chained PFASs (e.g., PFOA) raised further concerns on their long-term toxicities over generations. Interestingly, the impacts of PFOA over generations were accompanied with disturbances on the lipid metabolism (Li et al., 2020b). This point is still important for the short-chained PFASs substitutes because they also persist in environment, and can directly influence offspring via uterus or breast milk (Liew et al., 2020). Previous studies have shown that exposure of mother (F0) to PFHxS can cause the offspring (F1) to gain weight, and obesity later in life (Chen et al., 2017; Mora et al., 2018). So far, it remained unclear how the effects of PFBS and PFHxS on lipid metabolism would change over generations.

The purpose of this study was to explore whether short-chain PFASs substitutes (i.e., PFBS and PFHxS) have effects on lipid metabolism over generations. *C. elegans* was chosen as the model organism due to its feasibility in generational effect studies (Li et al., 2019). The multi-generational effects in continuous four generational exposure (i.e., F1 to F4) were used to illustrate potential adaptive response. The *trans*-generational effects in the offspring of F1 (i.e., T1-T3) and F4 (i.e., T1′-T3′) which were not continuously exposed were used to demonstrate the long-term residual consequences. Key enzymes/proteins in the lipid metabolism and the expression of regulating genes including the insulin and insulin-like signaling (IIS) pathway (e.g., *daf-2*, *daf-16* and *daf-7*) were measured to explore potential mechanisms.

2. Materials and methods

2.1. Chemical

Perfluorobutane sulfonate (PFBS, CAS NO: 29420-49-3, $C_4F_9KO_3S$, purity≥97%) and perfluorohexane sulfonate (PFHxS, CAS NO: 3871-99-6, $C_6F_{13}KO_3S$, purity≥97%) were purchased from Sigma-Aldrich (USA). Stock solution (10.0 mg/L) was prepared with dimethyl sulfoxide (DMSO, Sigma) and stocked at 4 °C. The stock solution was diluted with 1% DMSO to obtain a work solution with a concentration of 100.0 ng/L.

2.2. Preparation of nematode

The cultivation of the wild-type N2 *C. elegans* was based on previous studies. Briefly, the nematodes were cultured on nematode growth medium (NGM) with *E. coli* OP50 as food source (Brenner, 1974). Age synchronized eggs for toxicity test were obtained with fresh Clorox solution (1% NaClO and 0.5 mol/L NaOH) (Emmons et al., 1979).

2.3. Multi- and trans-generational exposure

The exposure of nematodes to PFBS and PFHxS was through the NGM. Briefly, 1% work solutions (i.e., with a dilution factor of 100) were added into NGM when it cooling down to 55 °C. The medium was poured into plates and cooled down to form agar. That is to say, the actual exposure concentrations of PFBS or PFHxS were 1.0 ng/L based on their environmental concentrations (Cui et al., 2020; Pizzurro et al., 2019). At the same time, 1% DMSO was also added with a dilution factor of 100 into NGM to form agar, and the final DMSO concentration in exposure was as low as 0.01%. Then, *E. coli* OP50 suspension from the same culture medium was spread across each NGM agar to form a lawn of bacteria. Each group including the solvent control had 10 replicates.

The multi-generational experiment on *C. elegans* was designed as described in earlier studies (Li et al., 2019), and the scheme is shown in Fig. 1. Age-synchronized eggs were used in this study to ensure exposure covered the entire life cycle of the nematode. After synchronization, eggs were pipetted onto the NGM containing target chemicals or solvent control solutions. The first exposed generation was marked as F1. After 72 h incubation at 15 °C, approximately 500 mature nematodes in each group were collected to measure effects in F1. For the multi-generational exposure effect, approximately 100 mature nematodes were picked onto new NGM agars with the same target chemicals or solvent control solutions. On the next day, the mature nematodes were removed and the newly hatched eggs were marked as F2 to start the exposure to a new generation. After 72 h, part of the mature F2 nematodes were used to measure effects, and another part of it were used to reproduce F3. The procedure was repeated until F4. For the *trans*-generational effect, approximately 100 mature nematodes from F1 were picked onto new NGM agars without any compounds. The newly hatched eggs within the first 24 h were marked as T1 generation. The T1 nematodes were used to hatch T2 which was used to obtain T3. The same procedure was employed to gain the offspring (T1′-T3′) of F4 generation.

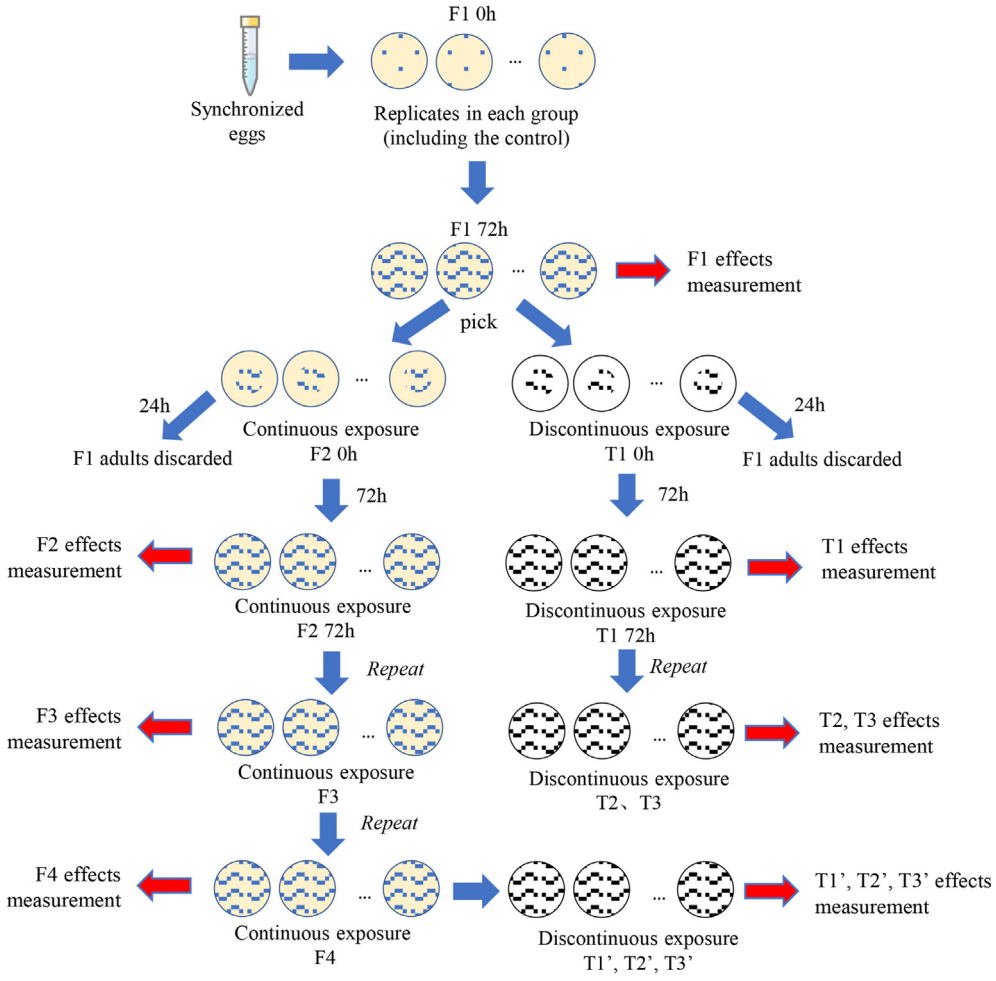

Fig. 1. Diagram for studying the multi-generational effects with continuous exposure from F1 to F4 and *trans*-generational effects in T1 to T3 (offspring of F1) and T1′ to T3' (offspring of F4) without continuous exposure.

2.4. Fat content measurement

Fat content (FC) were measured according to the Quick oil red O (qORO) method (Chen et al., 2018b; Wahlby et al., 2014). Briefly, the nematodes were washed twice with S buffer which contained 0.05 mol/L K_2HPO_4, 0.05 mol/L KH_2PO_4 and 0.1 mol/L NaCl. The nematodes were then transferred to 1.5 mL Eppendorf tubes and centrifuged at 2500 rpm for 3 min. After the supernatant was discarded, 500 μL isopropanol were added into the tubes followed by a centrifugation at 2500 rpm for 3 min. After the supernatant was discarded, ORO dye was added to stain the nematodes for 8−16 h. Finally, photos were obtained using microscope imaging system. The area being stained and total nematodes were calculated by using photoshop, and the ratio between them was used to characterize fat storages.

2.5. Biochemical indices measurement

The biochemical indices were chosen and measured according to earlier reports (Yu et al., 2020). Firstly, the nematode samples were homogenized with phosphate buffered solution (PBS) in ice bath, and the homogenates were centrifuged at 4000 rpm at 4 °C. The supernatants were used in measuring key enzymes/proteins. Among them, GPAT and FAS were chosen to represent enzymes that facilitate fat accumulation; Lipase, FATP, ACS, CPT, ACO and ACC were chosen as enzymes that promote fat consumption. The total protein (TP) in each sample was also measured using BCA Protein Assay Kit (Beyotime Biotechnology). All biochemical indices are presented as a proportion of TP in corresponding samples.

2.6. Gene expression levels detection

The expression levels of target genes were determined via qRT-PCR according to earlier report (Chen et al., 2018b). Firstly, total RNA was isolated from nematode samples using TRIzol reagent. Next, the total RNA was reversely transcribed into cDNA according to the manufacturer's instructions. Then, SYBR green Master Mix was used to perform RT-PCR reactions on Applied Biosystems 7900HT Fast Real-Time PCR System (USA). The relative expression levels of the chosen genes were quantified using the $2^{-\triangle\triangle CT}$ method. The expression levels *act-1* were treated as an internal standard (Zhang et al., 2012). The primers used for qRT-PCR are listed in Table S1.

2.7. Data presentation and statistical analysis

The data presentation and statistical analysis were based on previous research (Li et al., 2020b). The data of fat storage and lipid metabolism were expressed as percentage of the control (POC). Each exposure group had its own control group. The POC value of

less than 100% indicated inhibition, and the value of higher than 100% indicated stimulation. The results of expression levels of genes were showed as $\log_2$ (fold change against the control) (Wu et al., 2019). Statistical analysis was carried out using Origin software (Origin Lab Corp., USA). One-way ANOVA with post hoc Tukey's test ($p < 0.05$) was employed to analyze the significant differences among indices. Venn diagram was also employed to identify the shared changes in indices across generations.

3. Results and discussion

3.1. Effects of PFBS and PFHxS on lipid metabolism in F1

Results showed that PFBS did not significantly influence FC (Fig. 2a). However, it significantly influenced the key enzymes in lipid metabolism. For example, PFBS significantly stimulated FAS, Lipase, ACS and CPT with POC values as 115.0%, 134.2%, 113.4% and 120.8% ($p < 0.05$), respectively. At the same time, it significantly inhibited GPAT and ACC with the POC values as 69.3% and 61.8% ($p < 0.05$). At the same time, PFBS significantly up-regulated the expressions of *daf-2, daf-16, pod*-2 that regulate fat synthesis in wild type nematodes (Fig. 2c). It also significantly down-regulated *fat*-5 that encodes delta-9 desaturases in regulation of unsaturated fatty acids (Brock et al., 2006). Such results showed that PFBS significantly disturbed the lipid metabolism.

It was found that PFHxS significantly increased the FC with a POC value of 112.8% ($p < 0.05$, Fig. 2b). It also significantly stimulated ACC, ACS and CPT while inhibited FAS and ACO. Notably, PFHxS showed opposite effects on ACC and FAS compared to PFBS. In the results of gene expression levels (Fig. 2d), PFHxS significantly up-regulated *daf-2*, *daf-16*, *daf-7* and *ech-1*, and also *cebp-2* which was not influenced by PFBS. Moreover, PFHxS down-regulated *fat-5* more significantly than PFBS. Such results showed that PFHxS disturbed the overall lipid metabolism and also demonstrated the difference from those of PFBS.

In previous research, PFOA significantly increased FC (Li et al., 2020b), and the increased levels were greater than those of PFHxS in the present study. That is to say, the obesogenic potentials followed an order of PFOA > PFHxS > PFBS. Such order was consistent with the dependence on the chain length in earlier studies on developmental and reproductive toxicities (Gomis et al., 2018). These results may be explained by the greater lipophilicity in PFASs with longer chains. Such chain-dependence was also observed in toxicity and bioaccumulation of PFASs (Hagenaars et al., 2011; Liao et al., 2009). At the same time, both PFHxS and PFBS were correlated with body weight and obesity index in cats (Bost et al., 2016), which was different from the present finding where only PFHxS showed the obesogenic effects in nematodes. Such differences indicated different baselines of lipid content or metabolism in various animals, which might influence their responses to environmental obesogens.

The IIS pathway was the core pathway for lipid accumulation in *C. elegans*. In this pathway, both *daf-2* and *daf-16* genes were important. The former one was the main receptor, and the latter one was in the down-stream to regulate metabolism (Watkins et al., 2015). In this study, the upregulation of *daf-2* and *daf-16* indicated the involvement of IIS pathway in the effects of PFBS and PFHxS on lipid metabolism. Such involvement also explained the connection between PFAS exposure and diabetes which also closely relates with the IIS pathway (Schillemans et al., 2021). Moreover, such close involvement of IIS pathway in obesogenic effects was also founded in lindane (Chen et al., 2018b), BPA (Chen et al., 2016), PFOA (Li et al., 2020b) and antibiotics (Li et al., 2020a; Yu et al., 2020).

The *cebp-2* gene encodes CCAAT/enhancer-binding proteins (C/EBPs) in *C. elegans*, which was related to the adipocyte differentiation (Xu et al., 2015; Yang et al., 2006). Studies have shown that *cebp-2* mutation reduced fat accumulation (Xu et al., 2015). In the present study, PFHxS significantly up-regulated the expression of *cebp-2* and therefore affected the differentiation of fat cells. Despite of different up- or down-regulations on the expressions, both *ech-1* and *fat-5* were involved in the effects of PFBS and PFHxS. Notably, *ech-1* and *fat-5* participate in the fatty acid desaturation (Brock et al., 2007; Wang et al., 2018). Therefore, PFBS and PFHxS are

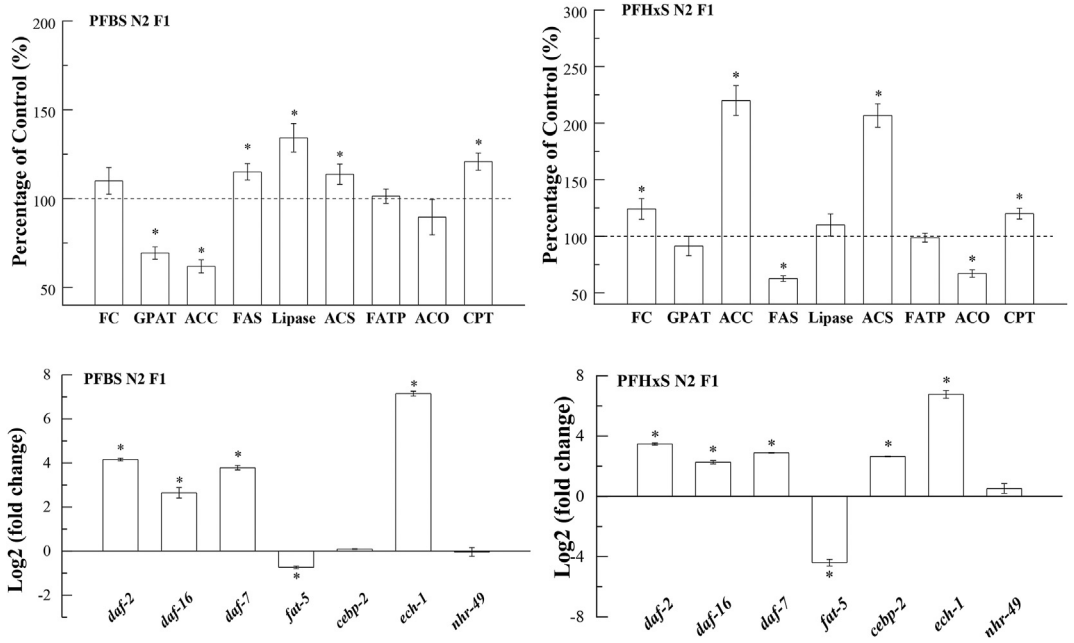

Fig. 2. Effects of perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) on fat content (FC), key enzymes and expression of target genes in lipid metabolism of *C. elegans* in the first exposed generation (F1). Asterisk (*) indicates significant difference from the control by one-way ANOVA ($p < 0.05$).

expected to influence the saturation levels of the fatty acids. Such concerns were consistent with the findings on the long-chained PFASs (PFOS and PFOA) which significantly influenced the composition of mono- and poly-unsaturated fatty acids (PUFAs) (Arukwe et al., 2013).

3.2. Multi-generational effects of PFBS and PFHxS on lipid metabolism

A multi-generational exposure was performed on three subsequent generations (from F2 to F4). Results showed that PFBS significantly increased FC in F2 (Fig. S1) and F4 (Fig. 3) which was not observed in F1 (Fig. 2). Such effects indicated that a multi-generational exposure might be necessary for PFBS to provoke obesogenic effects. In F4, PFBS stimulated FAS, lipase, ACS and ACO while inhibited FATP, and it down-regulated the expressions of *daf-16*, *ech-1* and *nhr-49*. The results showed both similarity and difference from those in F1, indicating continuous but different influences of PFBS on the lipid metabolism and the IIS pathway over generations. On the other hand, PFHxS stimulated FC in F2 (Fig. S1) and F4 (Fig. 3) without significant differences from those in F1 (Fig. 2). Such effects indicated that the obesogenic influence of PFHxS lasted over generations. The effects of PFHxS on the biochemical indices and expression levels of target genes were also similar to those in F1, indicating continuous influences of PFHxS on the lipid metabolism and the IIS pathway over generations.

One earlier review summarized six patterns in effects over generations (Bell and Hellmann, 2019). They included (1) bounce back, where the effects in F1 did not persist in F2 which did not show significant effects; (2) weaken, where the effects in F1 were still observed in F2 but with less or decreased levels; (3) persist, where the effects in F1 were observed in F2 with similar levels; (4) accumulate, where the effects in F1 were observed in F2 with increased levels; (5) delay, where the effects were not observed in F1 but in F2; and (6) reverse, where the effects in F1 changed to opposite directions in F2 (e.g., inhibition in F1 but stimulation in F2, or vice versa). In the present study, the alteration from no-effect to stimulation over generations in effects of PFBS belongs to the delay pattern. At the same time, the continuousness in the effects of PFHxS belongs to the persist pattern. Both patterns were consistent with the epidemiological studies that prenatal exposure to PFASs increased the obesity risk in offspring (Mora et al., 2017). Therefore, the long-term impacts of PFASs, especially the delayed results in PFBS, raised further concerns on the long-term outcome of PFASs exposure, which might be neglected in short-term studies.

The continuous disturbance of PFBS and PFHxS on the lipid metabolism and regulating pathways over generations indicated potential influences on the adaptation/fitness of organisms (Agathokleous and Calabrese, 2020; Westneat et al., 2019). Furthermore, nematodes are essential in the food web in soil environment. The nematodes containing more fat content indicated high-fat diet for their predators, and therefore showed implication to explain the widely observed overweight or obesity throughout the animal kingdom (Klimentidis et al., 2011), with potential disturbances on the ecological stability.

3.3. Trans-generational effects of PFBS and PFHxS on lipid metabolism

In T1 and T3, PFBS did not significantly influence the FC (Fig. 4), indicating no significant residual impacts on the overall lipid metabolism. In T1, PFBS significantly inhibited FAS and ACS and stimulated FATP and CPT. At the same time, it up-regulated the expressions of *daf-16*, *daf-7*, *fat-5*, *ech-1* and *nhr-49* and down-regulated those of *cebp-2*. In T3, PFBS significantly inhibited GPAT and FATP and stimulated ACC and FAS, while it showed up-regulation on the expressions of *daf-2*, *daf-16*, *fat-5*, *cebp-2* and *ech-1*. The results showed differences between F1 and T1 effects and also between T1 and T3 effects. The *trans*-generational effects of PFHxS in T1 and T3 showed similar pattern to those of PFBS, i.e., no significant residual impacts on FC with continuous influences on lipid metabolism, IIS pathway and also the adipocyte differentiation (*cebp-2*).

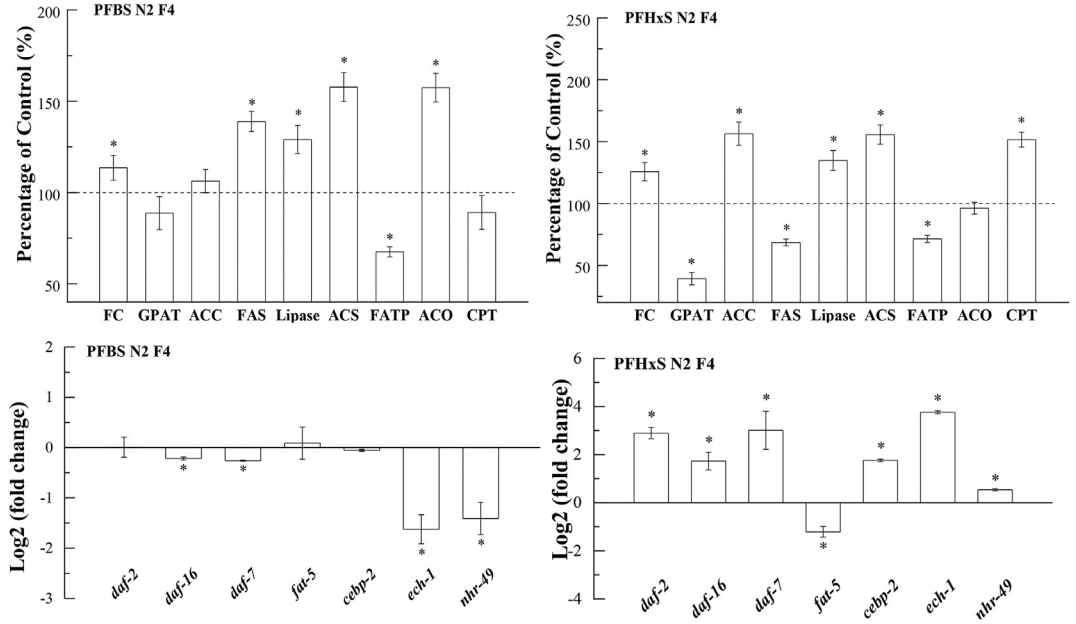

Fig. 3. Effects of perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) on fat content (FC), key enzymes and expression of target genes in lipid metabolism of *C. elegans* in the fourth consecutively exposed generation (F4). Asterisk (*) indicates significant difference from the control by one-way ANOVA ($p < 0.05$).

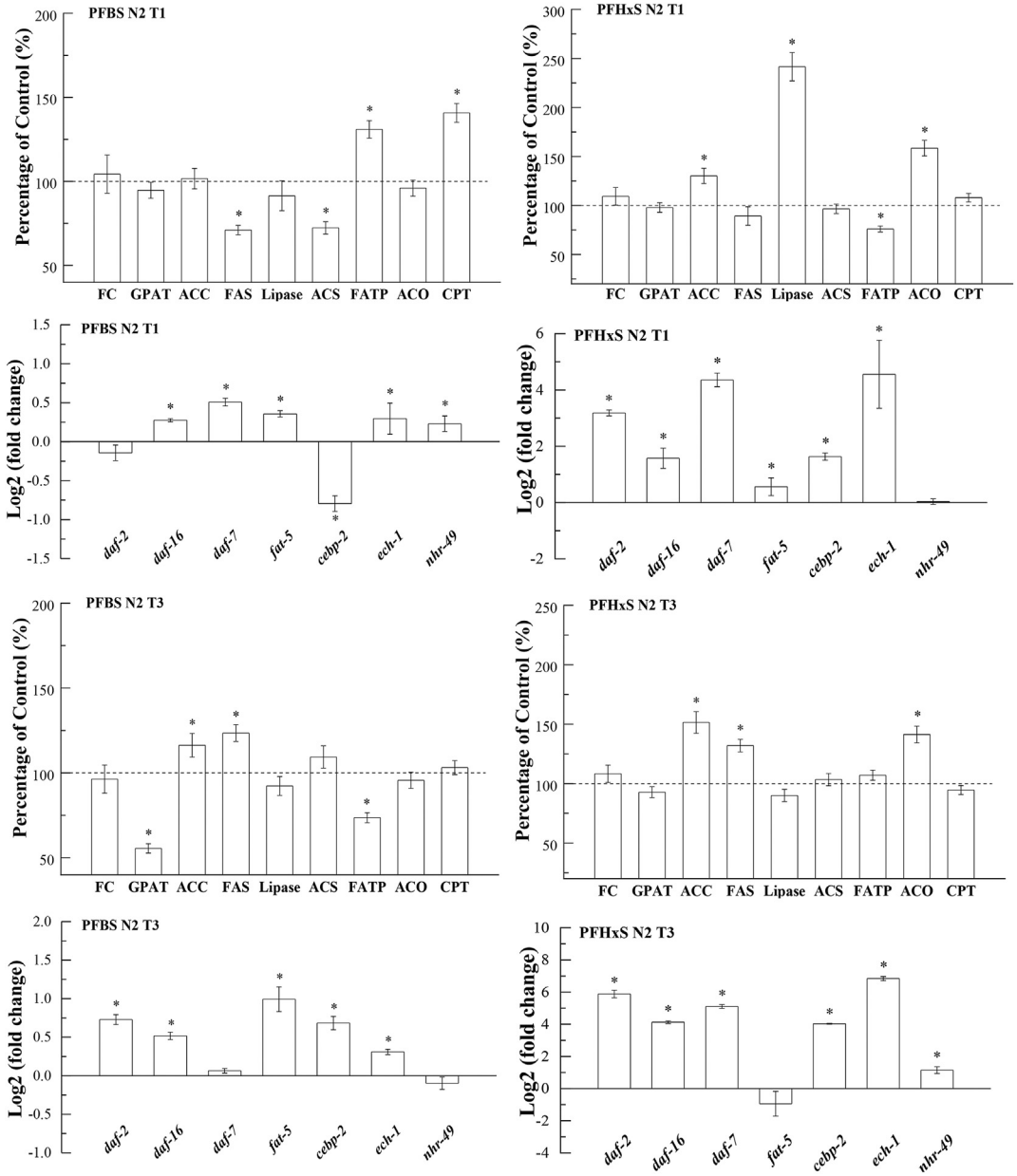

Fig. 4. Effects of perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) on fat content (FC), key enzymes and expression of target genes in lipid metabolism of *C. elegans* in the non-directly exposed offspring (T1 and T3) of the first exposed generation (F1). Asterisk (*) indicates significant difference from the control by one-way ANOVA ($p < 0.05$).

In T1′ and T3′, PFBS did not influence FC (Fig. 5). It stimulated GPAT, FATP, ACO and CPT and inhibited lipase in T1′, while it stimulated GPAT and inhibited ACS and FATP in T3'. At the same time, PFBS down-regulated the expressions of *daf-2*, *daf-16*, *daf-7*, *fat-5* and *cebp-2* and up-regulated that of *nhr-49* in both T1′ and T3′, showing similarities which were not observed between T1 and T3. The effects of PFBS in T1′ and T3′ on the biochemical indices and gene expressions were different from those in T1 and T3. Although the effects of PFHxS in T1′ and T3' showed differences from those of PFBS, they shared the same pattern that multi-generational exposure in F4 had different residual influences from those in F1.

In epidemiological studies, prenatal/maternal exposure to PFOS/PFOA and their influences on composition of PUFAs significantly influenced the health of the offspring (Kishi et al., 2015). In our previous laboratory studies, PFOA showed significant residual obesogenic effects in T1 (offspring of F1) and T3' (offspring of F4) (Li et al., 2020b). In the present study, although PFBS and PFHxS did not show significant residual effects on FC in T1 to T3 (offspring of F1) or T1′ to T3' (offspring of F4), they still possessed disturbances on the lipid metabolism, IIS pathway and adipocyte differentiation. Moreover, multi-generational exposure showed significant influences on the *trans*-generational residual impacts.

The Venn diagram (Fig. 6) was performed with an intention to explore common patterns that were shared in the multi- and *trans*-generational effects. Concerning the effects of PFBS in F1 and F4, there were 3 common responses including increases in lipase (marked as 0101), ACS (1101) and FAS (0111). Concerning the effects PFBS in T3 and T3′, there were 5 common responses including

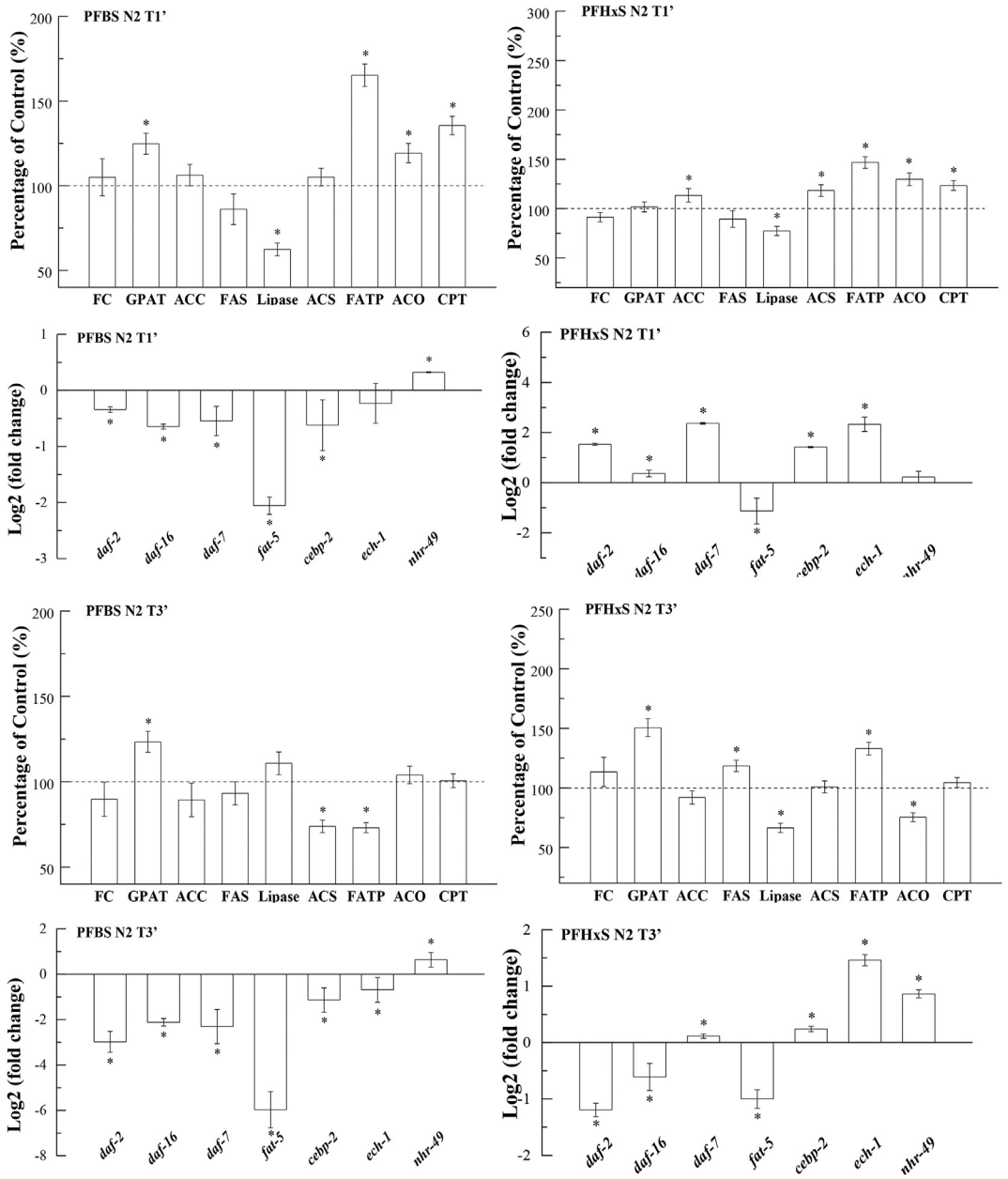

Fig. 5. Effects of perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) on fat content (FC), key enzymes and expression of target genes in lipid metabolism of *C. elegans* in the non-directly exposed offspring (T1′ and T3′) of the fourth consecutively exposed generation (F4). Asterisk (*) indicates significant difference from the control by one-way ANOVA ($p < 0.05$).

increases in ACC and up-regulation in *cebp-2* (marked as 1010) and up-regulation on daf-2, *daf-16* and *ech-1* (marked as 1011). However, there were no shared common responses in all the multi- and *trans*-generational effects of PFBS. On the other hand, PFHxS commonly up-regulated *daf-7* and *ech-1* (marked as 1111) in F1, F4, T3, and T3'. Such results demonstrated the side-chain influence on the multi- and *trans*-generational obesogenic effects with different potential mechanisms. Notably, the IIS pathway (including *daf-7*) is closely connected with epigenetic regulation (e.g., histone methylation) (Inoue et al., 2021). Moreover, the epigenetic regulations were already reported to be involved in the obesogenic effects of environmental pollutants (Li et al., 2020a). Therefore, the epigenetic regulation should be considered in future mechanism studies.

Since the DOHAD hypothesis (i.e., Developmental Origins of Adult Disease Hypothesis) by David Barker (Barker, 2003; Painter et al., 2006), increasing attentions fell on the *trans*-generational effects of environmental chemicals. Studies from rodents have demonstrated complex effects of environmental obesogens on body weight across generations, especially when exposures are only limited to the first generation (Heindel et al., 2015). The present study and also some earlier studies demonstrated the importance of multi-generational effects to provide a full picture of outcomes by long-term exposure to environmental pollution (Li et al., 2020a).

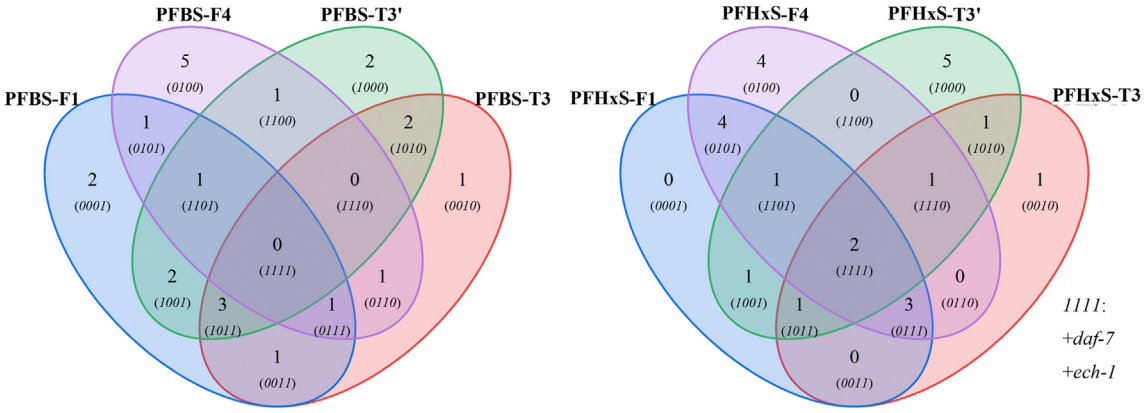

Fig. 6. Venn diagram for common pattern among the multi- and *trans*-generational effects of perfluorobutane sulfonate (PFBS) and perfluorohexane sulfonate (PFHxS) on the lipid metabolism of *C. elegans* in the first and fourth exposed generation (F1 and F4) and their non-exposed offspring (T3, and T3′).

4. Conclusion

PFBS (four-carbon) did not stimulate FC in F1 but in F4 in the multi-generational exposure. It showed continuous but different disturbances on the lipid metabolism and IIS pathway. PFHxS (six-carbon) stimulated FC in F1 and F4 with similar disturbances on the lipid metabolism and IIS pathway. The multi-generational exposure of PFBS and PFHxS influenced the residual effects in the following *trans*-generational experiment without continuous exposure. The multi- and *trans*-generational obesogenic effects demonstrated the long-term health risk of short-chained PFASs. The epigenetic regulation should be considered in future mechanism studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT author statement

Zhuo Li: Formal analysis, Investigation, Data curation, Writing – original draft. Zhenyang Yu: Conceptualization, Methodology, Software, Writing – review & editing, Supervision, Project administration, Funding acquisition. Daqiang Yin: Resources, Laboratory support, Project administration, Funding acquisition.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2021.130666.

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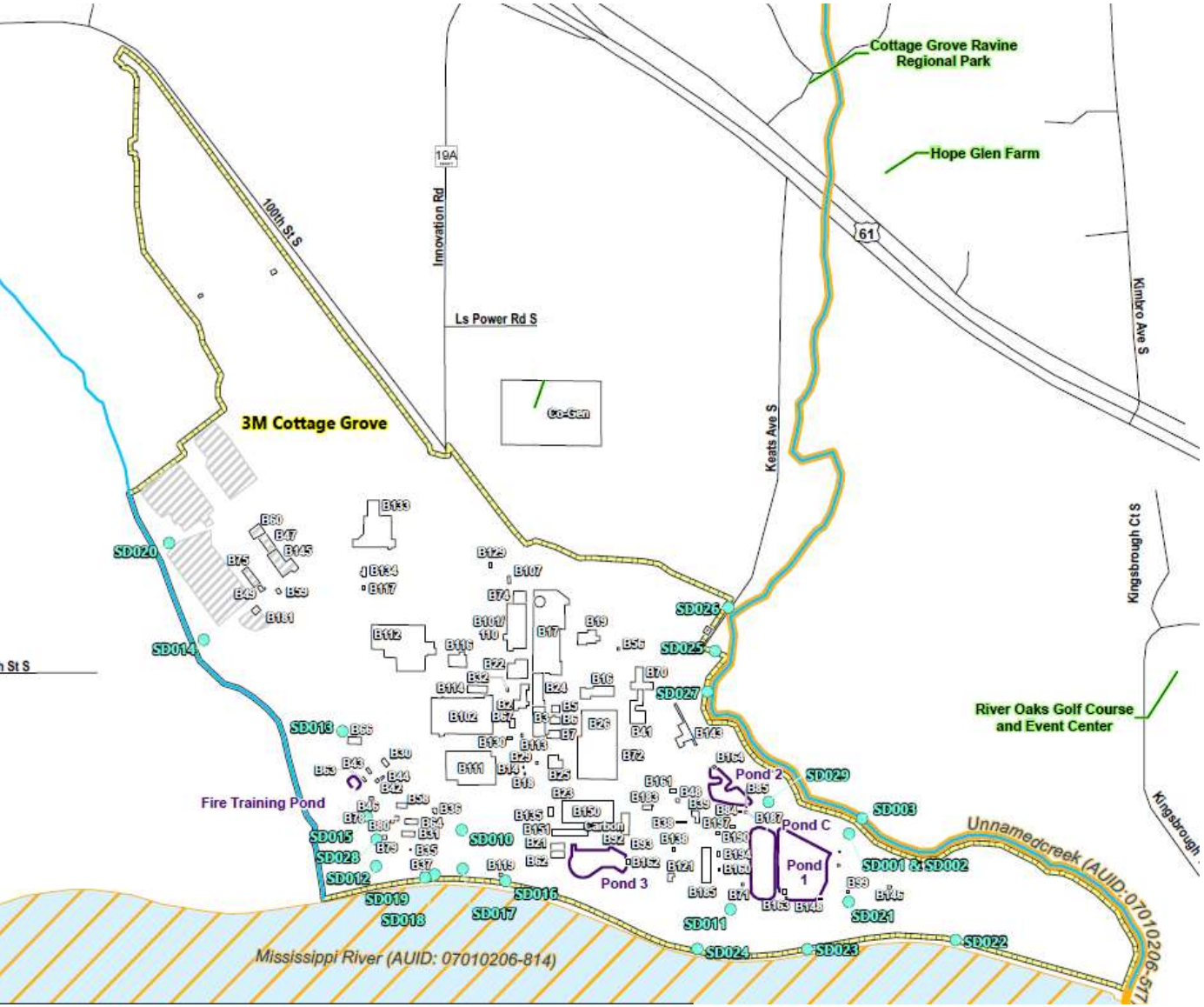
MINNESOTA POLLUTION CONTROL AGENCY

Draft wastewater permit for 3M Cottage Grove

July 2024

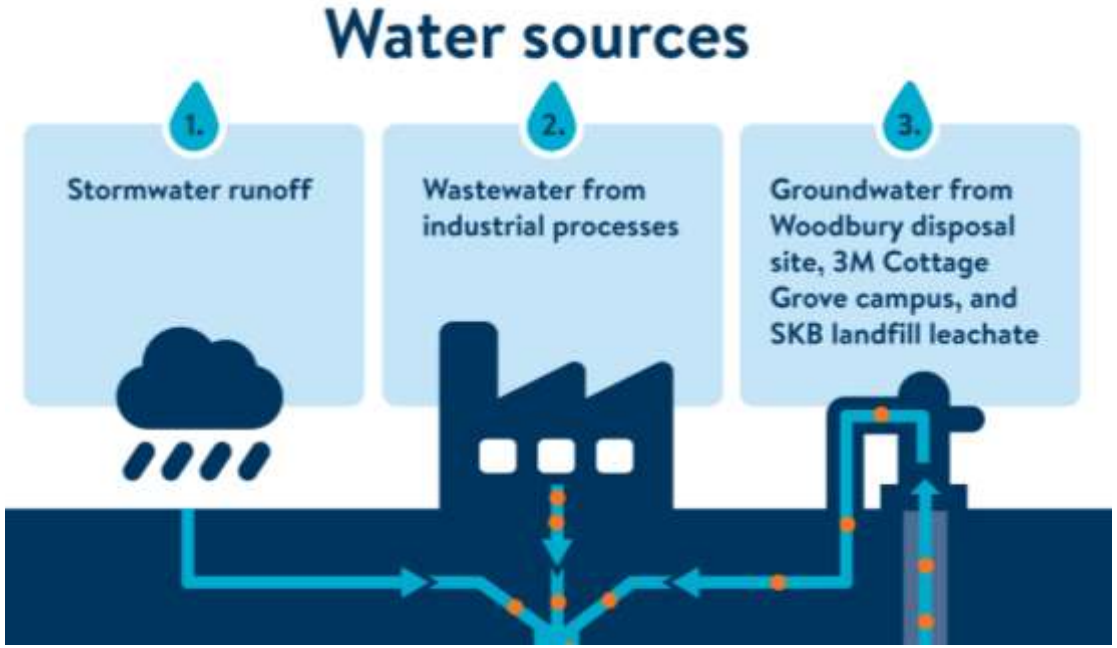
Agenda



1. Background
2. Pollution limits
3. Monitoring
4. Treatment technology
5. Compliance schedule
6. Transparency and accountability
7. Public comment period
8. Questions

Background: Facility and permit overview

- The 3M Cottage Grove facility manufactures a diverse group of products, including PFAS
- History of PFAS pollution and enforcement actions
- Wastewater treated at this facility is from a variety of sources
- Draft permit includes over 70 new or lower discharge limits, new treatment technology, and comprehensive monitoring



Background: MPCA actions on PFAS in wastewater

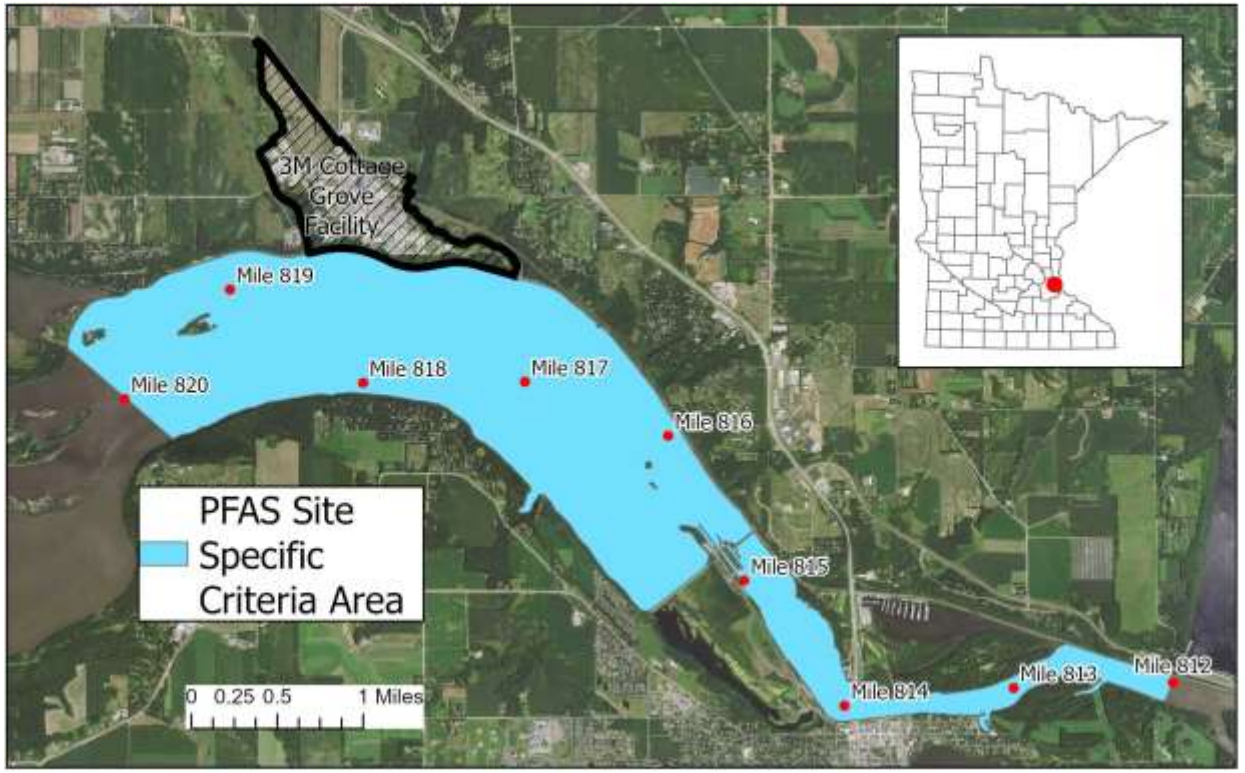
- 2003 permit with PFAS monitoring requirements
- 2007 Consent Order requires cooperation
- MPCA adds additional PFAS monitoring requirements in 2007 and 2020
- 2011 permit is paused due to the state's lawsuit, but onsite granular activated carbon treatment of incoming groundwater from both Woodbury and on-site wells is implemented in 2013
- 2018 Settlement does not directly address wastewater but leads to extensive groundwater testing
- Enforcement orders as part of an ongoing non-public investigation initiated in 2020, referenced in the draft permit fact sheet
- New (2024) site-specific water quality criteria for the Mississippi used in draft permit

Pollution limits: Non-PFAS

New limits in addition to PFAS

- Seven metals
- 55 semi-volatile or volatile organics
- Ammonia
- Phosphorus

Pollution limits: Role of PFAS site-specific criteria



- Site-specific criteria for Mississippi miles 820 – 812 were calculated in 2024 based on the 2023 assessment and factors like the latest toxicity research used by EPA
- This portion of the Mississippi is a Class 2 water
- Data was robust enough to establish criteria for six PFAS
- Criteria are used with other factors to set permit limits

Pollution limits: Factors contributing to PFAS limits

- 2024 site-specific water quality criteria
- Class 2 water designation
- “Unnamed creek” discharge point is recognized as a protected water

Pollution limits: PFAS

PFAS	Site-specific water quality criteria		Draft permit limits
	Surface water	Fish Tissue	Calendar month average
PFOS	0.027 ng/L	0.021 ng/g	0.038 ng/L (Detection limit: 2.2 ng/L)
PFOA	0.0092 ng/L	0.00036 ng/g	0.013 ng/L (Detection limit: 2.1 ng/L)
PFHxS	0.0023 ng/L	0.000043 ng/g	0.0032 ng/L (Detection limit: 2.1 ng/L)
PFHxA	4,400 ng/L	Not applicable	6,172 ng/L
PFBS	3,000 ng/L	Not applicable	4,208 ng/L
PFBA	25,000 ng/L	Not applicable	35,068 ng/L

Monitoring: More locations

Monitoring required in all treated wastewater and stormwater streams

- 19 industrial stormwater locations
- Seven internal waste streams, prior to treatment and between treatment locations
- Four locations on Mississippi river upstream and downstream of discharge
- Fish tissue up and downstream of discharge – required as part of instream study

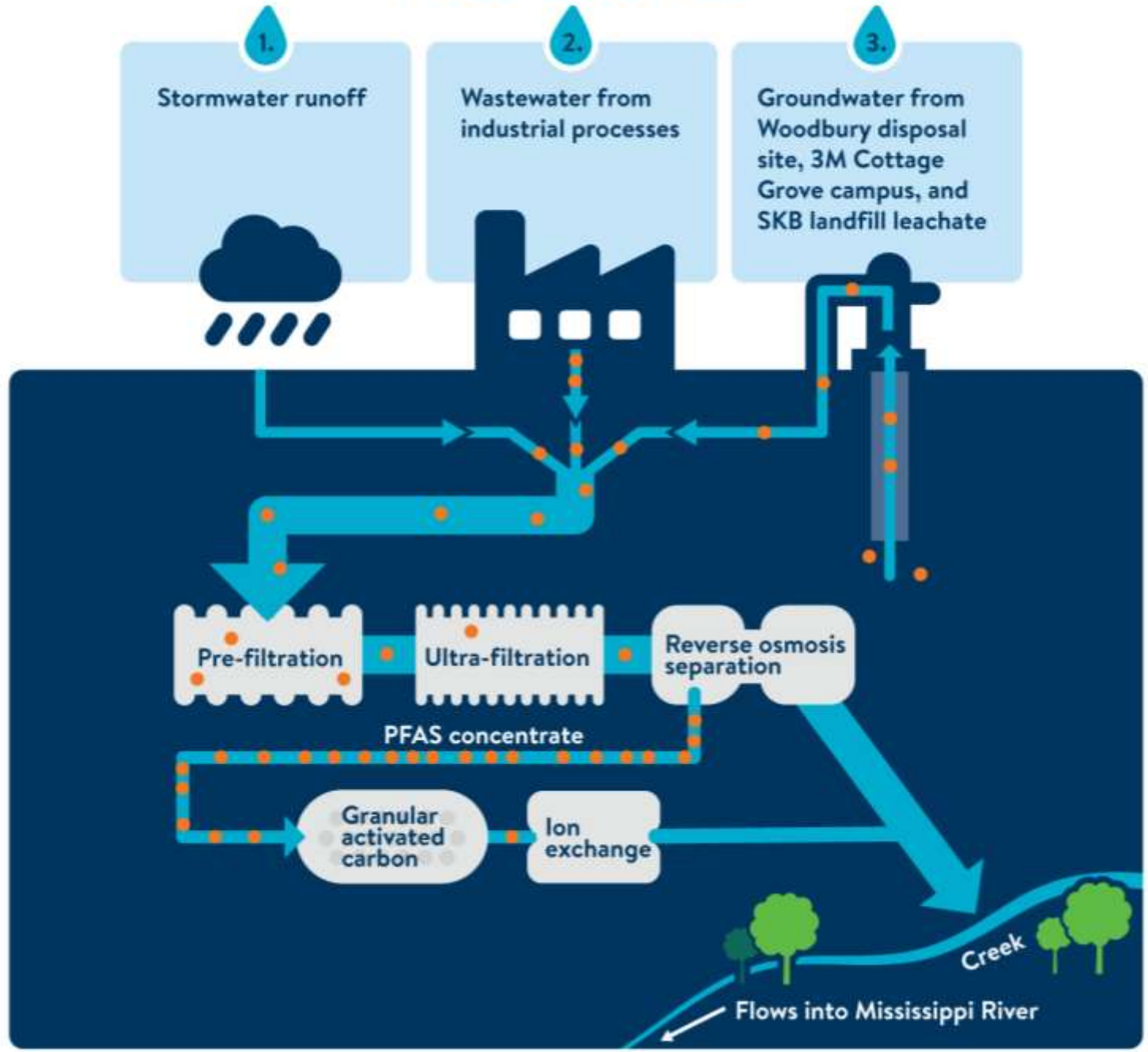
Monitoring: Greater detail and frequency

- Over 100 PFAS plus non-targeted analysis
- Semi-volatile and volatile organics, metals, nitrogen series, and salts
- Whole effluent testing changed from acute to chronic
- Added technology-based limits to SD 001 based on updates to 40 CFR 414 (Organic Chemicals, Plastics and Synthetic Fibers (OCPSF) Effluent Guidelines)
- Priority pollutant testing increased from twice to four times per year

Treatment technology

- 3M began construction of an Advanced PFAS Wastewater Treatment System in 2022
- Built in response to changing regulatory climate and in anticipation of this permit
- Two large buildings plus distillation towers
- Modelled on smaller facilities in Cordova, IL and Decatur, AL

Water sources

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Compliance schedule

PFAS/Advanced Wastewater Treatment System

- Initiation of operation by March 31, 2025
- Attain compliance with new limits by December 31, 2026
- Interim limits are in effect from permit issuance until then

Non-PFAS parameters

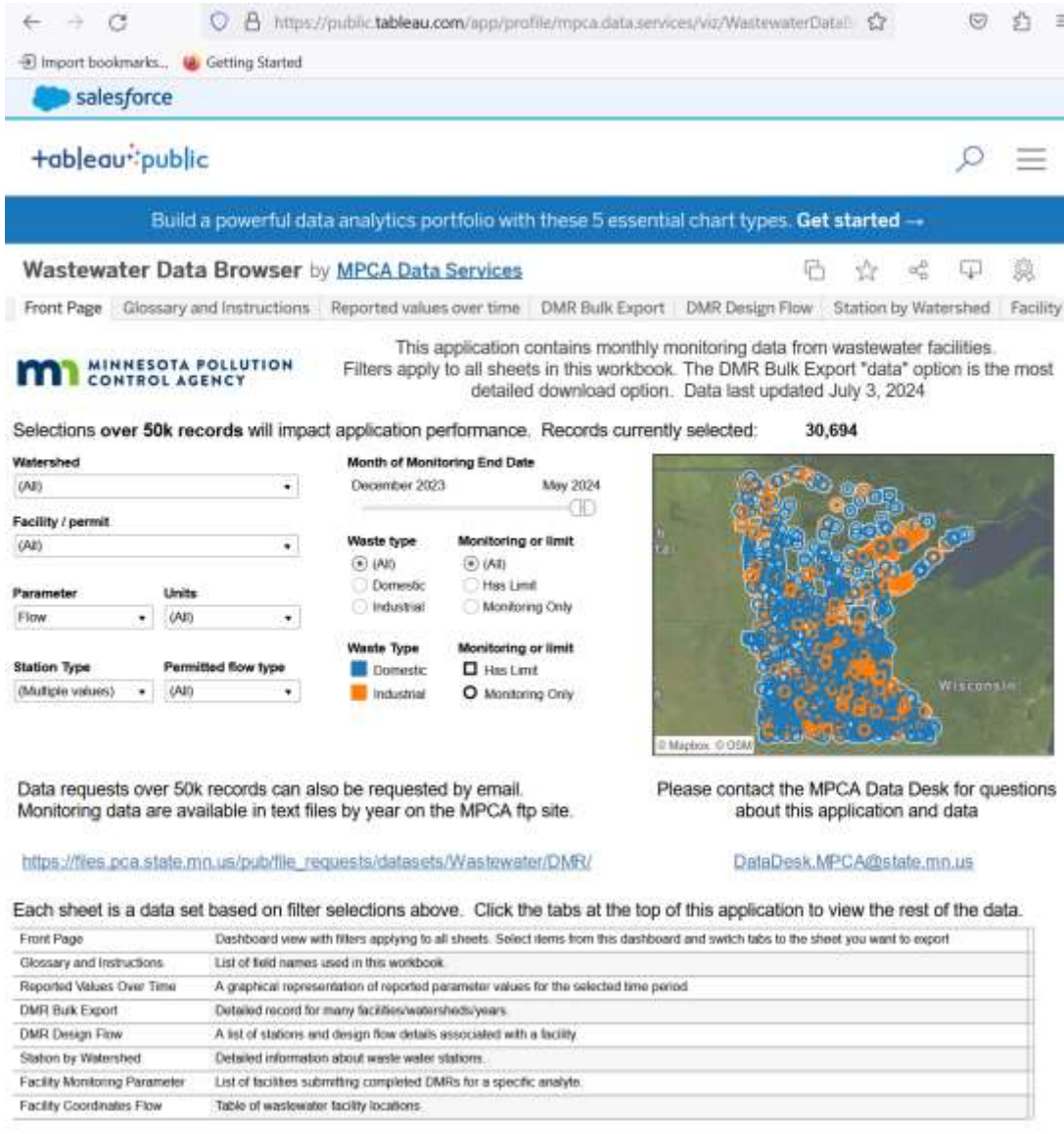
- By 5 years after permit issuance
- Interim limits in effect until final limits are met

Compliance schedule

- Individual subsurface/sewage treatment systems must be evaluated and upgraded by October 31, 2027
- Flow monitoring in creek upstream of discharging must begin by one year after permit issuance
- Incinerator closure water must receive comparable PFAS treatment by July 1, 2025

Transparency and accountability

- 3M must hold a community meeting once per year
- Monitoring data reported electronically and available online
- Annual disposal report



Public comment period: key dates

- July 1, 2024: Extended 45-day public notice begins with special outreach
- July 29, 2024: Virtual public information session
- July 31, 2024: In-person community meeting in Cottage Grove
- August 15, 2024: Deadline for public comments – public notice closes
- Post-August 2024: Review of public comments and finalization of the permit
- March 31, 2025: Under the draft permit, 3M Cottage Grove's advanced wastewater treatment system must be in operation.
- December 31, 2026: Under the draft permit, 3M must comply with new limits. Interim limits are in effect until this date.

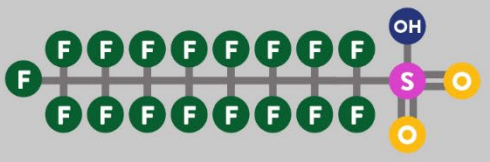
Thank you!

MINNESOTA POLLUTION CONTROL AGENCY

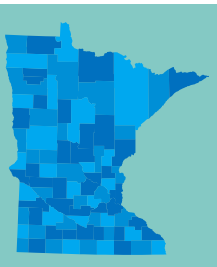
Water quality standards

May 2024

Human Health Protective Water Quality Criteria for Per- and Polyfluoroalkyl Substances (PFAS) in Mississippi River, Miles 820 to 812



MINNESOTA POLLUTION CONTROL AGENCY



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Minnesota Pollution Control

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May 29, 2024

- Minor, editorial errors were corrected

Minnesota Pollution Control Agency

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Document number: wq-s6-69a

Contents

Acronyms

ADAF	Age-Dependent Adjustment Factor
$AF_{lifetime}$	Lifetime Adjustment Factor
BAF	Bioaccumulation Factor
BCC	Bioaccumulative Chemical of Concern
CC_{FT}/CS_{FT}	Chronic Criterion or Standard – Fish tissue-based
CC_{FR}/CS_{FR}	Chronic Criterion or Standard – Fish consumption and recreation use class
CSF	Cancer Potency Slope Factor
CWA	Clean Water Act
EPA	United States Environmental Protection Agency
FCR	Fish Consumption Rate
HH-WQS	Human Health Water Quality Standards
IWR	Incidental Water Intake Rate
MDH	Minnesota Department of Health
MPCA	Minnesota Pollution Control Agency
Minn. R. ch.	Minnesota Rule chapter
NLC	Nonlinear Carcinogen
NPDES	National Pollutant Discharge Elimination System
PFAS	Per- and Polyfluoroalkyl Substances
PFBA	Perfluorobutanoic acid
PFBS	Pefluorobutane sulfonic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexane sulfonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
RfD	Reference Dose for noncancer toxicants and nonlinear carcinogens
ROS	Regression on Order Statistics
RSC	Relative Source Contribution factor
SSC	Site-Specific Water Quality Criteria
TSD	Technical Support Document
WQS	Water Quality Standard (refers to a pollutant-specific numeric standard in rule; also can refer to the three elements of a WQS)
WCBA	Women of Childbearing Age

Executive summary: site-specific water quality criteria for per- and polyfluoroalkyl substances

The Minnesota Pollution Control Agency (MPCA) has multiple programs monitoring and responding to per- and polyfluoroalkyl substance (PFAS) contamination in groundwater, surface water, and aquatic life, mainly fish. This technical support document (TSD) describes the derivation of site-specific water quality criteria (SSC) for six PFAS in the Mississippi River near Cottage Grove, MN.

The MPCA is the state agency responsible for setting water quality standards and criteria[1] under the Clean Water Act (CWA). Water quality standards (WQS) are used to:

- Protect water resources for uses such as: source for drinking water, fishing, swimming, and other aquatic recreation, and sustaining healthy communities of fish, bugs, plants, and other aquatic life.
- Identify polluted waters in need of restoration or healthy waters in need of additional protection.
- Guide the limits set on what regulated entities can discharge to surface water.

Minnesota's WQS are promulgated in Minn. R. ch. 7050 (Waters of the State), and 7052 (Lake Superior Basin Water Standards). Details of how WQS are implemented in point-source discharge permitting are contained in Minn. R. ch. 7053 (State Waters Discharge Restrictions), and parts of chapter 7052. WQS are the fundamental regulatory and policy foundation to preserve and restore the quality of all waters of the state. They consist of three elements:

- Water use classifications (beneficial uses) that identify how people, aquatic communities, and wildlife use our waters.
- Narrative and/or numeric standards to protect those uses by designating the specific amount of pollutants allowed in a body of water or making statements of unacceptable conditions in and on the water.
- Antidegradation policies to maintain existing uses, protect high quality waters, and preserve waters of outstanding value.

The federal CWA requires states to apply these three elements and other related protections as the framework for achieving the goals of this federal regulation.[2]

[1] In Minnesota, the term "water quality standard" or "WQS" refers to a promulgated narrative or numeric standard. A "water quality criterion/criteria" or "SSC" is a site-specific value(s) established for a specific toxic pollutant detected in surface water, fish, or effluents that lacks a numeric standard in rule.

[2] In the U.S. Environmental Protection Agency (EPA) guidance the numeric values that underpin application of water quality standards are called "water quality criteria" or "National Ambient Water Quality Criteria." Minnesota's water quality standards' rules use "criterion" or "criteria" to mean numeric values not listed in Minn. R. chs 7050 or 7052 but derived by EPA-approved methods in rule.

Minnesota's water quality rules establish the following seven beneficial uses for our waters:

Use class	Beneficial use
Class 1	Domestic consumption (i.e., drinking water and food processing)
Class 2	Aquatic life and recreation (including aquatic consumption)
Class 3	Industrial consumption
Class 4	Agricultural and wildlife
Class 5	Aesthetics and navigation
Class 6	Other uses
Class 7	Limited Resource Value Water (LRVW)

These use classes reflect the multiple beneficial uses that Minnesota's surface waters provide, and accordingly all surface waters are assigned multiple use classes. The MPCA also has the authority to protect groundwater for potable use in Minn. R. ch. 7060. Nearly all surface waters are designated Class 2 and require control of pollutants so that they are safe for people recreating and eating fish affected by contamination, and, if used as source waters for drinking, are also designated Class 1 for domestic consumption as described in Minn. R. chs. 7050 and 7052.[3]

Derivation of the PFAS SSC falls under the MPCA's authorities to protect human health from adverse impacts of toxic pollutants in in Class 2 surface waters and fish. PFAS are categorized as toxic pollutants that lack numeric WQS in rule; therefore, the MPCA has derived SSC that are as fully enforceable as WQS after allowing for the necessary opportunities for comment. The SSC are specific to protecting human health, and include several values, each specific to the surface water's designated beneficial uses. The CC for six PFAS applicable in surface water and/or fish-tissue are described in Table 1-1.

For purposes of this SSC derivation, the "site" is defined as the Mississippi River main channel between river miles 820 and 812 (referred to collectively as Pool 2 Section 4 and Pool 3 Section 1 in 3M's Instream PFAS Characterization Study Final Report, Mississippi River, Cottage Grove, Minnesota (Weston Solutions 2023)).

[3] The MPCA's Water Quality Standards also address impacts to aquatic life and fish-eating wildlife. Those evaluations are not covered in this TSD for human health-based SSC but should be reviewed in the future to determine if more stringent criteria are warranted to protect ecological species.

Table 1-1: Derived site-specific water quality criteria for PFAS for the protection of Class 2B surface water uses in Mississippi River, Miles 820 to 812

PFAS (CAS No. see Table 2-1)	Site-specific water quality criteria: Chronic Criteria (CC)		Health Risk Index Endpoints (Additive Risk)[4]
	Class 2B – fish consumption and recreational exposure (CC_{FR}) (30-day average)	**Class 2 fish-tissue** (CC_{FT}) (90^{th} percentile of 5 fish minimum per water body)	
PFOS	0.027 ng/L	0.021 ng/g	Developmental, Liver System, Immune System, Cancer
PFOA	0.0092 ng/L	0.00036 ng/g	Developmental, Liver System, Immune System, Cancer
PFHxS	0.0023 ng/L	0.000043 ng/g	Liver System, Thyroid (endocrine)
PFHxA	4,400 ng/L	Not applicable	Developmental, Thyroid (endocrine)
PFBS	3,000 ng/L	Not applicable	Thyroid (endocrine)
PFBA	25,000 ng/L	Not applicable	Liver System, Thyroid (endocrine)
Mixtures containing two or more of PFBA, PFBS, and PFHxA	≤ 1 (unitless) Health Risk Index	Not applicable	Thyroid (endocrine)

Definitions of CC:

CC_{FR}: Applied in Class 2B surface waters (F: Fish consumption and R: Recreational exposure)

CC_{FT}: Applied for Bioaccumulative Chemicals of Concern (BCC) in fish (fillet/muscle) for all Class 2 waters (FT: fish-tissue)

1. Introduction

Water quality standards (WQS) provide the minimum conditions for waters of the state to meet their designated beneficial uses. Numeric standards are a key foundation for ensuring that the regulatory goals of Minnesota's water quality statutes and rules and the Clean Water Act (CWA) are met.

WQS in Minn. R. chs. 7050 and 7052 provide the foundation for:

[4] When multiple chemicals are found in a water sample, those chemicals in combination may cause adverse effects that may not be equal to the effects that would be expected from exposure to a single chemical. When considering the effects from multiple chemicals, the health effects should be the same. The health risk index endpoints provided here are the currently known organ or body systems impacted by the chemical listed, that could be used to determine any additive effects of the chemicals when exposed in mixtures (MDH 2008). These endpoints are not necessarily what the CC_{FR} or CC_{FT} were based off of, though generally, at least one of the endpoints drive the criteria.

- Effluent limits in National Pollutant Discharge Elimination System (NPDES) wastewater and stormwater permits.
- Remedial cleanup goals.
- Assessment of available pollutant-specific monitoring data in surface waters and fish for the CWA 303(d) Impaired Waters List.

WQS are derived to be protective of both human health and aquatic life.[5] Minnesota's human health-based WQS protect the beneficial uses of drinking water, fish consumption, and recreation. Human health-based WQS are adopted into rule and are applicable to Class 2 surface waters across the state. For pollutants that do not have a human health-based WQS, human health-based water quality criteria may be derived and applied at a specific site or sites, based on methods already adopted into rule and approved by the United States Environmental Protection Agency (EPA). To summarize:

- WQS: Chronic Standards (CS) – derived for Class 2 waters; pollutant-specific standards adopted into rule.
- SSC: Chronic Criteria (CC) – derived and applied on a site-specific basis; based on methods adopted into rule (Minn. R. 7050.0217 to 7050.0219; 7052.0100 for the Lake Superior Basin).

CS and CC are derived based on the potential for adverse effects to human health and do not consider economic impacts or the availability of treatment technologies. Exceedance of a CS or CC is considered indicative of a polluted condition, which is actually or potentially deleterious, harmful, detrimental, or injurious with respect to the designated uses of the waters of the state (Minn. R. 7050.0150; 7050.0210, subp. 13). CS and CC refer to human health throughout the remainder of this document.

For purposes of SSC derivation, the "site" is defined as the Mississippi River main channel between river miles 820 and 812 (referred to collectively as Pool 2 Section 4 and Pool 3 Section 1 in 3M's Instream PFAS Characterization Study Final Report, Mississippi River, Cottage Grove, Minnesota (Weston Solutions 2023). This area is immediately adjacent to and downstream of 3M Cottage Grove manufacturing facility and demonstrably impacted by discharge from 3M (Figure 1-1). Several PFAS that are indicative of 3M production, including PFOSA, MeFOSA, MeFOSAA, MeFOSE, EtFOSA, EtFOSAA, EtFOSE, and HQ-115 are all detected in fish and water collected in this segment of the river. Fish and water data used for this SSC derivation were collected at the site in 2021.

[5] The MPCA's Water Quality Standards also address impacts to aquatic life and fish-eating wildlife. Those evaluations are not covered in this TSD for human health-based SSC.

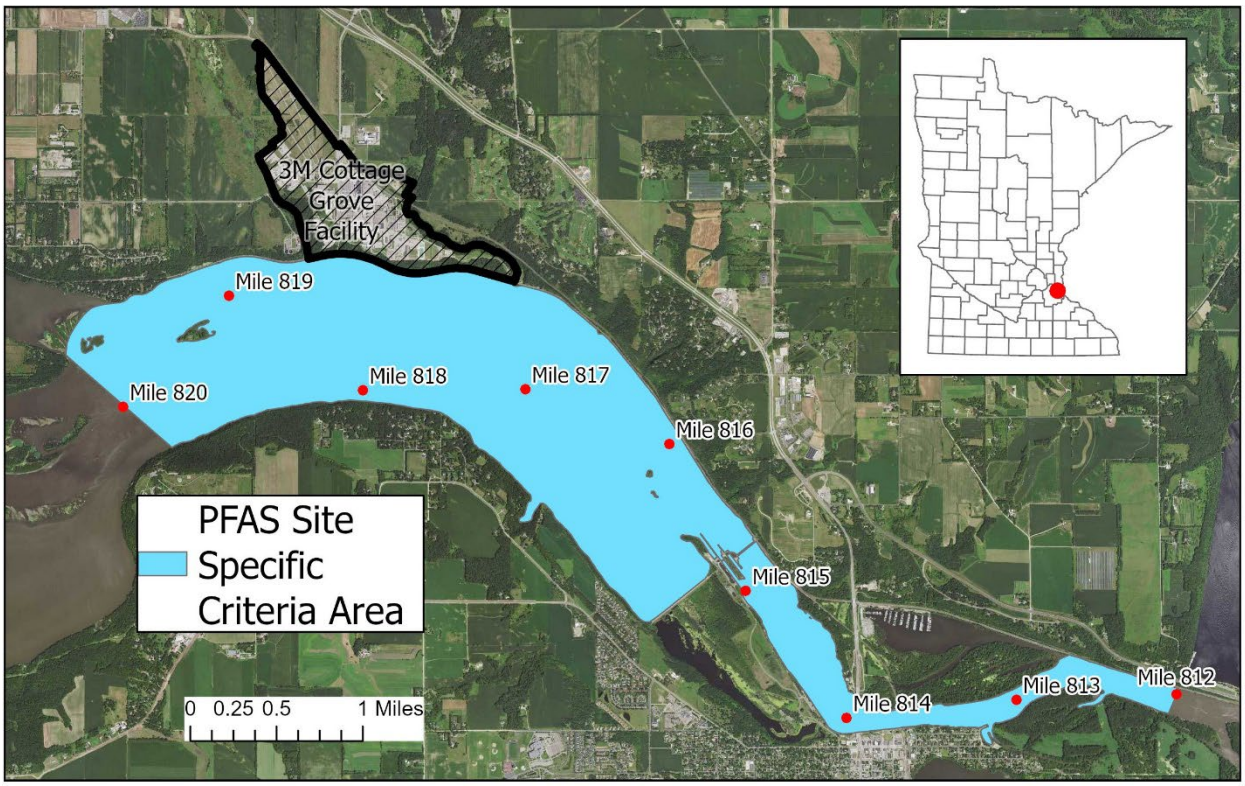

Figure 1-1: Location of site-specific water quality criteria development for Mississippi River, River Miles 820 to 812.

This TSD includes the derivation of a site-specific CC for perfluorobutane sulfonic acid (PFBS), perfluorobutanoic acid (PFBA), perfluorohexane sulfonic acid (PFHxS), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), and perfluorooctane sulfonic acid (PFOS). Class 2 CC are developed for application in fish-tissue and surface waters. The CC are based on the most recent toxicity information and the most recent site data, along with MCPA's 2017 human health-based WQS/SSC derivation methods as adopted in Minn. R. chs. 7050 and 7052.

Only the most recent site data were used in the SSC derivation because fish can rapidly take up and depurate PFAS (Hassel et al. 2020), leading to relatively rapid changes in fish tissue concentrations that follow changes in water concentration. Additionally, the most recent site data came from an extensive evaluation of site conditions that was designed to collect the type of data needed to develop criteria based on site-specific conditions. These data were determined to be the most reliable data that MPCA had available to best reflect current site conditions.

2. Problem formulation

2.1 Per-and polyfluoroalkyl substances

Minnesota defines PFAS as a class of fluorinated organic chemicals containing at least one fully fluorinated carbon atom (Minn. R. ch. 325F.075 subd. 1.3c). PFAS is a very large and diverse class of chemicals with a range of physicochemical properties and toxicity. Extensive sampling of both fish tissue and water have been completed in the Mississippi River for a subset of PFAS, allowing for the calculation of SSC for PFAS with sufficient environmental data and toxicity information. CC for PFAS are needed to

evaluate the risk of these toxic pollutants to human health and to use as a basis to remediate and control known and potential sources of PFAS contamination in the Mississippi River near Cottage Grove.

Table 2-1: PFAS evaluated for site-specific water quality criteria development for Mississippi River, Miles 820 to 812 (Acronyms, carbon/chain lengths, and CAS numbers)

PFAS by Acronyms		Aliphatic Carbon No. (Chain length)	CAS Numbers
PFBA	perfluorobutanoic acid	4	375-22-4 (acid) 45048-62-2 (anion)
PFBS	perfluorobutane sulfonic acid	4	375-73-5 (acid) 45187-15-3 (anion)
PFHxA	perfluorohexanoic acid	6	307-24-4 (acid) 92612-52-7 (anion)
PFHxS	perfluorohexane sulfonic acid	6	108427-53-8 (anion) 355-46-4 (acid) 3871-99-6 (potassium salt)
PFOA	perfluorooctanoic acid	8	45285-51-6 (anion) 335-67-1 (free acid) 335-66-0 (acid fluoride) 3825-26-1 (ammonium salt, APFO) 2395-00-8 (potassium salt) 335-93-3 (silver salt) 335-95-5 (sodium salt)
PFOS	perfluorooctane sulfonic acid	8	45298-90-6 (anion) 1763-23-1 (acid) 29081-56-9 (ammonium salt) 70225-14-8 (diethanolamine salt) 2795-39-3 (potassium salt) 29457-72-5 (lithium salt)

PFAS CC are derived based on the methods in Minn. R. chs. 7050 and 7052 for protecting human health from toxic pollutants in surface water and fish tissue.[6] The specific algorithms used, and subpopulations of concern depend on the use classification of the surface water and the toxicological profile of the pollutant. Details regarding the SSC methods and how they were applied to the PFAS CC are described in Sections 3 through 9.

[6] WQS methods are described in Minn. R. 7050.0217 through 7050.0219 for statewide application and Minn. R. 7052.0110 for the Lake Superior Basin. Derived site-specific CC have the same regulatory applications as the CS listed in Minn. R. 7050.0220 through 7050.0222 or 7052.0100 after allowing for comment as specified in Minn. R. 7050.0218, subp. 2, or 7052.0110, respectively.

2.2 Overview of fish and water data

Data used to develop the SSC were collected as part of an extensive site characterization conducted in 2021 by 3M and its contractors at the request of MPCA (Weston Solutions 2023). The study covered roughly 41 river miles of the Mississippi River, starting upstream of 3M Cottage Grove (Pool 2 Section 3, River Mile 833) and continuing downstream to River Mile 792 (Pool 4 Section 1) near Red Wing, MN. Environmental samples collected included fish, benthic macroinvertebrates, surface water, surface microlayer, pore water, and sediment. Fish and water data were used to derive the SSC. Data generated by this study are the most recent data available for this site and are likely representative of current environmental contamination.

Data used for BAF and SSC derivation were limited to the "site", that is, the area adjacent to and immediately downstream of 3M's Cottage Grove manufacturing facility (River Miles 820 – 812). Only samples collected in the main channel of the river were used. Only fillet data were used (whole fish were excluded) in BAF calculation.

Table 2-2: Summary of PFAS detected and geometric mean water concentrations from Mississippi River, Miles 820 to 812

PFAS	Percent detected in water	Mean detected water concentration (ng/L)	Percent detected in fish tissue	Mean detected fish tissue concentration (ng/g)
PFBS	93	43.7[2]	70	0.18[1]
PFBA	97	153.2[2]	50	0.31[1]
PFHxS	97	5.7[2]	40	0.19[1]
PFHxA	100	13.6[2]	41	0.12[1]
PFOA	83	37.4[1]	37	0.45[1]
PFOS	100	26.9	100	11.7

[1]Calculated using Regression on Order Statistics (ROS) method; geometric means

[2]Calculated using ½ detection limit in place of non-detects; geometric mean.

3. Analysis plan: site-specific chronic criteria derivation

3.1 WQS: chronic criteria

In Class 2 designated surface waters, State and CWA goals are integrated as stated in 7050.0140, subp. 3:

Class 2 waters, aquatic life and recreation. Aquatic life and recreation includes all waters of the state that support or may support aquatic biota, bathing, boating, or other recreational purposes and for which quality control is or may be necessary to protect aquatic or terrestrial life or their habitats or the public health, safety, or welfare.

Development of Class 2 WQS are more specifically cited in rule as:

- WQS: Chronic Standards (CS) – derived for Class 2 waters; pollutant-specific standards adopted into rule.
- SSC: Chronic Criteria (CC) – derived and applied on a site-specific basis; based on methods adopted into rule (Minn. R. 7050.0217 to 7050.0219; 7052.0100 for the Lake Superior Basin).

Use classifications for surface water are found in Minn. R. 7050.0400 through 7050.0470. The applicable Class 2 subclass for the Mississippi River Miles 820 to 812 is 2B. Therefore, a CC_{FR} is derived, which includes the following exposure pathways:

- Fish consumption (F).
- Recreation, which includes an incidental water intake rate (R).

The algorithms for derivation of CC_{FR} are found in Minn. R. 7050.0219, subp. 14, and can include a noncarcinogenic value only (for noncarcinogenic chemicals or for nonlinear carcinogens (NLC)), or both a noncarcinogenic and a carcinogenic value for linear carcinogenic chemicals. When both noncarcinogenic and carcinogenic values are derived, the lowest value is used as the final CC_{FR}. All final calculations are rounded to two significant figures as the final site-specific CC.

The algorithm for noncarcinogens or NLCs for CC_{FR} in Class 2B surface waters is:

$$CC_{FR} = \frac{RfD * RSC * 1x10^6\ ng/mg}{\{IWR + FCR\ [(0.24 * BAF_{TL3}) + (0.76 * BAF_{TL4})]\}}$$

where: CC_{FR} = fish consumption and recreation chronic criterion in nanograms (ng) per liter (L)
RfD = reference dose in milligrams (mg) per kilogram (kg)-day (d)
RSC = relative source contribution (no units)
$1x10^6$ ng/mg = a factor used to convert milligram to nanogram; there are 1,000,000 nanograms per milligram
IWR = 0.0013 L/kg-d; assumed incidental water intake rate based on minimum chronic duration
FCR = fish consumption intake rate. For chemicals with developmental toxicity, MPCA has calculated an interim FCR for women of childbearing age of 0.00094 kg/kg-d (see Section 3.3 for further discussion)
BAF_{TL3} = final BAF for TL_3 fish in L/kg; accounts for 24 percent of fish consumed
BAF_{TL4} = final BAF for TL_4 fish in L/kg; accounts for 76 percent of fish consumed

There are two linear cancer algorithms for Class 2B surface waters (Minn. R. 7050.0219, subp. 14). One algorithm uses a lifetime adjustment factor ($AF_{lifetime}$), while the other uses age-dependent adjustment factors (ADAF). The two equations allow the user to address any age-dependent cancer risk that may be known for a given chemical. Exposure to some carcinogens pose a higher risk for cancer development in infants and children, and those higher risks are accounted for with adjustment factors. Where the exact degree of risk is unknown for a chemical, default ADAFs may be used. Alternatively, if chemical-specific data are available to estimate higher lifetime potency associated with exposure in early life stages, this additional risk is included as a single $AF_{lifetime}$. If there is no additional early-life stage susceptibility, the $AF_{lifetime}$ may equal one (MPCA 2017).

Of the PFAS chemicals being evaluated for SSC, only PFOS and PFOA have been determined to be carcinogenic by the EPA (USEPA 2024a, 2024b). The other four PFAS currently have insufficient evidence to make a determination on carcinogenicity. The EPA made the determination that for both PFOS and PFOA, these chemicals do not pose additional cancer risk for early life stages, due to no evidence of a mutagenic mode of action, and a lack of evidence to determine whether exposure during early life stages increases cancer risks. Because of this, the linear carcinogen algorithm that does not utilize ADAF was chosen to develop a CC_{FR}. Because it is assumed that there is no additional cancer risk for early life stages, a $AF_{lifetime}$ of one was chosen to represent no additional risk.

The algorithm for linear carcinogenic chemicals with a lifetime $AF_{lifetime}$ for Class 2B surface waters:

$$CC_{FR} = \frac{CR\ (1x10^{-5})}{CSF\ x\ AF_{lifetime}} * \frac{1x10^6\ ng/mg}{\{IWR + FCR\ [(0.24 * BAF_{TL3}) + (0.76 * BAF_{TL4})]\}}$$

where: CC_{FR} = fish consumption and recreation chronic criterion in micrograms (ng) per liter (L)

CR = cancer risk level or an additional excess cancer risk equal to 1 x 10^{-5}
AF = lifetime adjustment factor (no units)
CSF = cancer potency slope factor in $(mg/kg\text{-}d)^{-1}$
Other factors are as described above

In addition to a CC_{FR}, a fish tissue-based CC (CC_{FT}) is derived for contaminants that are bioaccumulative contaminants of concern (BCC) to protect fish consumers. A BCC is defined as having a bioaccumulation factor (BAF) greater than 1,000 L/kg. While the mean PFOS BAF at this site, which is based on a relatively small set of site data, is less than 1,000 L/kg for trophic levels 3 and 4, individuals and certain species in the dataset exhibited PFOS BAFs greater than 1,000 L/kg (Appendix A). Furthermore, PFOS is widely recognized as a bioaccumulative chemical of concern as demonstrated by the development of fish consumption guidance in many states (including Minnesota), presence in 100% of fish at this site, and published BAFs that can be greater than 7,000 L/kg (Burkhard 2021, ITRC 2018, UNEP 2007, UNEP 2017, UNEP 2018). Because of this, a CC_{FT} was developed for PFOS.

Fish tissue-based SSC were also derived for PFHxS and PFOA. While the mean BAFs for PFHxS and PFOA were less than 1,000 L/kg at this site, both PFAS have demonstrated BAFs > 1,000 L/kg in fish in other field studies (ITRC 2018). PFHxS and PFOA are known to be highly bioaccumulative in humans with long half-lives (5.3 years and 2.7 years, respectively) (Li et al., 2018), and exhibit potential toxic effects at exceptionally low concentrations. In addition, PFHxS and PFOA are present in at least 40% and 37% of fish fillet at this site, respectively, presenting a likely route of exposure for people consuming fish collected in this area. These factors justify the BCC determination for PFHxS and PFOA.

Finally, when humans consume fish caught in this area, they are not just exposed to one type of PFAS. Rather, they are exposed to a mixture of numerous PFAS with overlapping toxic endpoints. These conditions and considerations support the development of fish-tissue based SSC.

The algorithm for Class 2 noncarcinogens or NLCs for CC_{FT} is:

$$CC_{FT} = \frac{RfD * RSC * 1x10^{6}\ ng/mg}{FCR}$$

where: CC_{FT} = fish consumption and recreation chronic criterion in nanograms (mg) per kilogram (kg)
Other factors are as described above

The algorithm for linear carcinogenic chemicals with lifetime adjustment factors ($AF_{lifetime}$) applicable to class 2 waters to calculate CC_{FT} is:

$$CC_{FT} = \frac{CR\ (1x10^{-5})}{CSF\ x\ AF_{lifetime}} * \frac{1x10^{6}\ ng/mg}{FCR}$$

where: CC_{FT} = fish consumption and recreation chronic criterion in nanograms (mg) per kilogram (kg)
Other factors are as described above

3.2 Bioaccumulation factor derivation

A bioaccumulation factor (BAF) is the ratio of a toxic pollutant's concentration in fish tissue to its concentration in ambient surface water at steady-state (in L/kg) and is used to set water column values (CC_{FR}) that if met, will also result in compliance with the fish-tissue criterion (CC_{FT}). The methods and data needs for developing a BAF are described in Minn. R. 7050.0219 and MPCA 2017. The preferred

procedure for developing a BAF is the use of field studies. The general approach to developing a BAF for application in CC is as follows:

- Internal review of quality assurance and control information provided by the lab.
- Consolidate paired surface water and fish datasets.
- Develop geometric mean water concentrations for a specific water body (lake or river segment).
- Calculate BAF for each individual fish by dividing reported concentrations in fillet tissue by water concentration. Combine BAF for geometric means for each species in a water body (if data warrant, there may be BAF by trophic level 3 and 4).

Evaluate these BAF to develop the final site-BAF (a "site" may be defined as narrowly as portion or stretch of a single water body or as broadly as all statewide surface waters), typically the geometric mean of all the species- or water body-geometric means.

BAFs are the ratio of the contaminant concentration in fish to the contaminant concentration in water (Minn. R. 7050.0219, subp. 8):

$$measured\ BAF = C_t/C_w$$

where: BAF = field-measured BAF based on total concentration in tissue and water (L/kg)
C_t = total concentration of the chemical in the specified wet tissue (µg/kg)
C_w = total concentration of the chemical in water (µg/L)

BAFs were calculated for fish in trophic levels 3 and 4 using the geometric mean concentration of PFAS in fish from each trophic level (Appendix A, Table 6). For compounds with 100% detection (like PFOS), a geometric mean was calculated using all data. For compounds with some non-detects, Regression on Order Statistics (ROS) were used, where possible, to calculate a geometric mean. Where the available data did not meet the criteria for ROS, ½ the detection limit was used for non-detects in geometric mean calculation. MPCA conducted an evaluation of multiple approaches to mean calculation, demonstrating that using ½ the detection limit in place of non-detects is a reasonable approach to mean calculation (Appendix A), but these methods are also generally accepted as reasonable approaches for addressing non-detect data (Mikkonen et al. 2018, USEPA 1991). The R script for mean calculation and all raw data used in BAF development are available upon request.

3.3 Fish consumption rate

The human health WQS (HH-WQS) methods include a default fish consumption rate (FCR) for adults, but this rate was not based on data specific to women of child-bearing age (WCBA). The HH-WQS TSD stated that if a pollutant affects development and prenatal to postnatal (gestational to lactational) exposure is relevant to the toxicity profile of the pollutant, the MPCA would review available fish consumption survey and exposure data to determine if the default adult FCR was representative of WCBA, or if an alternative rate was needed (MPCA 2017).

Using the best available and reliable data for this limited review to meet MPCA and EPA's protective goals for HH-WQS, an interim FCR for WCBA (FCR_{WCBA}) of 66 g/d and 70 kg bodyweight (0.94 g/kg-d or 0.00094 kg/kg-d) will be applied to account for reasonable maximum exposure to WCBA (ages 16 to 50) in Minnesota that consume freshwater fish. This FCR is based on the Minnesota Department of Health (MDH) Fish are Important for Superior Health (FISH) survey of North Shore Minnesotans (MDH 2017) and reflects similar rates found in other surveys of Minnesotan WCBA.

MPCA document number wq-s6-60, *Interim fish consumption rate for women of childbearing age* (2022) describes the derivation of the interim FCR in detail.

Some of the PFAS for which SSC were derived have direct evidence of developmental toxicity (Table 1-1). Other PFAS for which SSC were derived demonstrate thyroid toxicity. Thyroid hormones are critical to the fetal brain development, metabolism, and oxygen consumption (Bernal 2022; Forhead and Fowden 2014; Liu et al., 2023). Therefore, all 6 PFAS for which SSC were derived are determined to have developmental impacts, so the interim FCR_{WCBA} was applied in the calculation of each SSC.

3.4 Incidental intake rate

The incidental ingestion exposure parameter applies for human health standards or criteria developed for waters not designated as sources of drinking water, where the beneficial uses are narrowed to fish consumption and recreation, which applies to the Mississippi River, Miles 820 to 812 (Class 2B). The incidental intake rate of 0.0013 L/kg-d was used as the exposure factor in the calculation of the chronic criteria for the PFAS evaluated. This value is presented in Minnesota Rule 7050.0219, subp. 14.

3.5 Relative source contribution

The RSC factor is used to account for exposures to the same toxic pollutant from other sources unrelated to those addressed by the CC. Methods in Minn. R. 7050.0219, subp. 5 indicate that the RSC should be a default value of 0.2 (20%) for most pollutants, unless:

A. There are no significant known or potential sources other than those addressed for the designated use (then 0.5 must be used).
B. Sufficient exposure data are available to support an alternative pollutant-specific value between 0.2 and 0.8.

Use of an RSC of 20% assumes that 20% of a person's exposure to a specific chemical comes from the exposure pathways used to derive the CC, while the other 80% of the person's exposure to that pollutant comes from other sources. The RSC methods in Minn. R. 7050.0219 follow the EPA's RSC Decision Tree for deriving the RSC as described in MPCA 2017. Multiple lines of evidence are used to develop RSCs: availability of biomonitoring datasets, food and environmental media monitoring, physical-chemical properties, and fate and transport of the pollutant (USEPA 2000a).

For the PFAS in this evaluation, the evidence available supports use of 0.2 as the RSC in the CC_{FR}. The MPCA determined that exposure from eating freshwater fish should be limited to 20% of total exposure because of the presence of these PFAS and their precursors in other environmental media, food, drinking water, and consumer products. The CC RSC methods require use of the 0.2 RSC if there are other significant sources of exposure to the toxic pollutant.

4. SSC: Perfluorooctane sulfonic acid (PFOS)

PFOS is an eight-carbon chemical with a sulfonate functional group. Because PFOS has a long half-life and transgenerational transfer, even short durations of exposure can lead to significant increases in chronic duration or lifetime body burdens (Goeden et al. 2019, MDH 2022b).

4.1 Toxicological values and health risk index endpoints

The MPCA used the most recent EPA toxicity values for PFOS (USEPA 2024a) (Table 4-1).

Table 4-1 PFOS Toxicity values and health endpoints

PFAS	RfD or CSF	Health Risk Index Endpoints	Reference
PFOS RfD	1 x 10^{-7} mg/kg-d	Developmental, Liver System, Immune system	USEPA 2024a, MDH 2024b
PFOS CSF	39.5 $(mg/kg\text{-}d)^{-1}$	Cancer	USEPA 2024a

Use of these additivity endpoints for mixtures analyses is further described in Section 10.2.

4.2 Exposure factors

Exposure factors are based on the algorithms in Minn. R. 7050.0219. Because PFOS is characterized for CC as a developmental toxicant (USEPA 2024a), higher intake rates may need to be applied to protect developmental life stages (MPCA 2017).

Table 4-2 PFOS Exposure parameters

Exposure parameter	Rate or value	Basis
IWR	0.0013 L/kg-d	The default WQS incidental water intake rate is applied. The rate is based on children ages one through eight.
FCR	0.00094 kg/kg-d	The most stringent RfD is based on developmental impacts affecting prenatal to neonatal health endpoints (USEPA 2024a). Because of this, the use of the higher interim FCR_{WCBA} for this subpopulation of fish consumers is warranted and will protect other Minnesota fish consumers as well.
BAF_{TL3}	648.2 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. PFOS was detected in every water and fish tissue sample, so BAFs were calculated without further statistical analysis.
BAF_{TL4}	817.8 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. PFOS was detected in every water and fish tissue sample, so BAFs were calculated without further statistical analysis.
RSC	0.2	For the CC_{FR}, the default RSC is 0.2 because other routes of exposure beside recreation and freshwater fish consumption are significant to people's total exposure to PFOS. Aside from other sources, drinking water is a known source, with several drinking water sources in Minnesota having detectable levels of PFOS (USEPA 2024d).
$AF_{lifetime}$	1	The EPA determined that PFOS does not pose additional cancer risk for early life stages. Because it is assumed that there is no additional cancer risk for early life stages, a $AF_{lifetime}$ of one was chosen to represent no additional risk.

4.3 Chronic criteria calculation

PFOS is also a carcinogen (USEPA 2024a) and so is evaluated using both the noncarcinogenic and linear carcinogenic algorithms for that toxicological profile in Minn. R. 7050.0219, as described previously. The fish consumption and recreational exposure (CC_{FR}) values use the RfD and CSF in Table 4-1 paired with the exposure factors in Table 4-2.

Noncarcinogenic CC_{FR} calculation:

$$CC_{FR} = 0.027\ ng/L = \frac{1\ x\ 10^{-7} * 0.2\ * 1x10^{6}\ ng/mg}{\{0.0013\ + 0.00094\ [(0.24 * 648.2) + (0.76 * 817.8)]\}}$$

Linear carcinogenic CC_{FR} calculation:

$$CC_{FR} = 0.35\ ng/L = \frac{CR\ (1x10^{-5})}{39.5\ x\ 1} * \frac{1x10^{6}\ ng/mg}{\{0.0013\ + 0.00094\ [(0.24 * 648.2) + (0.76 * 817.8)]\}}$$

Because the noncarcinogenic CC_{FR} is more protective than the linear carcinogenic CC_{FR}, the noncarcinogenic CC_{FR} is the site-specific fish consumption and recreational exposure criterion.

In addition to a CC_{FR}, a fish-tissue based CC (CC_{FT}) was derived for PFOS due to its bioaccumulation potential. The fish tissue (CC_{FT}) values use the RfD and CSF in Table 4-1 paired with the exposure factors in Table 4-2.

Noncarcinogenic CC_{FT} calculation:

$$CC_{FT} = 21\ ng/kg = \frac{1\ x\ 10^{-7} * 0.2 * 1x10^{6}\ ng/mg}{0.00094}$$

Linear carcinogenic CC_{FT} calculation:

$$CC_{FT} = 269\ ng/kg = \frac{1x10^{-5}}{39.5\ x\ AF_{lifetime}} * \frac{1x10^{6}\ ng/mg}{0.00094}$$

Because the noncarcinogenic CC_{FT} is more protective than the linear carcinogenic CC_{FT}, the noncarcinogenic CC_{FT} is the site-specific fish consumption and recreational exposure criterion. Calculations were rounded to two significant figures.

Table 4-3: Derived site-specific water quality criteria for PFOS for the protection of Class 2B surface water uses in Mississippi River, Miles 820 to 812

PFAS (CAS No. see Table 2-1)	Site-specific water quality criteria: Chronic Criteria (CC)		Health Risk Index Endpoints (Additive Risk)
	Class 2B – fish consumption and recreational exposure (CC_{FR}) (30-day average)	Class 2 fish-tissue (CC_{FT}) (90^{th} percentile of 5 fish minimum per water body)	
PFOS	0.027 ng/L	0.021 ng/g	Developmental, Liver System, Immune System (MDH 2023a)

Definitions of CC:

CCFR: Applied in Class 2B surface waters (F: Fish consumption and R: Recreational exposure)

CCFT: Applied for Bioaccumulative Chemicals of Concern (BCC) in fish (fillet/muscle) for all Class 2 waters (FT: fish-tissue

5. SSC: Perfluorooctanoic acid (PFOA)

PFOA is an eight-carbon chemical with a carboxylate (oxygen) functional group. Because PFOA has a long half-life and transgenerational transfer, even short durations of exposure can lead to significant increases in chronic duration or lifetime body burdens (Goeden et al. 2019, MDH 2024a).

5.1 Toxicological values and health risk index endpoints

The MPCA used the most recent EPA toxicity values for PFOA (USEPA 2024b) (Table 5-1).

Table 5-1 PFOA Toxicity values and health endpoints

PFAS	RfD or CSF	Health Risk Index Endpoints	Reference
PFOA RfD	3×10^{-8} mg/kg-d	Developmental, Liver System, Immune system	USEPA 2024b, MDH 2024a
PFOA CSF	$29{,}300\ (mg/kg\text{-}d)^{-1}$	Cancer	USEPA 2024b

Use of these additivity endpoints for mixtures analyses is further described in Section 10.2.

5.2 Exposure factors

Exposure factors are based on the algorithms in Minn. R. 7050.0219. Because PFOA is characterized for CC as a developmental toxicant (USEPA 2024b), higher intake rates may need to be applied to protect developmental life stages (MPCA 2017).

Table 5-2 PFOA Exposure parameters

Exposure parameter	Rate or value	Basis
IWR	0.0013 L/kg-d	The default WQS incidental water intake rate is applied. The rate is based on children ages one through eight.
FCR	0.00094 kg/kg-d	The most stringent RfD is based on developmental impacts affecting prenatal to neonatal health endpoints (USEPA 2024b). Because of this, the use of the higher interim FCR_{WCBA} for this subpopulation of fish consumers is warranted and will protect other Minnesota fish consumers as well.
BAF_{TL3}	22.9 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. Non-detect values in fish tissue and water samples were evaluated using ROS (see discussion in Section 3.2, and analyses in Appendix A).
BAF_{TL4}	42.8 L/kg	Paired fish and water samples from the Mississippi River Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. Non-detect values in fish tissue and water samples were evaluated using ROS. (see discussion in Section 3.2, and analyses in Appendix A).
RSC	0.2	For the CC_{FR}, the default RSC is 0.2 because other routes of exposure beside recreation and freshwater fish consumption are significant to people's total exposure to PFOA. Aside from other sources, drinking water is a known source, with several drinking water sources in Minnesota having detectable levels of PFOA (USEPA 2024d).
$AF_{lifetime}$	1	The EPA determined that PFOA does not pose additional cancer risk for early life stages. Because it is assumed that there is no additional cancer risk for early life stages, a $AF_{lifetime}$ of one was chosen to represent no additional risk.

5.3 Chronic criteria calculation

PFOA is a carcinogen (USEPA 2024b) and so is evaluated using both the noncarcinogenic and linear carcinogenic algorithms for that toxicological profile in Minn. R. 7050.0219, as described earlier. The CC_{FR} values use the RfD and CSF in Table 5-1 paired with the exposure factors in Table 5-2.

Noncarcinogenic CC_{FR} calculation:

$$CC_{FR} = 0.16\,ng/L = \frac{3\,x\,10^{-8} * 0.2 * 1x10^{6}\,ng/mg}{\{0.0013 + 0.00094\,[(0.24 * 22.9) + (0.76 * 42.8)]\}}$$

Linear carcinogenic CC_{FR} calculation:

$$CC_{FR} = 0.0092\,ng/L = \frac{CR\ (1x10^{-5})}{29{,}300\,x\,1} * \frac{1x10^{6}\ ng/mg}{\{0.0013 + 0.00094\,[(0.24 * 22.9) + (0.76 * 42.8)]\}}$$

Because the linear carcinogen CC_{FR} is more protective than the noncarcinogenic CC_{FR}, the carcinogenic CC_{FR} is the site-specific fish consumption and recreational exposure criterion.

In addition to a CC_{FR}, a fish-tissue based CC (CC_{FT}) was derived for PFOA due to its bioaccumulation potential. The fish tissue (CC_{FT}) values use the RfD and CSF in Table 5-1 paired with the exposure factors in Table 5-2.

Noncarcinogenic CC_{FT} calculation:

$$CC_{FT} = 6.4\ ng/kg = \frac{3\ x\ 10^{-8} * 0.2 * 1x10^{6}\ ng/mg}{0.00094}$$

Linear carcinogenic CC_{FT} calculation:

$$CC_{FT} = 0.36\ ng/kg = \frac{1x10^{-5}}{29{,}300\ x\ 1} * \frac{1x10^{6}\ ng/mg}{0.00094}$$

Because the noncarcinogenic CC_{FT} is more protective than the linear carcinogenic CC_{FT}, the carcinogenic CC_{FT} is the site-specific fish consumption and recreational exposure criterion. Calculations were rounded to two significant figures.

Table 5-3: Derived site-specific water quality criteria for PFOA for the protection of Class 2B surface water uses in Mississippi River Miles 820 to 812

PFAS (CAS No. see Table 2-1)	**Site-specific water quality criteria: Chronic Criteria (CC)**		**Health Risk Index Endpoints (Additive Risk)**
	Class 2B – fish consumption and recreational exposure (CC_{FR}) (30-day average)	**Class 2 fish-tissue** (CC_{FT}) (90th percentile of 5 fish minimum per water body)	
PFOA	0.0092 ng/L (Cancer)	0.00036 ng/g (Cancer)	Developmental, Thyroid (E), Cancer (MDH 2024b)

Definitions of CC:

CC_{FR}: Applied in Class 2B surface waters (F: Fish consumption and R: Recreational exposure)

CC_{FT}: Applied for Bioaccumulative Chemicals of Concern (BCC) in fish (fillet/muscle) for all Class 2 waters (FT: fish-tissue)

6. SSC: Perfluorohexane sulfonic acid (PFHxS)

PFHxS is a six-carbon chemical with a sulfonate functional group. PFHxS is characterized as a long-chain PFSA, with some properties similar to PFOS. Because PFHxS has a long half-life and transgenerational transfer, even short durations of exposure can lead to significant increases in chronic duration body burdens (MDH 2020a).

6.1 Toxicological values and health risk index endpoints

The MPCA used the most recent EPA toxicity values for PFHxS (USEPA 2023a) (Table 6-1).

Table 6-1 PFHxS Toxicity values and health endpoints

PFAS	RfD	Health Risk Index Endpoints	Reference
PFHxS	2 x 10^{-10} mg/kg-d	Liver System, Thyroid (E)	USEPA 2023a, MDH 2023b

Key: (E) stands for endocrine and means a change in circulating hormone levels or interactions with hormone receptors, regardless of the organ or organ system affected (Minn. R. 7050.0218, subp. 3 (X), based on 4717.7820, subp. 10)

Use of these additivity endpoints for mixtures analyses is further described in Section 10.2.

6.2 Exposure factors

Exposure factors are based on the algorithms in Minn. R. 7050.0219. PFHxS is characterized for CC as a developmental toxicant based on short-term effects to the thyroid including effects on offspring during gestational studies and developmental immune responses (USEPA 2023a). PFHxS also has a very long half-life in people, meaning that exposure at birth is influenced by the lifetime exposure of the mother. Therefore, higher intake rates may need to be applied to protect developmental life stages when exposure to a toxic pollutant is greater on a per body weight basis (MPCA 2017).

Table 6-2 PFHxS Exposure parameters

Exposure parameter	Rate or value	Basis
IWR	0.0013 L/kg-d	The default WQS incidental water intake rate is applied. The rate is based on children ages one through eight.
FCR	0.00094 kg/kg-d	The toxicological profile of PFHxS demonstrated evidence of developmental impacts, including effects to thyroid in offspring after gestational exposure. Because of this, the use of the higher interim FCR_{WCBA} for this subpopulation of fish consumers is warranted and will protect other Minnesota fish consumers as well.
BAF_{TL3}	30.2 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. Non-detect values in fish tissue samples were evaluated using ROS. For water samples, ROS could not be calculated, therefore ½ of the detection limit was used in place of all non-detects (see discussion in Section 3.2, and analyses in Appendix A).
BAF_{TL4}	13.3 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. Non-detect values in fish tissue samples were evaluated using ROS. For water samples, ROS could not be calculated, therefore ½ of the detection limit was used in place of all non-detects (see discussion in Section 3.2, and analyses in Appendix A).
RSC	0.2	For the CC_{FR}, the default RSC is 0.2 because other routes of exposure beside recreation and freshwater fish consumption are significant to people's total

		exposure to PFHxS. Aside from other potential sources, drinking water is a known source, with several drinking water sources in Minnesota having detectable levels of PFHxS (USEPA 2024d).

6.3 Chronic criteria calculation

The EPA has concluded that there is inadequate information to assess carcinogenic potential for PFHxS (USEPA 2023a). Because of this, for criteria development, it is considered a noncarcinogen, and is evaluated using the noncarcinogenic algorithms for that toxicological profile in Minn. R. 7050.0219, as described earlier. The CC_{FR} uses the RfD (Table 6-1) paired with the exposure factors in Table 6-2.

$$CC_{FR} = 0.0023\, ng/L = \frac{2\,x\,10^{-10} * 0.2 * 1x10^{6}\, ng/mg}{\{0.0013 + 0.00094\,[(0.24 * 30.2) + (0.76 * 13.3)]\}}$$

In addition to a CC_{FR}, a fish-tissue based CC (CC_{FT}) was derived for PFHxS due to its bioaccumulation potential. The fish tissue (CC_{FT}) values use the RfD and CSF in Table 6-1 paired with the exposure factors in Table 6-2.

Noncarcinogenic CC_{FT} calculation:

$$CC_{FT} = 0.043\, ng/kg = \frac{2\,x\,10^{-10} * 0.2 * 1x10^{6}\, ng/mg}{0.00094}$$

Table 6-3: Derived site-specific water quality criteria for PFHxS for the protection of Class 2B surface water uses in Mississippi River Miles 820 to 812

PFAS (CAS No. see Table 2-1)	Site-specific water quality criteria: Chronic Criteria (CC)		Health Risk Index Endpoints (Additive Risk)
	Class 2B – fish consumption and recreational exposure (CC_{FR}) (30-day average)	**Class 2 fish-tissue** (CC_{FT}) (90th percentile of 5 fish minimum per water body)	
PFHxS	0.0023 ng/L	0.000043 ng/g	Liver System, Thyroid (E) (MDH 2023b)

Definitions of CC:

CC_{FR}: Applied in Class 2B surface waters (F: Fish consumption and R: Recreational exposure)

CC_{FT}: Applied for Bioaccumulative Chemicals of Concern (BCC) in fish (fillet/muscle) for all Class 2 waters (FT: fish-tissue)

7. SSC: Perfluorohexanoic acid (PFHxA)

PFHxA is a six-carbon chemical with a carboxylate (oxygen) functional group. PFHxA has characteristics similar to PFBA, so is described as a short-chain PFAS. PFHxA has much shorter half-lives in people and laboratory animals than PFOA (MDH 2021).

7.1 Toxicological values and health risk index endpoints

The MPCA used the most recent EPA toxicity values for PFHxA (USEPA 2023b) (Table 7-1).

Table 7-1 PFHxA Toxicity values and health endpoints

PFAS	RfD	Health Risk Index Endpoints	Reference
PFHxA	5 x 10^{-4} mg/kg-d	Developmental, Thyroid (E)	USEPA 2023b, MDH 2023c

Key: (E) stands for endocrine and means a change in circulating hormone levels or interactions with hormone receptors, regardless of the organ or organ system affected (Minn. R. 7050.0218, subp. 3 (X), based on 4717.7820, subp. 10)

Use of these additivity endpoints for mixtures analyses is further described in Section 10.2.

7.2 Exposure factors

Exposure factors are based on the algorithms in Minn. R. 7050.0219. Because PFHxA is characterized for CC as a developmental toxicant (USEPA 2023b), higher intake rates need to be applied to protect developmental life stages (MPCA 2017).

Table 7-2 PFHxA Exposure parameters

Exposure parameter	Rate or value	Basis
IWR	0.0013 L/kg-d	The default WQS incidental water intake rate is applied. The rate is based on children ages one through eight.
FCR	0.00094 kg/kg-d	The most stringent RfD is based on developmental impacts affecting prenatal to neonatal health endpoints (USEPA 2023b). Because of this, the use of the higher interim FCR_{WCBA} for this subpopulation of fish consumers is warranted and will protect other Minnesota fish consumers as well.
BAFTL3	24.4 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. For fish tissue samples, ROS could not be calculated, therefore ½ of the detection limit was used in place of all non-detects (see discussion in Section 3.2, and analyses in Appendix A). There were no non-detects in the water samples.
BAFTL4	22.5 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. For fish tissue samples, ROS could not be calculated, therefore ½ of the detection limit was used in place of all non-detects (see discussion in Section 3.2, and analyses in Appendix A). There were no non-detects in the water samples.
RSC	0.2	For the CC_{FR}, the default RSC is 0.2 because other routes of exposure beside recreation and freshwater fish consumption are significant to people's total exposure to PFHxA. Aside from other potential sources, drinking water is a known source, with drinking water sources in Minnesota having detectable levels of PFHxA (USEPA 2023b).

7.3 Chronic criteria calculation

The EPA has concluded that there is inadequate information to assess carcinogenic potential for PFHxA (USEPA 2023b). Because of this, for criteria development, it is considered a noncarcinogen, and is evaluated using the noncarcinogenic algorithms for that toxicological profile in Minn. R. 7050.0219, as described earlier. The CC_{FR} uses the RfD (Table 7-1) paired with the exposure factors in Table 7-2.

$$CC_{FR} = 4{,}371\, ng/L = \frac{5\ x\ 10^{-4}\ *0.2\ *1x10^{6}\ ng/mg}{\{0.0013\ +0.00094\ [(0.24*24.4)+(0.76*22.5)]\}}$$

Calculations were rounded to two significant figures for setting the CC_{FR}.

Table 7-3: Derived site-specific water quality criteria for PFHxA for the protection of Class 2B surface water uses in Mississippi River Miles 820 to 812

PFAS (CAS No. see Table 2-1)	Site-specific water quality criteria: Chronic Criteria (CC)		Health Risk Index Endpoints (Additive Risk)
	Class 2B – fish consumption and recreational exposure (CC_{FR}) (30-day average)	**Class 2 fish-tissue** (CC_{FT}) (90^{th} percentile of 5 fish minimum per water body)	
PFHxA	4,400 ng/L	Not applicable	Developmental, Thyroid (E) (MDH 2023c)

Definitions of CC:

CC_{FR}: Applied in Class 2B surface waters (F: Fish consumption and R: Recreational exposure)

CC_{FT}: Applied for Bioaccumulative Chemicals of Concern (BCC) in fish (fillet/muscle) for all Class 2 waters (FT: fish-tissue)

8. SSC: Perfluorobutane sulfonic acid (PFBS)

PFBS is characterized as a four-carbon chain PFAS or short-chain perfluoroalkane sulfonate or sulfonic acid (PFSA) based on its carbon number and sulfonate (sulfur and oxygen) functional group. PFOS is also a perfluorosulfonic acid (PFSA) but has different characteristics than PFBS mainly due to its longer carbon chain and more hydrophobic properties. PFBS has much shorter half-lives in people and laboratory animals than PFOS (MDH 2023a).

8.1 Toxicological values and health risk index endpoints

The MPCA used the most recent EPA toxicity values for PFBS (USEPA 2021, 2024c) (Table 8-1).

Table 8-1 PFBS Toxicity values and health endpoints

PFAS	RfD	Health Risk Index Endpoints	Reference
PFBS	3 x 10^{-4} mg/kg-d	Thyroid (E)	USEPA 2021, USEPA 2024c, MDH 2023a

Key: (E) stands for endocrine and means a change in circulating hormone levels or interactions with hormone receptors, regardless of the organ or organ system affected (Minn. R. 7050.0218, subp. 3 (X), based on 4717.7820, subp. 10)

Use of these additivity endpoints for mixtures analyses is further described in Section 10.2.

8.2 Exposure factors

Exposure factors are based on the algorithms in Minn. R. 7050.0219. Because the EPA determined that evidence supports PFBS as a developmental toxicant (USEPA 2021), higher intake rates need to be applied to protect developmental life stages (MPCA 2017).

Table 8-2 PFBS Exposure parameters

Exposure parameter	Rate or value	Basis
IWR	0.0013 L/kg-d	The default WQS incidental water intake rate is applied. The rate is based on children ages one through eight.
FCR	0.00094 kg/kg-d	The toxicological profile of PFBS demonstrated evidence of developmental impacts, including effects to thyroid function. Because of this, the use of the higher interim FCR_{WCBA} for this subpopulation of fish consumers is warranted and will protect other Minnesota fish consumers as well.
BAF_{TL3}	29.8 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. Non-detect values in fish tissue samples were evaluated using ROS. For water samples, ROS could not be calculated, therefore ½ of the detection limit was used in place of all non-detects (see discussion in Section 3.2, and analyses in Appendix A).
BAF_{TL4}	16.8 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. Non-detect values in fish tissue samples were evaluated using ROS. For water samples, could not be calculated, therefore ½ of the detection limit was used in place of all non-detects (see discussion in Section 3.2, and analyses in Appendix A).
RSC	0.2	For the CC_{FR}, the default RSC is 0.2 because other routes of exposure beside recreation and freshwater fish consumption are significant to people's total exposure to PFBS. Aside from other potential sources, drinking water is a known source, with several drinking water sources in Minnesota having detectable levels of PFBS (MDH 2022b).

8.2 Chronic criteria calculation

The EPA has concluded that there is inadequate information to assess carcinogenic potential for PFBS (USEPA 2022). Because of this, for criteria development, it is considered a noncarcinogen, and is evaluated using the noncarcinogenic algorithms for that toxicological profile in Minn. R. 7050.0219, as described earlier. The CC_{FR} uses the RfD (Table 8-1) paired with the exposure factors in Table 8-2.

$$CC_{FR} = 2{,}996\,ng/L = \frac{3x10^{-4} * 0.2 * 1x10^{6}\,ng/mg}{\{0.0013 + 0.00094\,[(0.24 * 29.8) + (0.76 * 16.8)]\}}$$

Calculations were rounded to two significant figures for setting the CC_{FR}.

Table 8-3: Derived site-specific water quality criteria for PFBS for the protection of Class 2B surface water uses in Mississippi River Miles 820 to 812

PFAS (CAS No. see Table 2-1)	Site-specific water quality criteria: Chronic Criteria (CC)		Health Risk Index Endpoints (Additive Risk)
	Class 2B – fish consumption and recreational exposure (CC_{FR}) (30-day average)	**Class 2 fish-tissue** (CC_{FT}) (90th percentile of 5 fish minimum per water body)	
PFBS	3,000 ng/L	Not applicable	Thyroid (endocrine) (MDH 2023a)

Definitions of CC:

CC_{FR}: Applied in Class 2B surface waters (F: Fish consumption and R: Recreational exposure)

CC_{FT}: Applied for Bioaccumulative Chemicals of Concern (BCC) in fish (fillet/muscle) for all Class 2 waters (FT: fish-tissue)

9. SSC: Perfluorobutanoic acid (PFBA)

Like PFBS, PFBA is also a four-carbon or short-chain PFAS. This chemical has a carboxylate (oxygen) functional group (ITRC 2020c). This category of PFAS, perfluoroalkyl carboxylates or carboxylic acids (PFCA) also includes PFOA. PFOA as a long-chain PFCA has properties that differ from PFBA. PFBA has much shorter half-lives in people and laboratory animals than PFOA (MDH 2018).

9.1 Toxicological values and health risk index endpoints

The MPCA used the most recent EPA toxicity values for PFBA (USEPA 2022) (Table 9-1).

Table 9-1 PFBS Toxicity values and health endpoints

PFAS	RfD	Health Risk Index Endpoints	Reference
PFBA	1 x 10^{-3} mg/kg-d	Liver System, Thyroid (E)	USEPA 2022, MDH 2018
Key: (E) stands for endocrine and means a change in circulating hormone levels or interactions with hormone receptors, regardless of the organ or organ system affected (Minn. R. 7050.0218, subp. 3 (X), based on 4717.7820, subp. 10)			

Use of these additivity endpoints for mixtures analyses is further described in Section 10.2.

9.2 Exposure factors

Exposure factors are based on the algorithms in Minn. R. 7050.0219. Because PFBA is characterized for CC as a developmental toxicant (USEPA 2022), higher intake rates need to be applied to protect developmental life stages (MPCA 2017).

Table 9-2 PFBA Exposure parameters

Exposure parameter	Rate or value	Basis
IWR	0.0013 L/kg-d	The default WQS incidental water intake rate is applied. The rate is based on children ages one through eight.
FCR	0.00094 kg/kg-d	The subchronic RfD is based on developmental impacts affecting prenatal to neonatal health endpoints (USEPA 2022). Because of this, the use of the higher interim FCR_{WCBA} for this subpopulation of fish consumers is warranted and will protect other Minnesota fish consumers as well.
BAF_{TL3}	5.1 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. Non-detect values in fish tissue samples were evaluated using ROS. For water samples, ROS could not be calculated, therefore ½ of the detection limit was used in place of all non-detects (see discussion in Section 3.2, and analyses in Appendix A).
BAF_{TL4}	7.6 L/kg	Paired fish and water samples from the Mississippi River, Miles 820 to 812 yielded sufficient data to develop BAFs for two trophic levels. Non-detect values in fish tissue samples were evaluated using ROS. For water samples, ROS could not be calculated, therefore ½ of the detection limit was used in place of all non-detects (see discussion in Section 3.2, and analyses in Appendix A).
RSC	0.2	For the CC_{FR}, the default RSC is 0.2 because other routes of exposure beside recreation and freshwater fish consumption are significant to people's total exposure to PFBA. Aside from other potential sources, drinking water is a known source, with several drinking water sources in Minnesota having detectable levels of PFBA (MDH 2022a).

9.3 Chronic criteria calculation

The EPA has concluded that there is inadequate information to assess carcinogenic potential for PFBA (USEPA 2022). Because of this, for criteria development, it is considered a noncarcinogen, and is evaluated using the noncarcinogenic algorithms for that toxicological profile in Minn. R. 7050.0219, as described earlier. The CC_{FR} uses the RfD (Table 9-1) paired with the exposure factors in Table 9-2.

$$CC_{FR} = 25{,}381\, ng/L = \frac{1\, x\, 10^{-3} * 0.2 * 1x10^{6}\, ng/mg}{\{0.0013 + 0.00094\, [(0.24 * 5.1) + (0.76 * 7.6)]\}}$$

Calculations were rounded to two significant figures for setting the CC_{FR}.

Table 9-3: Derived site-specific water quality criteria for PFBA for the protection of Class 2B surface water uses in Mississippi River Miles 820 to 812

PFAS (CAS No. see Table 2-1)	Site-specific water quality criteria: Chronic Criteria (CC)		Health Risk Index Endpoints (Additive Risk)
	Class 2B – fish consumption and recreational exposure (CC_{FR}) (30-day average)	**Class 2 fish-tissue** (CC_{FT}) (90th percentile of 5 fish minimum per water body)	
PFBA	25,000 ng/L	Not applicable	Liver System, Thyroid (E) (MDH 2018)

Definitions of CC:

CC_{FR}: Applied in Class 2B surface waters (F: Fish consumption and R: Recreational exposure)

CC_{FT}: Applied for Bioaccumulative Chemicals of Concern (BCC) in fish (fillet/muscle) for all Class 2 waters (FT: fish-tissue)

10. Risk characterization

10.1 Application

It is appropriate to use the PFAS CC in the following ways:

- CC_{FT}: compare to concentration of PFAS in fish-tissue to evaluate potential risks at those water bodies for which this site-specific CC was derived.
- CC_{FR}: compare to PFAS concentrations in Class 2B surface waters to evaluate potential risks at those water bodies for which this site-specific CC was derived.

A sufficient number of samples should be used when comparing water and fish monitoring data to the CC. The CC_{FR} is applied as a 30-day average concentration that should not be exceeded more than once in a water body in a three-year window. The CC_{FT} requires at least five fish of the same species or a lesser number of fish from at least three species from a water body. Calculation of a 90th percentile PFAS concentration by species with the minimum number of individuals or average across species in the fillet tissue for comparison to the fish tissue CC. These details are found in the assessment methods in the most recent MPCA *Guidance for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment: 305(b) and 303(d) Impaired Waters List* (2024).

In addition, not all PFAS can be evaluated at this time due to analytical method limitations or lack of available toxicological values. The methods to protect human health do incorporate additive risk from

mixtures of two or more toxic pollutants in fish or water samples. Additive risks for noncancer effects are based on toxic pollutants that have numeric WQS or SSC and the same Health Risk Index Endpoints (Section 10.2, MPCA 2017).

10.2 Additive risks

Methods to develop CC require evaluation of additive risk when more than one toxic pollutant is present in surface water or fish tissue (Minn. R. 7050.0222, subp. 7 D). Additive risks are evaluated for both noncancer and cancer effects. The PFAS CC presented in this document are derived based on noncancer effects (except for PFOA). PFBA, PFBS, PFHxA, and PFHxS all impact the thyroid health endpoint. PFHxA, PFOS, and PFOA all impact the developmental health endpoint. PFOS and PFOA both impact the immune health endpoint. And PFBA, PFHxS, PFOA, and PFOS all impact the liver health endpoint.

To evaluate additive risks from noncancer effects, hazard quotients are calculated by dividing a water concentration by the CC for each individual contaminant present. All of the hazard quotients for individual chemicals that affect the same health endpoint are summed to calculate a health risk index. If the health risk index is equal to or less than 1, it is not likely that exposure to those contaminants involved in the evaluation will lead to a health risk. Concentrations above would exceed the SSC for mixtures.

$$Noncancer\ Health\ Risk\ Index\ by\ Common\ Health\ Risk\ Index\ Endpoint =$$

$$\frac{C_1}{CC_1} + \frac{C_2}{CC_2} + \cdots + \frac{C_n}{CC_n} \leq 1$$

Where:

$C_1 \ldots C_n$ is the concentration in water (as a 30-day average) for the first through the n^{th} noncancer pollutant with the same Health Risk Index Endpoints. These health endpoints for PFAS are found in Table 1-1.

$CC_1 \ldots CC_n$ is the fish consumption and recreation chronic criteria for the first to the n^{th} noncancer pollutant.

The CC for PFHxS, PFOS, and PFOA are well below their respective detection limits. Any detection of PFHxS, PFOS, or PFOA would automatically result in a health risk index greater than 1. As such, PFHxS, PFOS, and PFOA should be excluded from the health risk index calculation.

Additivity and health risk index calculation should be considered for PFBA, PFBS, and PFHxA, all of which have CC well above their respective detection limits and share the thyroid health endpoint. The equation to apply the health risk index to these PFAS is:

$$\frac{C_{PFBA}}{25{,}000\ ng/L} + \frac{C_{PFBS}}{3{,}000\ ng/L} + \frac{C_{PFHxA}}{4{,}400\ ng/L} \leq 1$$

10.3 Tribal and Environmental Justice communities

Fishing patterns and fish consumption from Minnesota's water bodies are likely not the same among all populations living within the borders of Minnesota. Fortunately, the MDH has conducted or partnered with many researchers, communities, and healthcare providers to gain important information on Minnesota and Great Lakes regional fish consumers and provide guidance to ensure balanced and healthy fish consumption (MDH 2020).

In developing WQS for pollutants in fish, the MPCA considers the need to address subsistence fishing by communities or populations and to ensure those populations are adequately protected. The MDH FISH study was specifically used as the basis for an interim FCR for WCBA because it was conducted in communities on the North Shore of Minnesota with a high rate of freshwater fishing (MDH 2017). Specific demographics of the women that participated were kept confidential, except for the age range for participation of 16 to 50 years; the survey results indicated that 73% of the women consumed freshwater-caught fish. By contrast, most surveys of Minnesota as a whole estimate consumption for WCBA at around 40%. Because more research and outreach is needed to finalize a FCR for WCBA, the rate being used for SSC is considered "interim."

Tribal nations have reserved fishing rights in many water bodies across the state, and therefore members of Tribal nations are important fish consumers. They are likely to consume fish at higher rates than the "average" Minnesotan. For water bodies in the Lake Superior Basin, there are Tribal Water Quality Standards that have different human health-based methods and intake rates. For example, the Fond du Lac Band of Lake Superior Chippewa use a FCR of 60 g/d and Grand Portage Band of Lake Superior Chippewa use a FCR of 142.5 g/d. These rates have provided important context to the MPCA's decision on an interim FCR. If the MPCA considers a statewide WQS for PFAS in fish tissue or develops criteria for water resources that are important tribal fisheries, the MPCA will engage with affected Tribes to consider the appropriate fish consumption rates.

The MPCA also has a published story map of areas of potential environmental justice concern in the state –areas where the number of people of color exceed 50% and/or more than 40% of the households have a household income of less than 185% of the federal poverty level (MPCA 2019). The map also includes Tribal areas. As PFAS CC are applied on a site-specific basis, information specific to environmental justice areas will be considered, particularly specific to exposure parameters.

Environmental justice also considers populations that may be more susceptible to adverse effects from environmental pollutants or may be more highly exposed. For PFOS, PFHxS, and PFOA the combination of bioaccumulation, developmental toxicity, cancer risk, and high exposure during infancy means protecting these early-life stages is dependent on a mother's lifetime body burden.

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Appendix A – Summary of PFAS Data and Non-Detection Analysis

In order to determine PFAS site-specific criteria for the Mississippi River, Miles 820 to 812, the Minnesota Pollution Control Agency (MPCA) calculated bioaccumulation factors (BAFs) with PFAS surface water and fish measurements from the Mississippi River near Cottage Grove, MN. BAF calculation requires geometric means per PFAS compound for surface water and fish. Since some measurements are less than the reported detection limit of the analytical method, the MPCA applied non-detection analyses to the data.

Prior to calculating PFAS surface water and fish means and BAFs, we processed the data. We converted data to be in a consistent unit (ng/L and ng/g), removed measurements from quality control samples, retained data resulting from a single analytical method (method 537.1 and ETS-8-045 for surface water and fish samples, respectively), and addressed duplicate data.

We calculated PFAS means using six different non-detection approaches and PFAS geometric means with five different non-detection methods. The six methods are described in Table 1. We did not calculate means via the Kaplan-Meier and Regression on Order Statistics (ROS) for PFAS compounds when certain criteria were not met: (1) two or fewer values in the given dataset were detected or (2) two or fewer values in the given dataset were not detected. Application of the Kaplan-Meier or ROS methods is not appropriate when datasets are composed of nearly all detected or non-detected measurements. We did not determine geometric means via the Kaplan-Meier technique since it is not possible with negative values, which results when using log-transformed data in geometric mean calculations. All data processing and statistical calculations were performed in R statistical software (2024).[7] R scripts and raw data used for this analysis are available upon request.

Table 3. Non-detection analyses.

Method	Description
Raw	Geometric mean of raw data with non-detection values excluded
Detection Limit	Geometric mean of data with non-detection values replaced with the reported detection limit
Half Detection Limit	Geometric mean of data with non-detection values replaced with half of the reported detection limit
Zero	Geometric mean of data with non-detection values replaced with zero
ROS	Semi-parametric method for calculating a probability distribution and estimating statistics, including means; utilizes the detection limit dataset
Kaplan-Meier	Nonparametric method for calculating a probability distribution and estimating statistics, including means; utilizes the detection limit dataset

Surface Water PFAS Data

For surface water data, calculated geometric means are comparable between all non-detection methods (Table 2 and Figure 1). In many cases, means for raw, detection limit, half detection limit, and zero methods are equivalent since the dataset for the given PFAS compound contains only detected values

[7]R Core Team (2024). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

(Table 3). For most PFAS compounds in surface water, we have not calculated means with the Kaplan-Meier or ROS methods since the datasets contained too few non-detection values (Table 3).

Table 2. Surface water geometric means (ng/L) per PFAS compound.

Method	PFBA	PFBS	PFHxA	PFHxS	PFOA	PFOS
Raw	91.3	10.7	11.3	5.03	26.4	16.4
Detection Limit	89.4	9.49	11.3	4.86	25.2	16.4
Half Detection Limit	87.3	9.05	11.3	4.74	22.3	16.4
Zero	NA	NA	11.3	NA	NA	16.4
ROS	NA	NA	NA	NA	23.0	NA
Kaplan-Meier	NA	NA	NA	NA	NA	NA

Table 3. Surface water sample composition. *ND* abbreviates *non-detected*.

	PFBA	PFBS	PFHxA	PFHxS	PFOA	PFOS
Total Count	29	29	29	29	29	29
ND Count	1	2	0	1	5	0
Percent ND (%)	3.4	6.9	0	3.4	17.2	0

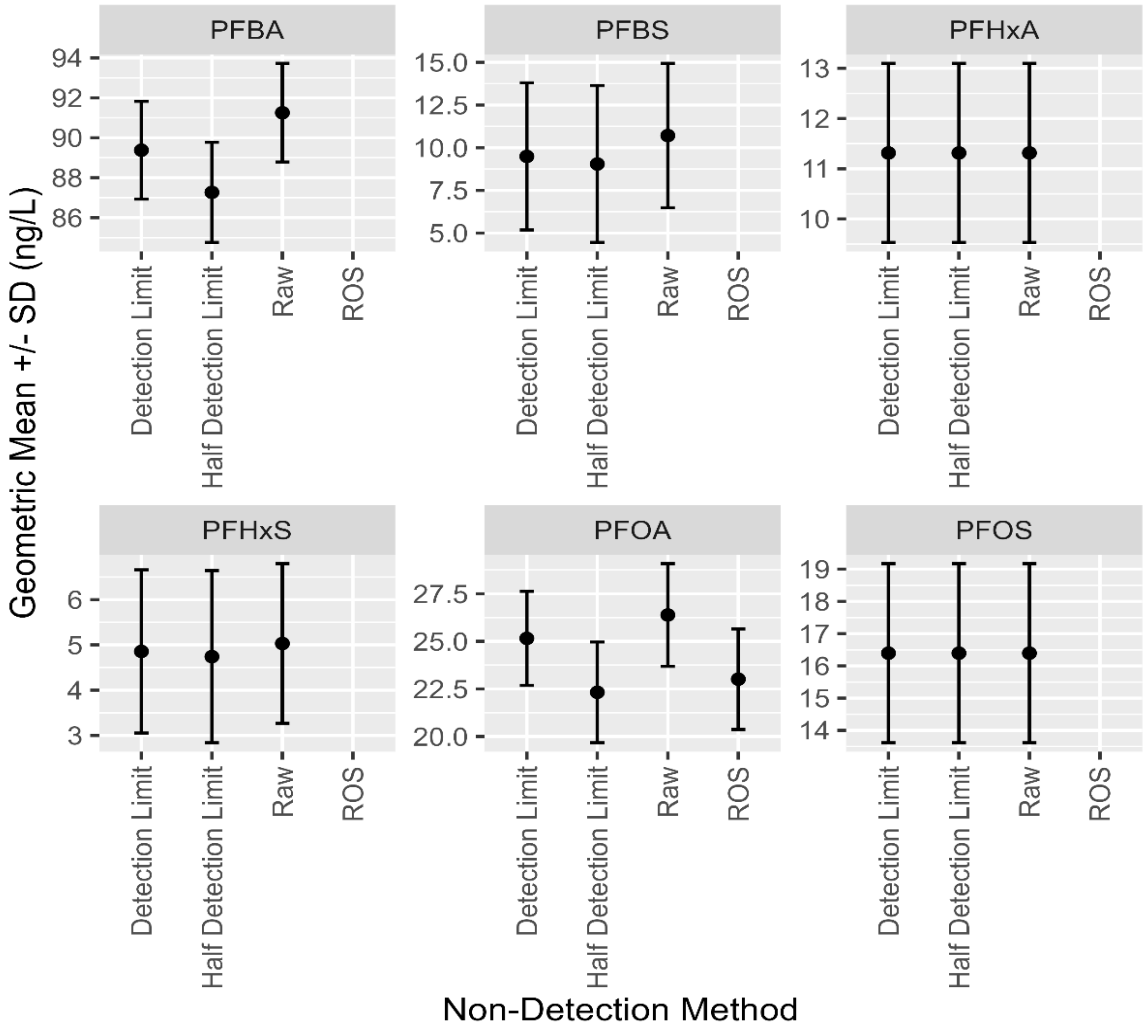


Figure 1. Surface water geometric means ± standard deviation (SD) for PFAS compounds.

Fish PFAS data

All combined fish data

We have calculated PFAS geometric means for all combined fish samples, as well as per trophic level and taxa. For combined fish, calculated means are comparable between all non-detection methods as with surface water results (Table 4 and Figure 2). PFOS means are equivalent among raw, detection limit, half detection limit, and zero methods since this dataset contains only detected values (Table 5). We have not calculated Kaplan-Meier and ROS methods for PFOS since the dataset does not contain non-detection values (Table 5). Additionally, we have not calculated geometric means for the zero and Kaplan-Meier non-detection methods for any PFAS compound. Calculation of geometric means is not possible with a dataset that contains zeros. The Kaplan-Meier non-detection requires positive values and log-transforming this dataset, as done for geometric mean calculation, produces negative values.

Table 4. Fish geometric means (ng/g) per PFAS compound.

Method	PFBA	PFBS	PFHxA	PFHxS	PFOA	PFOS
Raw	0.410	0.137	0.220	0.119	0.557	11.7
Detection Limit	0.328	0.137	0.136	0.071	0.306	11.7
Half Detection Limit	0.231	0.111	0.091	0.047	0.196	11.7
Zero	NA	NA	NA	NA	NA	11.7
ROS	0.550	0.211	0.269	0.123	0.668	NA
Kaplan-Meier	NA	NA	NA	NA	NA	NA

Table 5. Fish sample composition. *ND* abbreviates *non-detected*.

	PFBA	PFBS	PFHxA	PFHxS	PFOA	PFOS
Total Count	139	139	140	140	140	140
ND Count	70	42	82	84	88	0
Percent ND (%)	50.4	30.2	58.6	60.0	62.9	0

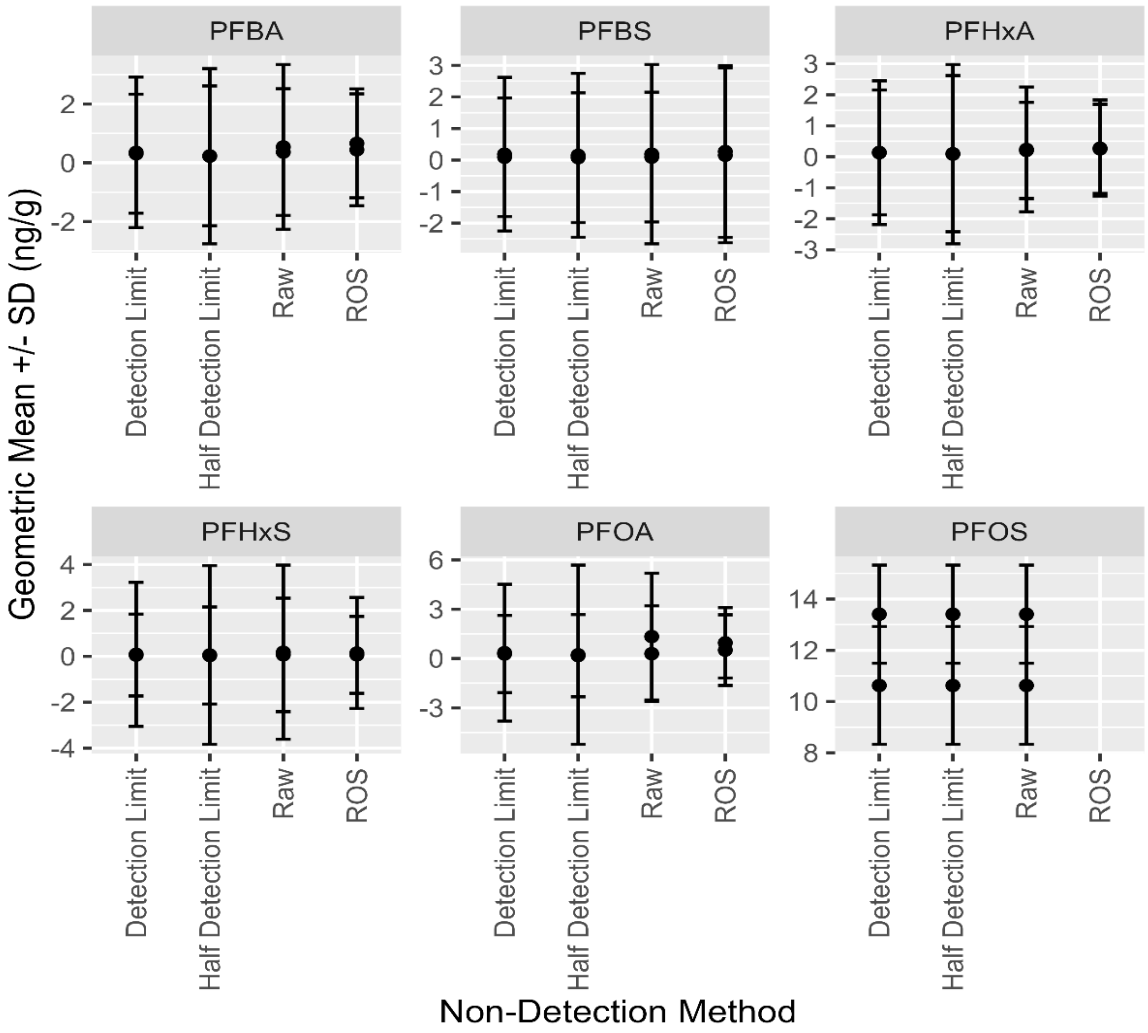


Figure 2. PFAS means ± standard deviation (SD) for all fish samples with six non-detection methods.

Fish data by trophic level

As with surface water and fish PFAS data, calculated geometric means are comparable among non-detection methods per fish trophic level (Table 6 and Figure 3). PFOS means are equivalent among raw, detection limit, half detection limit, and zero methods since this dataset contains only detected values (Table 7). We have not calculated Kaplan-Meier and ROS methods for PFOS since the dataset does not contain non-detection values (Table 7). Additionally, we have not calculated geometric means for the zero and Kaplan-Meier non-detection methods for any PFAS compound for reasons described above. Calculation of geometric means is not possible with a dataset that contains zeros. Means of PFAS compounds are generally comparable among trophic levels (Figure 3).

Table 6. Fish taxa geometric means (ng/g) per PFAS compound.

Trophic Level	Method	PFBA	PFBS	PFHxA	PFHxS	PFOA	PFOS
3	Raw	0.364	0.188	0.236	0.179	0.293	10.6
	Detection Limit	0.309	0.185	0.132	0.088	0.274	10.6
	Half Detection Limit	0.235	0.148	0.086	0.059	0.176	10.6
	Zero	NA	NA	NA	NA	NA	10.6
	ROS	0.441	0.270	0.276	0.143	0.511	NA
	Kaplan-Meier	NA	NA	NA	NA	NA	NA
4	Raw	0.539	0.093	0.204	0.064	1.34	13.4
	Detection Limit	0.356	0.090	0.141	0.054	0.355	13.4
	Half Detection Limit	0.225	0.075	0.098	0.034	0.226	13.4
	Zero	NA	NA	NA	NA	NA	10.6
	ROS	0.661	0.152	0.255	0.063	0.955	NA
	Kaplan-Meier	NA	NA	NA	NA	NA	NA

Table 4. Fish trophic level sample composition. *ND* abbreviates *non-detected*.

Trophic Level	Statistic	PFBA	PFBS	PFHxA	PFHxS	PFOA	PFOS
3	Total Count	79	80	80	80	80	80
	ND Count	31	26	50	46	50	0
	Percent ND (%)	39.2	32.5	62.5	57.5	62.5	0
4	Total Count	60	59	60	60	60	60
	ND Count	39	16	32	38	38	0
	Percent ND (%)	65	27.1	53.3	63.3	63.3	0

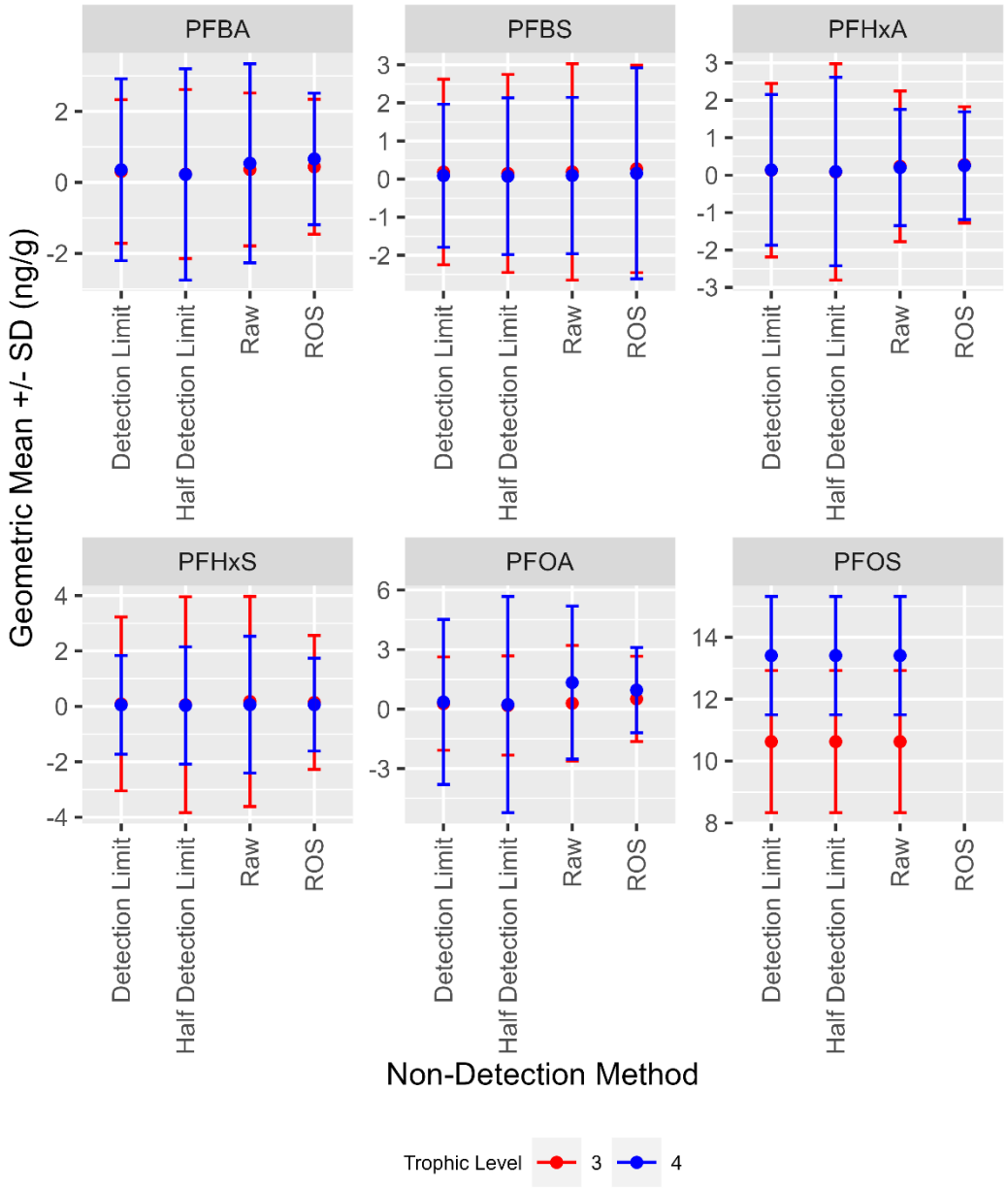


Figure 3. PFAS geometric means ± SD by fish trophic level for six non-detection methods.

Fish data by taxa

Among the non-detection methods, geometric means per taxa and PFAS compound are comparable (Figs. 4 - 7). Calculations for some methods were limited as described above. Figures 4-7 show box and whisker plots per PFAS compound among all taxa for raw, detection limit-substituted, half detection limit substituted, and zero substituted data.

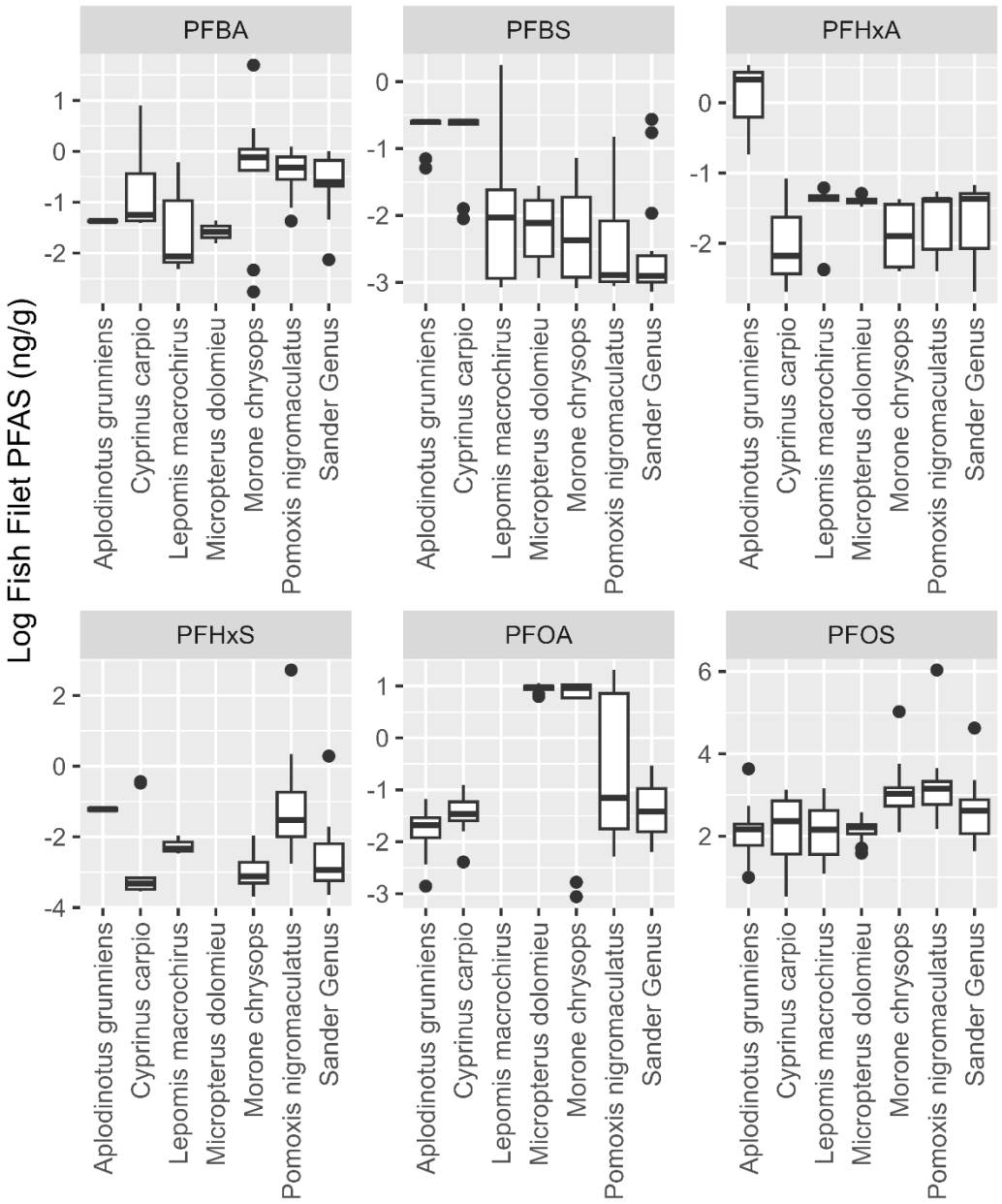


Figure 4. Box and whisker plots for natural log transformed raw PFAS data (ng/g) by taxa.

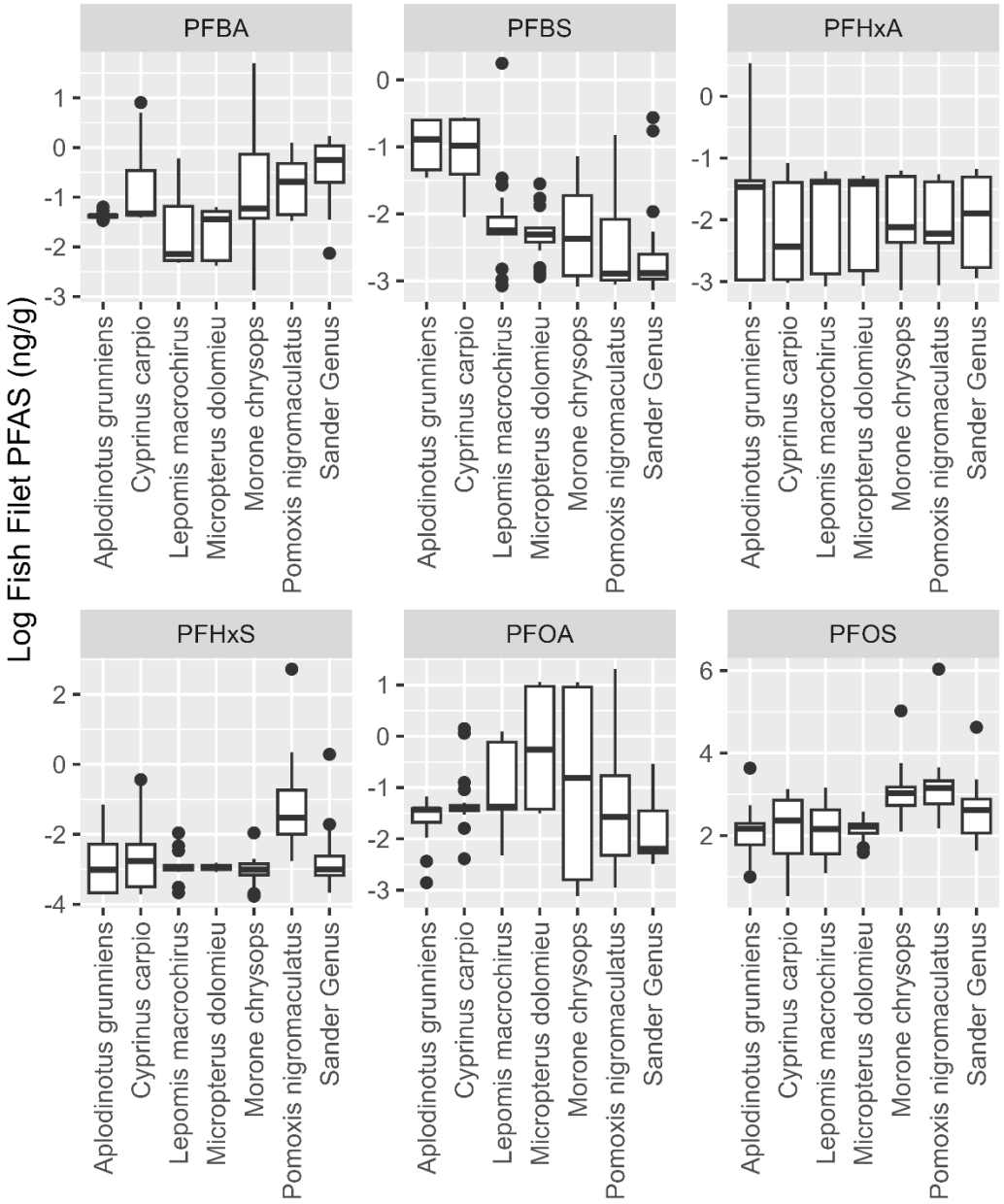


Figure 5. Box and whisker plots for detection limit substituted PFAS data with natural log transformation (ng/g) by taxa.

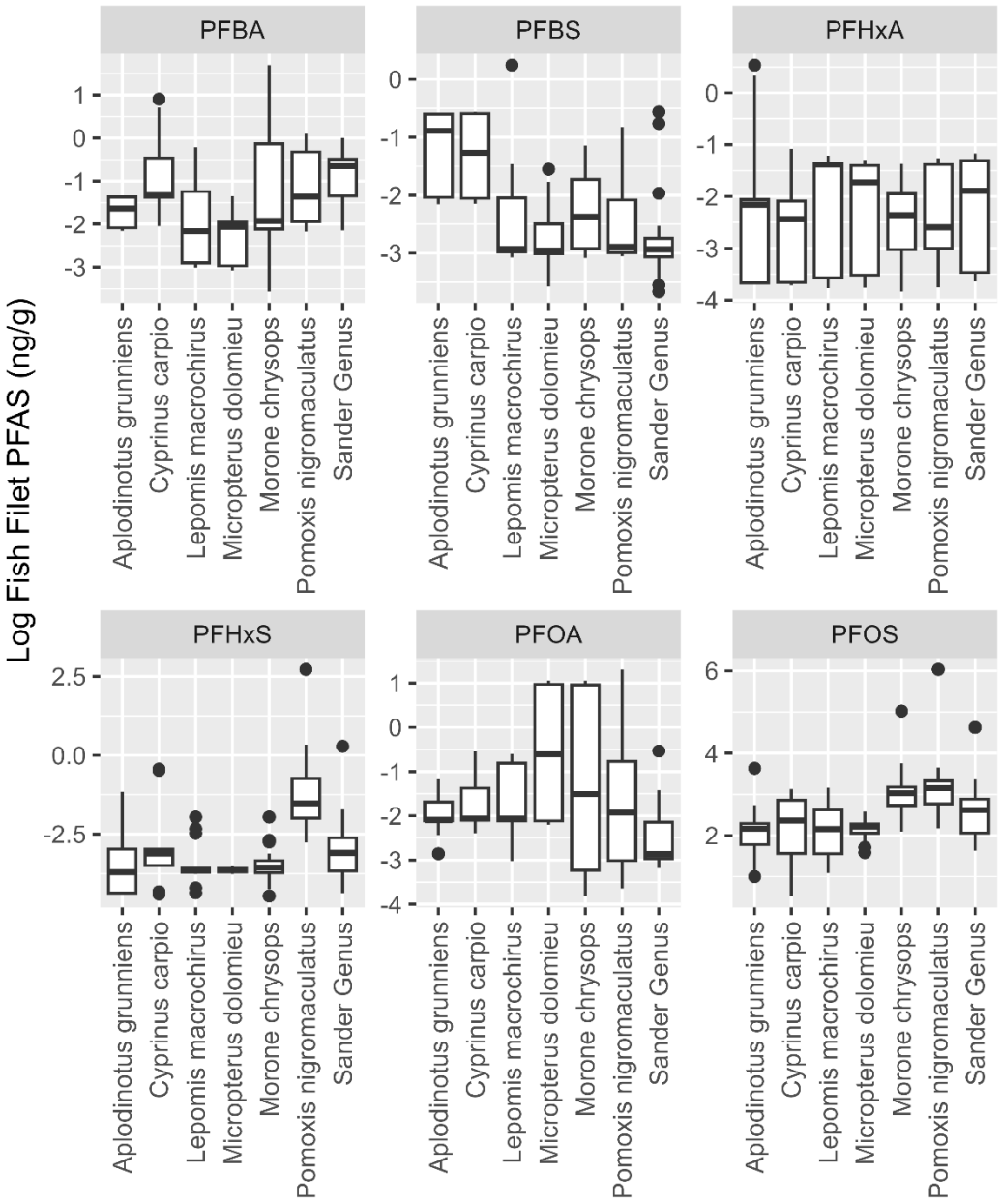


Figure 6. Box and whisker plots for half detection limit substituted PFAS data with natural log transformation (ng/g) by taxa.

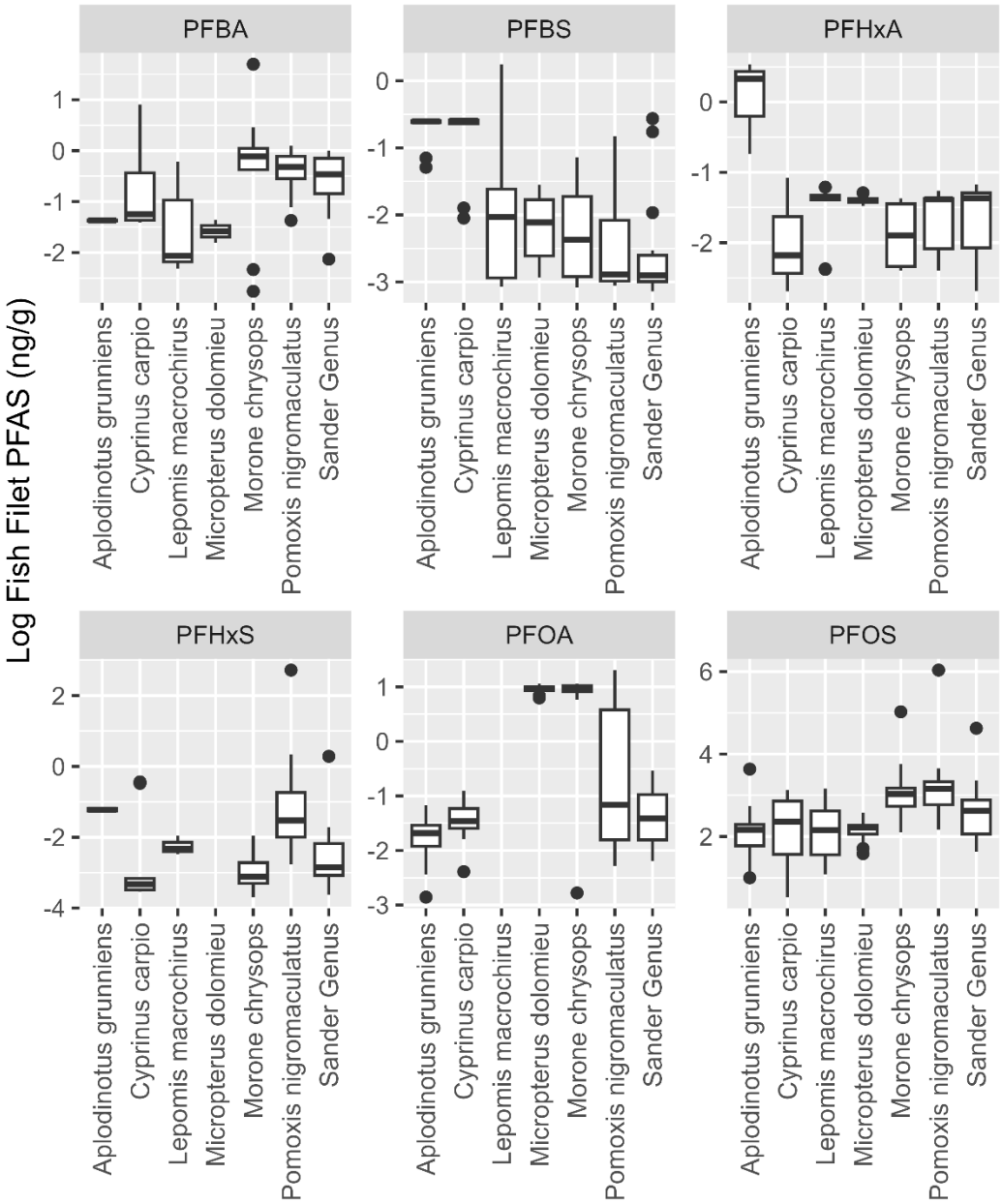


Figure 7. Box and whisker plots for zero substituted PFAS data with natural log transformation (ng/g) by taxa.

Discharger: 3M Cottage Grove
Permit Number: MN0001449
AI Number: 1163
Outfall Number: SD001
Date: 05-22-2024
Dann White and Scott Kyser

Background

The discharge is located on an unnamed creek (07010206-517) that flows into Pool 2 of the Mississippi River (07010206-814). The unnamed creek and the Mississippi River are class 2Bg, 3, 4A, 4B, 5, 6 waters. The unnamed creek is listed as an impaired water for fish bioassessments. Pool 2 of the Mississippi River is listed as impaired for total mercury, PCBs, PFOS in surface water and fish tissue, aluminum, and fecal coliform. Effluent limits were set to protect water quality in Unnamed Creek, Pool 2 of the Mississippi River, and all downstream waters. Effluent limitations for PFAS were set to protect Class 2Bg PFAS site-specific criteria in the Mississippi River and effluent limitations for every other parameter were set to protect class 2Bg water quality standard in the immediate receiving water of Unnamed Creek.

This discharge is process wastewater from their main plant and sanitary sewer water from the facility. The Maximum Design Flow (MDF) is used to calculate water quality-based effluent limits (WQBELs) under critical low flow stream conditions. The MDF flow for this station is 6.5 mgd. The low flow condition is defined by the once in ten year weekly average flow ($7Q_{10}$), which is determined to be 2,167 CFS for the Mississippi River and 0.0 CFS for the unnamed creek. The analysis below is based on the most recent five years of data submitted to date.

Under the previous permit, SD001 discharged to Unnamed Creek. In that permit, the assumption was made that Unnamed Creek was a “discharge ravine” that functioned as a direct conduit to the Mississippi River and was, thereupon, completely, and instantaneously mixed. In this permit, Unnamed Creek is protected as a water of the state where surface water quality standards apply and is not treated as a direct conduit to the Mississippi River. Based on available surface water monitoring data and the hydrology of Pool 2 of the Mississippi River, it is unreasonable to assume that the 3M discharge is completely and instantaneously mixed into Pool 2 of the Mississippi River. Pool 2 of the Mississippi River downstream of the confluence of Unnamed creek is at least 0.7 miles wide, has a minimum volume of 3.46 billion gallons and has a large $7Q_{10}$ flow rate. The large flow and volume makes complete mixing impossible. Additionally, Pool 2 PFAS concentrations in the sediment, surface water and surface water microlayer of the Mississippi River tend to increase as you get closer to 3M, which is an indicator that the PFAS discharged by 3M are not completely and instantaneously mixed into Pool 2 of the Mississippi River.

Reasonable Potential Analyses for Chemical Specific Pollutants

Federal regulations (40CFR122.44(d)(1)) require the Minnesota Pollution Control Agency (MPCA) to evaluate the discharge to determine whether it has the reasonable potential to cause or contribute to a violation of water quality standards. The agency must use acceptable technical procedures, accounting for variability (coefficient of variation, or CV), when determining whether the effluent causes, has the reasonable potential to cause, or contribute to an excursion of an applicable water quality standard. Projected Effluent Quality (PEQ) derived from effluent monitoring data is compared to Preliminary Effluent Limits (PELs) determined from mass balance inputs. Both determinations account for effluent variability. Where PEQ exceeds the PEL, there is reasonable potential to cause or contribute to a water quality standards excursion. When reasonable potential is indicated the permit must contain a WQBEL for that pollutant.

Per- and Polyfluorinated (PFAS) Substances

The PFAS effluent limits in Table 1 should be included in the draft permit. A summary of the derivation of these limits is explained further below. Mass limits were calculated based on the monthly average limit and the max design flow. PFBA, PFBS, PFHxA, PFHxS, PFOA and PFOS abbreviate perfluorbutanoic acid, perfluorobutnesulfonoic acid, perfluorhexanoic acid, perfluorohexanesulfonic acid, perfluorooctanoic acid, and perfluorooctanesulfonic acid, respectively.

Table 1. PFAS effluent limit summary.

Limit Type	Units	PFBA	PFBS	PFHxA	PFHxS	PFOA	PFOS	Hazard Index
Daily Max	ng/L	60,752	7,290	10,692	0.0056	0.022	0.066	Monitor Only
Monthly Average	ng/L	35,068	4,208	6,172	0.0032	0.013	0.038	Monitor Only
Monthly Average	g/day	861,622	103,394	151,645	0.079	0.32	0.93	Monitor Only
Compliance Limit for a WQBEL that is below the detection limit		Not Applicable	Not Applicable	Not Applicable	2.1 ng/L as a daily max and monthly average	2.1 ng/L as a daily max and monthly average	2.2 ng/L as a daily max and monthly average	Not Applicable

PFAS Site-Specific Criteria

No Per- and Polyfluorinated Substance (PFAS) compound has a statewide water quality standard listed in MN rule and Minnesota has no PFAS site-specific standard for any water. Since PFAS are discharged by 3M Cottage Grove to waters of the state and PFAS have the potential to cause toxic effects, the MPCA derived site-specific criteria for six PFAS compounds (Table 2) using the procedures outlined in Minn. R. 7050.0217, Minn. R. 7050.0218 and Minn. R. 7050.0219. These PFAS site-specific criteria were derived to be specific to the point source being addressed and to protect water quality in Pool 2 of the Mississippi River for human health. The permittee must be

given notice of any specific effluent limitation derived from these criteria and given opportunity to request a hearing as provided in Minn. R. 7000.1800.

Table 2. Summary of PFAS site-specific criteria.

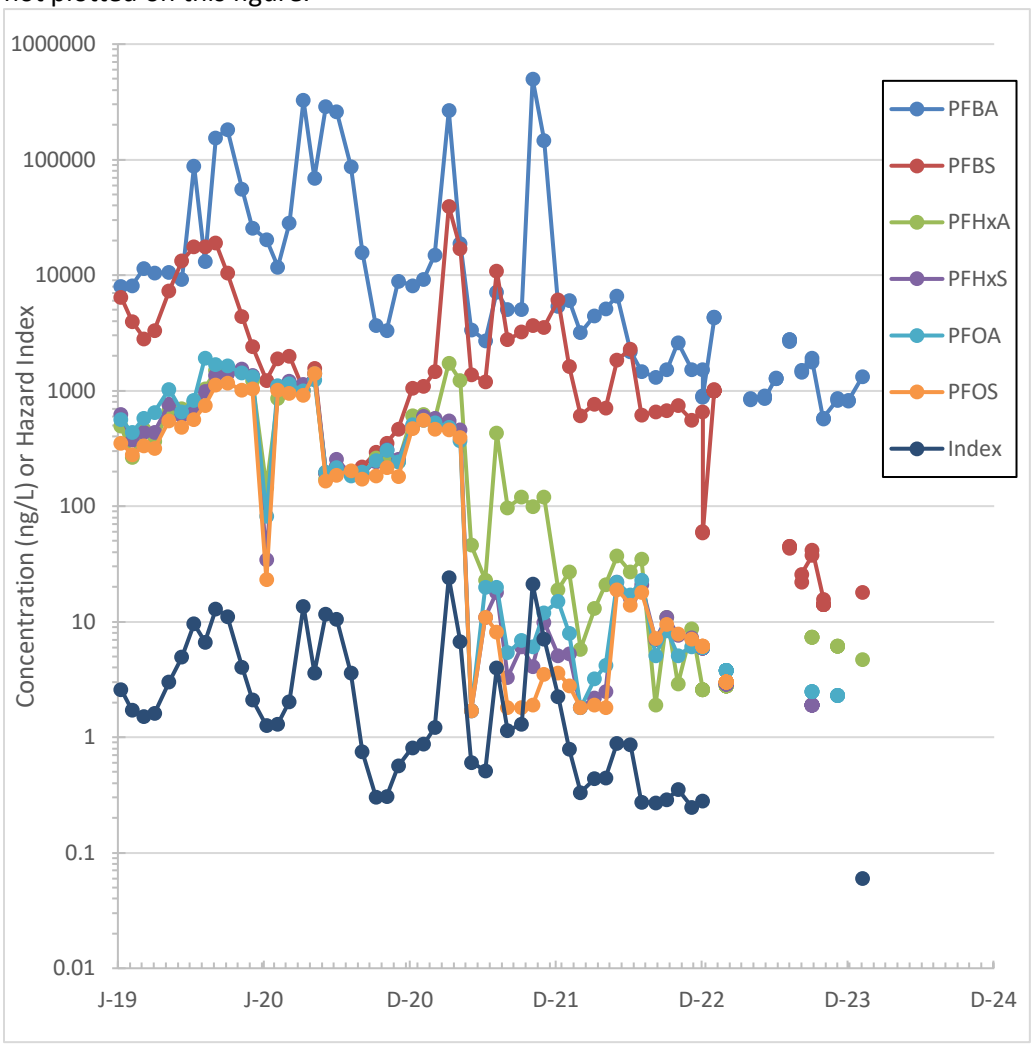
PFAS (CAS No. see Table 2-1)	**Site-specific water quality criteria: Chronic Criteria (CC)**		***Health Risk Index Endpoints* (Additive Risk)**
	Class 2B – fish consumption and recreational exposure (CC_{FR}) (30-day average)	**Class 2 fish-tissue** (CC_{FT}) (90^{th} percentile of 5 fish minimum per water body)	
PFOS	0.027 ng/L	0.021 ng/g	Developmental, Liver System, Immune System, Cancer (MDH 2024b)
PFOA	0.0092 ng/L	0.00036 ng/g	Developmental, Liver System, Immune System, Cancer (MDH 2024a)
PFHxS	0.0023 ng/L	0.000043 ng/g	Liver System, Thyroid (endocrine) (MDH 2023b)
PFHxA	4,400 ng/L	Not applicable	Developmental, Thyroid (endocrine) (MDH 2023c)
PFBS	3,000 ng/L	Not applicable	Thyroid (endocrine) (MDH 2023a)
PFBA	25,000 ng/L	Not applicable	Liver System, Thyroid (endocrine) (MDH 2018)
Mixtures containing two or more of PFBA + PFBS + PFHxA	≤ 1 (unitless) Health Risk Index	Not applicable	Thyroid (endocrine)
Definitions of CC: CC_{FR} : Applied in Class 2B surface waters (F: Fish consumption and R: Recreational exposure) CC_{FT}: Applied for Bioaccumulative Chemicals of Concern (BCC) in fish (fillet/muscle) for all Class 2 waters (FT: fish-tissue)			

The proposed PFAS site-specific criteria are applicable to the Mississippi River between river miles 812-820 and do not apply to the immediate receiving water of Unnamed Creek. The site-specific criteria have a 30-day duration and a once in three-year allowable frequency of exceedance. Effluent limitations for PFAS were set to protect water quality in Pool 2 of the Mississippi River. MPCA's reasonable potential analysis was performed only for the six PFAS compounds with developed site-specific criteria (see section below).

3M SD001 PFAS Monitoring Data

A summary of 3Ms reported PFAS data for station SD001 from 2019 to February 2024 can be seen in Figure 1 below.

Figure 1. Reported SD001 PFAS concentration in ng/L. Note the log scale. Non-detect values are not plotted on this figure.



PFAS Surface Water Monitoring

There is sufficient data to characterize PFAS levels in the receiving waters for surface water, fish tissue and macro invertebrates. A summary of PFAS surface water monitoring found in the April 28, 2023, report title 'Instream PFAS Characterization Study Interim Report Mississippi River Cottage Grove, Minnesota' can be seen in the Figures 2 and 3 and Table 3. The samples in the report represented the most recent PFAS monitoring and were collected in July and August of 2021.

There is evidence that the 3M discharges are causing PFAS levels to increase in Unnamed Creek downstream of the discharges (Table 3). It is not possible to say exactly how much of that PFAS increase is attributable to SD001 versus SD002 because the two discharges have not been sampled on the days of the surface water sampling and the flow in Unnamed Creek on those days was not measured. Not every PFAS compound increased downstream of 3M on Unnamed Creek by the same amount, but this can be explained by the high variability of PFAS concentrations in 3M discharges (Figure 1). If Unnamed Creek had been sampled at a different moment when 3M was discharging a different mixture of PFAS, then different, but still elevated, concentrations of individual PFAS in Unnamed Creek would likely have been measured.

There is also evidence that the elevated levels of PFAS in Unnamed Creek (attributable to the 3M discharges) have the reasonable potential to cause an exceedance of a PFAS site-specific criteria in Pool 2 of the Mississippi River, especially since the 3M discharges have PFAS levels well above the site-specific criteria in Pool 2 of the Mississippi River (Figure 1 and Table 3). For example, PFBS concentrations in Unnamed Creek are several-fold higher than the Pool 2 PFBS concentrations and a the highest PFBS value in the Mississippi River was measured at the confluence of Unnamed Creek with the Mississippi River. This analysis of discharge and surface water monitoring data is a supplementary line of evidence in MPCA's reasonable potential analysis for PFAS compounds. The analysis justifies the assumptions that PFAS have a conservative fate and transport between the discharges and Pool 2 of the Mississippi River and that the 3M discharge is not completely and instantaneously mixed into Pool 2 of the Mississippi River.

It is uncertain whether PFAS contaminated groundwater in the East Cove is contributing PFAS into Unnamed Creek because of the nearby groundwater pump-system, local typography, soils, and depth to groundwater. More data explaining the flow of groundwater in the East Cove is available upon request in the report title '2021 Annual Perflourochemical (PFCs) Groundwater Report for the 3M Cottage Grove Site'.

Figure 2. Map of surface water PFAS sampling locations. Red dots are locations in Unnamed Creek and blue dots are in the Mississippi River. Crosses represent transect sample locations.

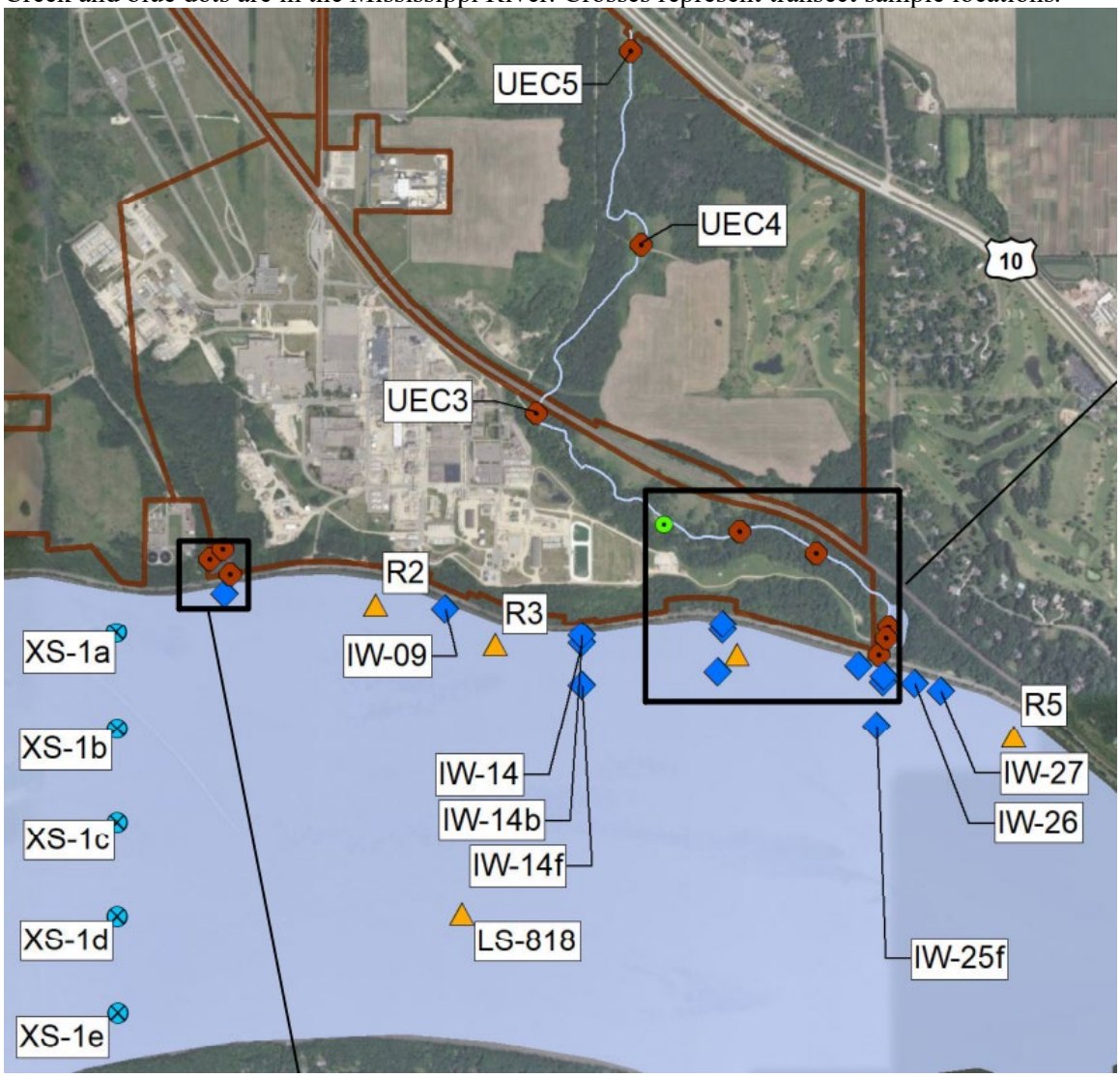


Figure 3. Close up of sample locations on Unnamed Creek and the East Cove of the Mississippi River. Red dots are locations in Unnamed Creek and blue dots are in the Mississippi River.

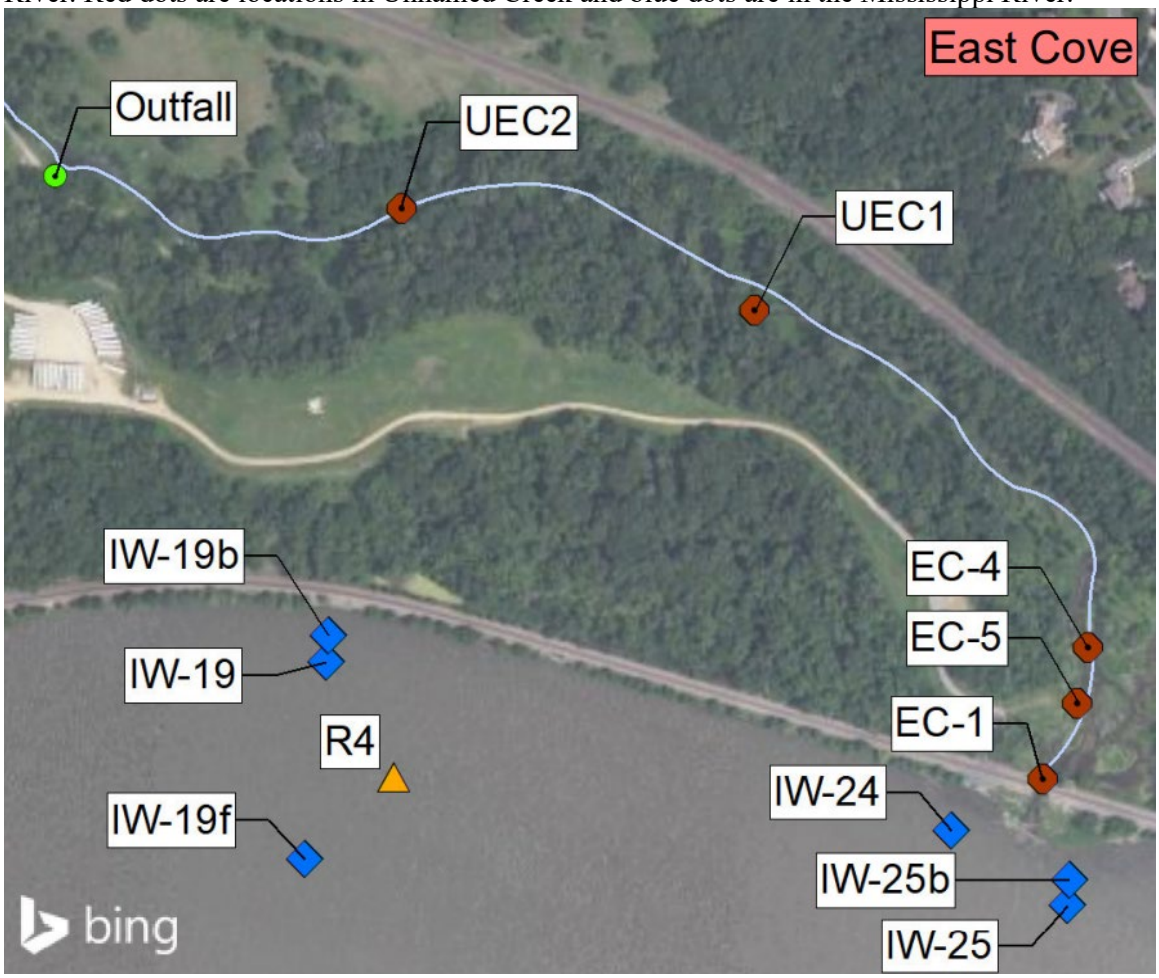


Table 3. PFAS surface water monitoring data points. Units are ng/L. Values above the criteria in the Mississippi (Miss.) River are in bold. Italics indicate that the values are from the 3M discharge.

			PFBS	PFBA	PFOS	PFOA	PFHxS	PFHxA
Site-Specific Criteria (ng/L)			350	10,000	0.05	88	36	950
Location	**Waterbody**	**Description**						
UEC5	Unnamed Creek	Upstream of discharge	16	1,800	3.9	63	10	60
UEC4	Unnamed Creek	Upstream of discharge	16	1,800	4.2	110	10	61
UEC3	Unnamed Creek	Upstream of discharge	17	1,900	4.6	110	11	65
Discharge	SD001	Projected Effluent Quality (max value)	*39,400*	*498,000*	*1,410*	*1,930*	*1,740*	*1,540*
Discharge	SD002	Projected Effluent Quality (max value)	7,720	20,600	6,300	11,100	9,380	6,200
UEC2	Unnamed Creek	Downstream of discharge	2,900	6,000	3.2	63	37	250
UEC1	Unnamed Creek	Downstream of discharge	2,500	5,400	45	76	39	210
EC-5	Unnamed Creek	Downstream of discharge	5,700	6,900	36	68	47	380
EC-4	Unnamed Creek	Downstream of discharge	5,500	7,000	28	74	42	380
EC-1	Unnamed Creek	Downstream of discharge	4,300	6,400	45	70	44	360
IW-24	Miss. River	Upstream of Unnamed Creek	17	190	**28**	**54**	**7.2**	15
IW-19b	Miss. River	Upstream of Unnamed Creek	20	130	**96**	**200**	**12**	28
IW-19	Miss. River	Upstream of Unnamed Creek	10	75	**39**	**70**	**7.2**	14
IW-19f	Miss. River	Upstream of Unnamed Creek	11	68	**47**	**52**	**6**	13
IW-25b	Miss. River	At confluence of Unnamed Creek	560	560	**21**	**34**	**5.9**	42
IW-25	Miss. River	At confluence of Unnamed Creek	240	1,200	**16**	**29**	**7.2**	24
IW-26	Miss. River	Immediately downstream of Unnamed Creek	180	470	**82**	**130**	**14**	30
IW-27	Miss. River	Immediately downstream of Unnamed Creek	110	42	**72**	**130**	**12**	130
XS-1a	Miss. River	Transect upstream of 3M Cottage Grove	3.3	39	**49**	**11**	**10**	7.1
XS-1b	Miss. River	Transect upstream of 3M Cottage Grove	3.5	42	**91**	**11**	**11**	8
XS-1c	Miss. River	Transect upstream of 3M Cottage Grove	2.9	31	**7.1**	**11**	**3.3**	6.4
XS-1d	Miss. River	Transect upstream of 3M Cottage Grove	4.1	130	**14**	**14**	**5.3**	9.2
XS-1e	Miss. River	Transect upstream of 3M Cottage Grove	4	97	**7.5**	**11**	**3.5**	8.1

Fish Tissue Monitoring and Dilution

PFAS are accumulating in fish tissue in the Mississippi River (Figure 4) and mean fish tissue are above the fish tissue criteria for the three PFAS with applicable fish tissue criteria (Table 4). This is strong line of evidence that no receiving water dilution should be allowed for PFOA, PFOS and PFHxS in the Mississippi River.

Table 4. Comparison of the fish tissue site-specific criteria to the in-stream measured mean fish tissue concentrations. The mean fish tissue concentrations were calculated using non-detection methodologies detailed in the PFAS site-specific criteria document.

	Fish Tissue Site-Specific Criteria (ng/g)	Mean Fish Tissue Concentration in SSC area (ng/g)
PFOS	0.021	17.9
PFOA	0.00036	0.454
PFHxS	0.000043	0.192
PFHxA	Not Calculated	0.147
PFBA	Not Calculated	0.31
PFBS	Not Calculated	0.175

The MPCA will allow no receiving water dilution for PFHxA, PFBA and PFBS when calculating limits, for the following reasons:

- The measured fish tissue concentrations of PFHxA, PFBA and PFBS are similar to the three PFAS with fish tissue site-specific criteria (Table 4). This means that all six PFAS are accumulating in fish tissue at similar, but still elevated concentrations.
- While there are no PFAS criteria for benthic macroinvertebrates, every single benthic macroinvertebrate in Pool 2 had a detectable level of PFOS, PFOA and many other PFAS were also present in benthic macroinvertebrates (Figure 5). This is another line of evidence that PFAS is generally accumulating in aquatic life in Pool 2 of the Mississippi River and that there is no assimilative capacity or dilution for PFAS in Pool 2 of the Mississippi River.
- Treating PFHxA, PFBA and PFBS similarly with respect to dilution increases consistency when considering limits to protect the hazard index site-specific criteria.

Figure 4. Box and whisker plots for natural log transformed raw PFAS data (ng/g) by taxa.

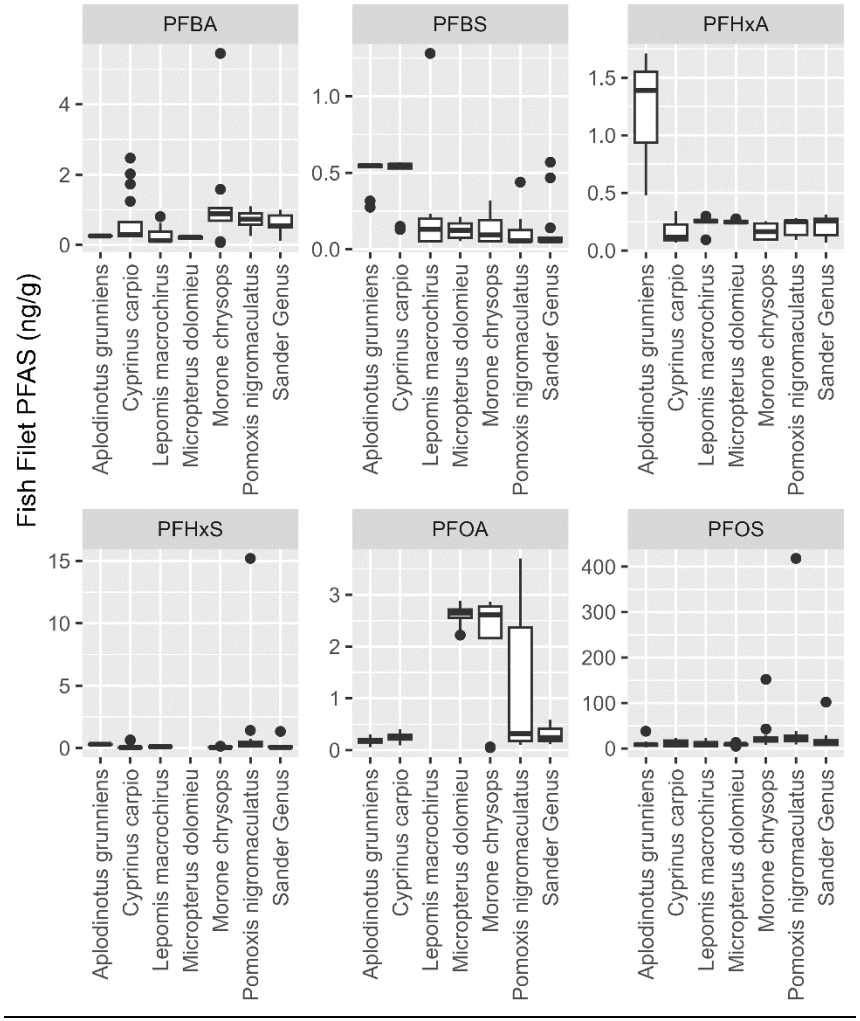
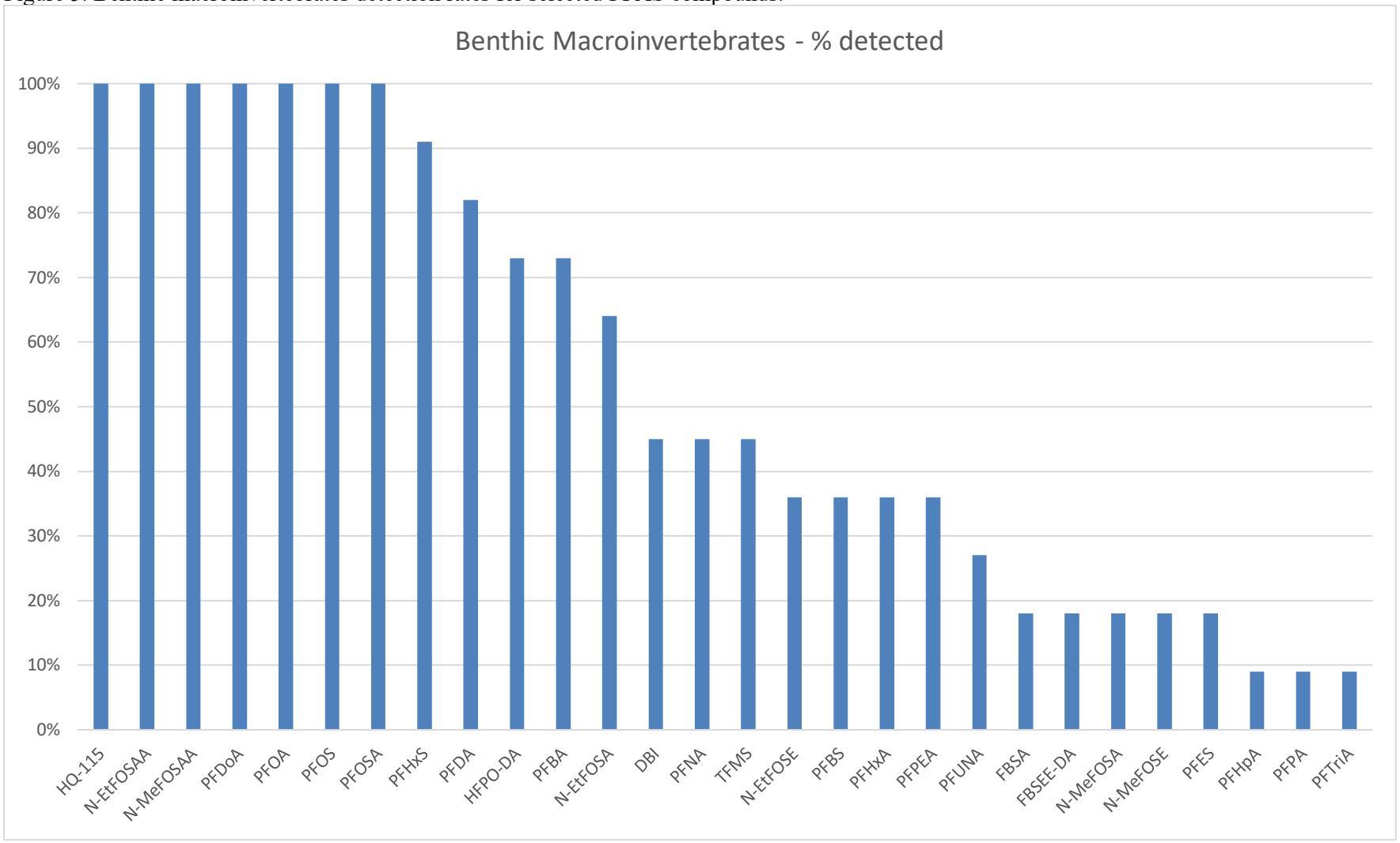


Figure 5. Benthic macroinvertebrates detection rates for selected PFAS compounds.



PFBS Reasonable Potential Analysis

Using the methodologies in the 1991 TSD, the 3M discharge has the reasonable potential to cause an exceedance of the PFBS site-specific criterion in the Mississippi River. The 3M PFBS effluent data are highly variable and have a CV greater than three. Since that variability is so high, the MPCA's default CV of 0.6 was used to set limits. The PEQ was based on the highest reported value (75,800 ng/L) and a PEQ factor of one. WQBELs were set to ensure that the 3,000 ng/L PFBS site-specific criterion was met at the confluence of Unnamed Creek and at the stream's confluence with the Mississippi River under a zero $7Q_{10}$ low flow condition.

PFBA Reasonable Potential Analysis

Using the methodologies in the 1991 TSD, the 3M discharger has the reasonable potential to cause an exceedance of the PFBA site-specific criterion in the Mississippi River. The 3M PFBA effluent data are highly variable and have a CV greater than three. Since that variability is so high, the MPCA's default CV of 0.6 was used to set limits. The PEQ was based on the highest reported value (498,000 ng/L) and a PEQ factor of one. WQBELs were set to ensure that the 25,000 ng/L PFBA site-specific criterion was met at the confluence of Unnamed Creek and at the Mississippi River under a zero $7Q_{10}$ low flow condition.

PFHxA Reasonable Potential Analysis

Using the methodologies in the 1991 TSD, the 3M discharger has the reasonable potential to cause an exceedance of the PFHxA site-specific criterion in the Mississippi River. The 3M PFHxA effluent data are highly variable and have a CV greater than three. Since that variability is so high, the MPCA's default CV of 0.6 was used to set limits. The PEQ was based on the highest reported value (1,740 ng/L) and a PEQ factor of one. WQBELs were set to ensure that the 4,400 ng/L PFHxA site-specific criterion was met at the confluence of Unnamed Creek and at the stream's confluence with the Mississippi River under a zero $7Q_{10}$ low flow condition.

PFHxS Reasonable Potential Analysis

Using the methodologies in the 1991 TSD, the 3M discharger has the reasonable potential to cause an exceedance of the PFHxS site-specific criterion in the Mississippi River. The 3M PFHxS effluent data are highly variable and have a CV greater than three. Since that variability is so high, the MPCA's default CV of 0.6 was used to set limits. The PEQ was based on the highest reported value (1,540 ng/L) and a PEQ factor of one. WQBELs were set to ensure that the 0.0023 ng/L PFHxS site-specific criterion was met at the confluence of Unnamed Creek and at the stream's confluence with the Mississippi River under a zero $7Q_{10}$ low flow condition.

PFOA Reasonable Potential Analysis

Using the methodologies in the 1991 TSD, the 3M discharge also has the reasonable potential to cause an exceedance of the PFOA site-specific criterion in the Mississippi River. The 3M PFOA effluent data are highly variable and have a CV greater than three. Since that variability is so high, the MPCA's default CV of 0.6 was used to set limits. The PEQ was based on the highest reported value (1,930 ng/L) and a PEQ factor of one. WQBELs were set to ensure that the 0.0092 ng/L PFOA site-specific criterion was met at the confluence of Unnamed Creek and at the stream's confluence with the Mississippi River under zero a $7Q_{10}$ low flow condition.

PFOS Reasonable Potential Analysis

Using the methodologies in the 1991 TSD, the 3M discharge also has the reasonable potential to cause an exceedance of the PFOS site-specific criterion in the Mississippi River. The 3M PFOS effluent data are highly variable and have a CV greater than three. Since that variability is so high, the MPCA's default CV of 0.6 was used to set limits. The PEQ was based on the highest reported value (1,410 ng/L) and a PEQ factor of one. WQBELs were set to ensure that the 0.027 ng/L PFOS site-specific criterion was met at the confluence of Unnamed Creek and at the confluence with the Mississippi River under a $7Q_{10}$ low flow condition.

PFAS Hazard Index Reasonable Potential Analysis

The 3M discharge does not have the reasonable potential to cause an exceedance of the PFAS site-specific criterion hazard index of 1.0 in the Mississippi River and no effluent limit for the hazard index is recommended. There is no additional monitoring needed because PFBA, PFBS and PFHxA are already required to be monitored.

Individual effluent limitations for PFBA, PFBS and PFHxA are being included and compliance with those limits, will bound the concentrations of PFBA, PFBS and PFHxA that can be discharged. These three individual limits significantly reduce the likelihood that the cumulative hazard index for these three compounds will be exceeded.

From an engineering perspective, the low-level limits for PFOS, PFOA and PFHxS will also force PFBA, PFBS and PFHxA to be treated to low levels. In order to comply with the PFOS, PFOA and PFHxS limits, a greater than 99.8% removal of those compounds is required. The reverse osmosis and media sorption treatment processes that remove PFOS, PFOA and PFHxS at a greater than 99.8% removal rate will also remove PFBA, PFBS and PFHxA at removal rate greater than 99% (Source: 2021 3M treatability study). A greater than 99% removal rate for PFBA, PFBS and PFHxA will lower PFBA, PFBS and PFHxA concentrations to low enough levels that it is unlikely that the 1.0 hazard unit will be exceeded in the receiving waters.

PFAS Compliance Limits

The PFOS, PFOA and PFHxS limits are below the conventional (<2-4 ng/L) reporting limit for currently available analytical technology such as EPA method 1633. These limits are so low that a separate compliance limit must be established for the purposes of reporting limit compliance to the MPCA.

On January 12, 2024 the MPCA sent 3M a pre-public notice permit that included daily max and monthly average PFOS water quality based effluent limits that had compliance limits below the detection limit. In that pre-public notice permit, the MPCA include a compliance limit of "below reporting limit" for both the daily max and monthly average PFOS effluent limits.

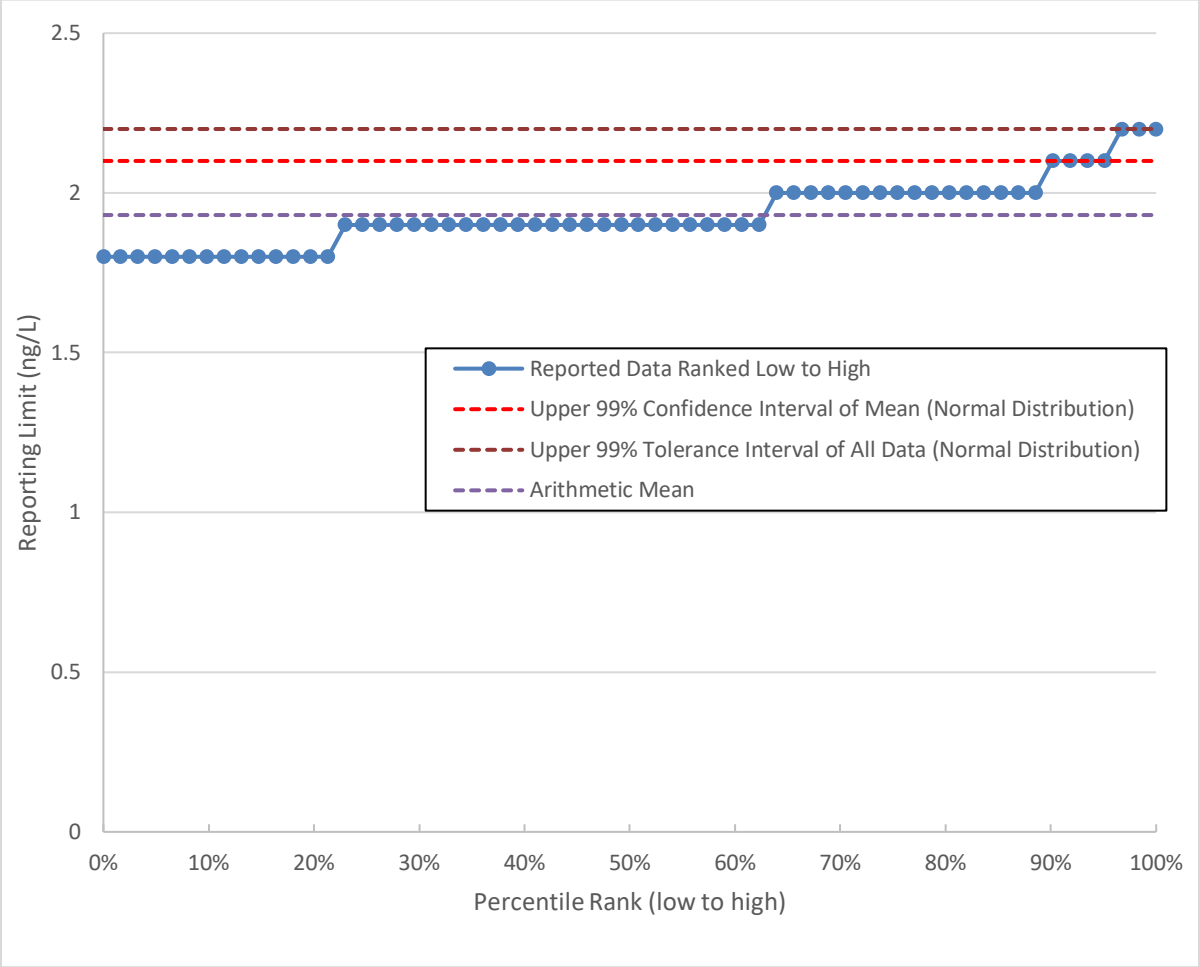
In a 5-7-24 response letter, 3M requested a compliance limit for PFOS of 2.2 ng/L expressed as a daily max and monthly average instead of "below reporting limit". 3M provided the data and calculations they used to derive the 2.2 ng/L value. 3M's 2.2 ng/L was calculated by compiling all PFOS reporting limit data for stations SD001 and SD002 from the calendar year 2023. In that dataset, diluted samples with high reporting limits were removed. 3M determined that the dataset was best fit using the SHASH (Sinh-Arcsinh) probability distribution and that a 99% tolerance interval of that distribution should be compliance limit of 2.2 ng/L.

The MPCA reviewed 3M's calculations and agrees with their compliance limit value (2.2 ng/L) but now with how it was calculated. Specifically, MPCA disagrees with how 3M assigned the SHASH probability distribution. To assign the SHASH distribution, 3M used a software package that evaluated 10 different probability distributions and then chose the one with the best coefficient of fit. The top six ranked distributions had coefficients of fits that were similarly good and could have been interchangeably selected. Especially because the other distributions are commonly used and are not obscure. (Note: The MPCA could not find a reference to the SHASH distribution in an intro to statistics textbook and the MPCAs internal statistics expert had never heard of the distribution).

In short, 3M should have used more statistical intuition in their analysis, selected a more commonly used probability distribution and framed their statistical decisions with greater context. The difference in absolute variance between the minimum and maximum value in this data set is very small (0.4 parts per trillion or 0.00000000004%). In addition, a simple eyeballing of the data also generates a value of 2.2 ng/L, as well several other statistical methods. And whatever statistical method used would generate a value that differed from the next method by at most 0.1 part per trillion (Figure 6). As a general rule, the MPCA prefers to use statistical analysis that focus on whether the statistical analysis answering the right question and is less focused on whether the lowest coefficient of fit being used.

Ultimately, the MPCA believes that the difference between assigning a compliance limit of "below reporting limit" and 2.2 ng/L is so small as to make little difference because both are protective of downstream water quality standards. In addition, a compliance value of 2.2 ng/L is similar to the value in EPA's recently promulgated PFAS drinking water rule, is simple to understand, is simple to enforce and provides the permittee regulatory certainty. During the next permit re-issuance, MPCA will re-review the compliance limit based on the current state of PFAS analytical abilities and revise it downward if reporting limits become lower over time.

Figure 6. PFOS reporting limit data supplied by 3M. The dashed lines represent statistical tests on the dataset.



On 5-17-24 3M sent in a similar reporting limit analysis for PFOA and PFHxS using a combination of complex statistical analysis and a simpler frequency analysis visible in Table 5. Using these statistical analyses, 3M proposed a compliance limit of 2.1 ng/L for both PFOA and PFHxS. The MPCA agrees with 3M's proposed compliance limits for PFOA and PFHxS because the value is similar to the proposed compliance value for PFOS, represents the current state of PFAS analytical chemistry and was chosen using reasonable statistical methods.

Table 5. Frequency analysis of 2023 reporting limit data supplied by 3M.

PFOS		PFOA		PFHxS	
RL (ng/L)	Frequency (n=62)	RL (ng/L)	Frequency (n=744)	RL (ng/L)	Frequency (n=75)
2.2	4.8%	2.1	1.1%	2.1	3.10%
2.1	6.5%	2.0	6.8%	2.0	21.40%
2.0	25.8%	1.9	40.9%	1.9	59.20%
1.9	40.3%	1.8	47.1%	1.8	16.30%
1.8	22.6%	1.7	4.1%		

The Permittee must sample and analyze PFAS compounds using methodology capable of detecting PFAS to the minimum reporting levels available and specifically below a 4 ng/L reporting limit for PFOS, PFOA, PFHxS such as EPA method 1633, a method equivalent to EPA method 1633 or a method better than EPA method 1633.

Note – Reporting limit compliance will be assessed by averaging all reporting limits at each individual monitoring station within a calendar year period and comparing against a 4 ng/L limit. The annual average of the reporting limit shall be included in the comments cell of the respective DMRs for all stations with the exception of WS 005 on the December reporting requirement. A violation of the annual average RL condition is not a WQBEL limit violation but is a permit violation at the specified station.
Note – Due to the variable stormwater characteristics, stormwater SD and WS stations may use all results from all stormwater stations when assessing compliance with the 4 ng/L reporting limit.

Note – Process control sampling does not have to meet the reporting limits established in item "A" above or any other quality assurance requirements otherwise required of the monitoring required in the Limits and Monitoring Requirement table of this permit.

DMR Requirements

An individual sample result that is below its reporting limit is considered to be in compliance with the associated daily maximum limit. [Minn. R. 7001]
Use the following instructions to determine a reportable value where sample values are less than the RL and the permit requires reporting of an average.
A. If some values are less than (<) the RL, substitute zero for all non-detectable values to report the average or summed concentration.
Example: The values for the month are: 5.0 ng/L, 4.0 ng/L, 3.0 ng/L and <2.0 ng/L. Report the monthly average or sum as (5.0 + 4.0 + 3.0 + 0.0) = 12.0 ÷ 4 = 3.0 ng/L
B. If all values are less than (<) the RL, use the RL for all non-detectable values to calculate the average or sum and report as < the RL calculated average or summed concentration.
Example: The values for the month are <0.2 ng/L, <0.4 ng/L, <0.2 ng/L, <2.0 ng/L. Report the monthly average or sum as (0.2 + 0.4 + 0.2 + 2.0) = 2.8 ÷ 4 = < 0.7 ng/L.
C. For calculating the average reporting limit: Average the numeric reporting limit for each PFOS or PFOA sample over the calendar year. If the average reporting limit is less than 4 ng/L, then the reporting limit is in compliance for that year.
Example: The reporting limits for four PFOS samples for a given year are: 1.8 ng/L, 3.2 ng/L, 4.0 ng/L, and 5.0 ng/L. This averages out to 3.5 ng/L as a yearly average and would be in compliance with the 4 ng/L value. [Minn. R. 7001]

Non-PFAS Water Quality Parameters

Reasonable Potential Summary for non-PFAS Pollutants

This outfall has shown reasonable potential (rp) for total cadmium, antimony, Di-2-ethyhexylphthalate (DEHP), total selenium, total zinc, and total mercury.

Table 6 contains the inputs to the reasonable potential analysis for 1,2 Dichloroethane, arsenic, cadmium, antimony, hexavalent chromium, copper, free cyanide, chloroform, Di-2-ethylhexylthalate, methylene chloride, nickel, lead, phenol, selenium, toluene, zinc and mercury. The analysis is made with effluent data that is expressed as total metal except hexavalent chromium. Table 6 also has reasonable potential calculations for perfluorbutanoic acid (PFBA), perfluorobutnesulfonoic acid (PFBS), perfluorhexanoic acid (PFHxA), perfluorohexanesulfonic acid (PFHxS), perfluorooctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS). These pollutants were evaluated on the basis of analytical measurements that made evident the need for a full determination. Where Projected Effluent Quality (PEQs) exceed Preliminary Effluent Limitations (PELs), a Water Quality-based Effluent Limit (WQBEL) is needed. Please note there is no dilution given for total mercury or for PFOS since pool 2 of the Mississippi River is listed as an impaired water for these two pollutants.

Table 6. Tabular summary of the RP calculations. The three tables below are actually one table but are split up for better reading.

Table 6. Reasonable Potential Results for 3M Cottage Grove (SD001).

Parameter	1,2-DCA (ug/L)	T. Arsenic (ug/L)	T. Cd (ug/L)	T. Sb (ug/L)	Cr6 (ug/L)	T. Cu (ug/L)	Free CN (ug/L)
Plant flow ADW (mgd)	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Rec. water flow, 7Q10(mgd)	0	0	0	0	0	0	0
Background Conc.	0	0	0	0	0	0	0
Chronic Std (cs)	190.00	53.00	2.62	31.00	11.00	19.02	5.20
290							
ppm hard							
Maximum Std (ms)	45050.00	360.00	111.12	90.00	16.00	48.34	22.00
290							
ppm hard							
Final Acute Value (FAV)	90100	720	222	180	32	97	45
290							
ppm hard							
Mass Balance -cs	190.00	53.00	2.62	31.00	11.00	19.02	5.20
Mass Balance -ms	45050.00	360.00	111.12	90.00	16.00	48.34	22.00
Coeff of Variation (CV)	0.60000	0.60000	0.60000	2.08951	0.60000	0.60000	0.60000
Long Term Avg-cs	148.26	41.36	1.38	6.08	5.80	10.03	2.74
Long Term Avg-ms	14465.35	115.59	35.68	10.23	5.14	15.52	7.06
Preliminary Effl limits:							
Daily Max	461.72	128.80	4.30	53.46	16.00	31.24	8.54
Monthly Ave (2x/month)	266.52	74.34	2.48	20.00	9.24	18.03	4.93
Max Measured Value	2.4100	5.3000	7.4000	1400.0000	67.0000	7.6000	41.0000
# data points	89	43	118	118	17	118	88
PEQ	2.166	5.755	6.218	931.966	96.438	7.220	36.900
Reasonable Potential							
PEQ>Daily max	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	TRUE
PEQ>Monthly Ave.	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	TRUE
PEQ> FAV	FALSE	FALSE	FALSE	TRUE	TRUE	FALSE	FALSE
Final Reasonable Potential	No	No	Yes	Yes	NO!	No	NO!

Notes

The unnamed stream is a class 2Bg, 3, 4A, 4B, 5, 6 water

The Mississippi River is a class 2Bg, 3, 4A, 4B, 5, 6 water

The unnamed stream ihas a 7Q10 of 0.0cfs

The Mississippi River 7Q10 = 2167 cfs

Max Design flow equals 6.5 mgd

The Mississippi River has aTMDL for PFOS and total mercury

No!-Chromium will need to be re-evaluated. The Chromium data is total chromium , not Cr 6.

No!- the cyanide data is based on total cyanide. The WQS is free CN. Monitoring for free or amendable CN will be needed

Phenol data is limited to non AAP method for phenol.

Phenol method ifor routine monitoring specifically did not include the

AAP method to measure phenol.

Routine monitoring was required for

T. cadmuim, T. antimony, T selenium, T. zinc, and phenol were routinely sampled

as part of the permit requirement for outfall SD001.

copper and nickel were re-done using DMR data

Zinc wasn't re-done with dMR data since it already had reasonable potential

Table 6. Reasonable Potential Results for 3M Cottage Grove (SD001).

Parameter	Chloroform (ug/L)	DEHP (ug/L)	Methylene Chloride (ug/L)	T. Ni (ug/L)	Pb (ug/L)	Phenol (ug/L)	T. Se (ug/L)
Plant flow ADW (mgd)	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Rec. water flow, 7Q10(mgd)	0	0	0	0	0	0	0
Background Conc.	0	0	0	0	0	0	0
Chronic Std (cs)	155.00	2.10	1940.00	388.08	12.34	123.00	5.00
290							
ppm hard							
Maximum Std (ms)	1392.00	210.00	13875.00	3490.92	316.64	2214.00	20.00
290							
ppm hard							
Final Acute Value (FAV)	2784	420	27749	3491	635	4428	40
290							
ppm hard							
Mass Balance -cs	155.00	2.10	1940.00	388.08	12.34	123.00	5.00
Mass Balance -ms	1392.00	210.00	13875.00	3490.92	316.64	2214.00	20.00
Coeff of Variation (CV)	0.60000	0.60000	0.60000	0.60000	0.60000	0.60000	0.60000
Long Term Avg-cs	81.75	1.64	1513.77	204.68	6.51	64.87	2.64
Long Term Avg-ms	446.96	67.43	4455.20	1120.92	101.67	710.91	6.42
Preliminary Effl limits:							
Daily Max	254.60	5.10	4714.40	637.46	20.27	202.04	8.21
Monthly Ave (2x/month)	146.96	2.95	2721.28	367.96	11.70	116.62	4.74
Max Measured Value	3.0100	57.2000	20.7000	55.0000	3.8000	2.6000	30.0000
# data points	89	88	89	118	117	88	118
PEQ	2.706	51.559	18.607	52.250	3.199	2.344	25.207
Reasonable Potential							
PEQ>Daily max	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE
PEQ>Monthly Ave.	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	TRUE
PEQ> FAV	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
Final Reasonable Potential	No	Yes	No	No	No	No	Yes

Notes

The unnamed stream is a class 2Bg, 3, 4A, 4B, 5, 6 water

The Mississippi River is a class 2Bg, 3, 4A, 4B, 5, 6 water

The unnamed stream ihas a 7Q10 of 0.0cfs

The Mississippi River 7Q10 = 2167 cfs

Max Design flow equals 6.5 mgd

The Mississippi River has aTMDL for PFOS and total mercury

No!-Chromium will need to be re-evaluated. The Chromium data is total chromium , not Cr 6.

No!- the cyanide data is based on total cyanide. The WQS is free CN. Monitoring for free or amendable CN will be needed

Phenol data is limited to non AAP method for phenol.

Phenol method ifor routine monitoring specifically did not include the

AAP method to measure phenol.

Routine monitoring was required for

T. cadmuim, T. antimony, T selenium, T. zinc, and phenol were routinely sampled

as part of the permit requirement for outfall SD001.

copper and nickel were re-done using DMR data

Zinc wasn't re-done with dMR data since it already had reasonable potential

Table 6. Reasonable Potential Results for 3M Cottage Grove (SD001).

Parameter	Toluene (ug/L)	Zn (ug/L)	Hg (ng/L)	PFBA (ng/L)	PFBS (ng/L)	PFHxA (ng/L)	PFHxS (ng/L)	PFOA (ng/L)	PFOS (ng/L)
Plant flow ADW (mgd)	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Rec. water flow, 7Q10(mgd)	0	0	0	0	2040	0	0	0	0
Background Conc.	0	0	6.9	13.5	0	15.63333	0	19.358	0.05
Chronic Std (cs)	253.00	261.28	6.90	25000.00	3000.00	4400.00	0.0023	0.0092	0.027
290									
ppm hard									
Maximum Std (ms)	1352.00	288.44	2400.00	NA	NA	NA	NA	NA	NA
290									
ppm hard									
Final Acute Value (FAV)	2703	577	4900	NA	NA	NA	NA	NA	NA
290									
ppm hard									
Mass Balance -cs	253.00	261.28	6.90	25000.00	3000.00	4400.00	0.00	0.01	0.03
Mass Balance -ms	1352.00	288.44	2400.00	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
Coeff of Variation (CV)	0.60000	0.60000	0.60000	0.60000	0.60000	0.60000	0.60000	0.60000	0.60000
Long Term Avg-cs	133.44	137.81	5.38	19507.37	2340.88	3433.30	0.00	0.01	0.02
Long Term Avg-ms	434.12	92.62	770.63	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!	#VALUE!
Preliminary Effl limits:									
Daily Max	415.57	288.44	16.77	60752.55	7290.31	10692.45	0.01	0.02	0.06561
Monthly Ave (2x/month)	239.88	166.50	9.68	35068.08	4208.17	6171.98	0.00	0.01	0.03787
Max Measured Value	2.3000	247.0000	120.0000	498000.0000	39400.0000	1740.0000	1540.0000	1930.0000	1410.0000
# data points	88	102	45	105	106	78	80	78	79
PEQ	2.067	214.823	128.687	498000.000	39400.000	1740.000	1540.000	1930.000	1410.000
Reasonable Potential									
PEQ>Daily max	FALSE	FALSE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE
PEQ>Monthly Ave.	FALSE	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE	TRUE	TRUE
PEQ> FAV	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
Final Reasonable Potential	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes

Notes

The unnamed stream is a class 2Bg, 3, 4A, 4B, 5, 6 water

The Mississippi River is a class 2Bg, 3, 4A, 4B, 5, 6 water

The unnamed stream ihas a 7Q10 of 0.0cfs

The Mississippi River 7Q10 = 2167 cfs

Max Design flow equals 6.5 mgd

The Mississippi River has aTMDL for PFOS and total mercury

No!-Chromium will need to be re-evaluated. The Chromium data is total chromium , not Cr 6.

No!- the cyanide data is based on total cyanide. The WQS is free CN. Monitoring for free or amendable CN will be needed

Phenol data is limited to non AAP method for phenol.

Phenol method ifor routine monitoring specifically did not include the

AAP method to measure phenol.

Routine monitoring was required for

T. cadmuim, T. antimony, T selenium, T. zinc, and phenol were routinely sampled

as part of the permit requirement for outfall SD001.

copper and nickel were re-done using DMR data

Zinc wasn't re-done with dMR data since it already had reasonable potential

The existing WQBEL limits for total copper, total nickel and total zinc were re-examined

These three WQBELs were reexamined by MPCA staff. At the time, they used best professional judgement and were based largely on FAV or acute LC50's. since the unnamed stream is now being protected, the acute LC50's and the best professional judgments for acute toxicity no longer apply. The chronic WQS for each of these three metals now drive any potential RP to protect the unnamed stream. As shown below, only total zinc illustrated RP to need a WQBEL.

Reasonable Potential Conclusions for total cadmium
Reasonable potential to cause or contribute to the excursion above a water quality standard has been indicated for total cadmium. The effluent limits were derived from water quality standards pursuant to 40 CFR 122.44 (d)(1)(vii)(A). The calculation of WQBELs are as follows:

Daily Max = 4.3 ug/L

Monthly Ave. = 2.5 ug/L (based on sampling 2x/month).

Reasonable Potential Conclusions for total antimony
Reasonable potential to cause or contribute to the excursion above a water quality standard has been indicated for total antimony. The effluent limits were derived from water quality standards pursuant to 40 CFR 122.44 (d)(1)(vii)(A). The calculation of WQBELs are as follows:

Daily Max = 53.5 ug/L

Monthly Ave. = 20 ug/L (based on sampling 2x/month).

Reasonable Potential Conclusions for DEHP
Reasonable potential to cause or contribute to the excursion above a water quality standard has been indicated for DEHP. The effluent limits were derived from water quality standards pursuant to 40 CFR 122.44 (d)(1)(vii)(A). The calculation of WQBELs are as follows:

Daily Max = 5.10 ug/L

Monthly Ave. = 3 ug/L (based on sampling 2x/month).

Reasonable Potential Conclusions for total selenium
Reasonable potential to cause or contribute to the excursion above a water quality standard has been indicated for total selenium. The effluent limits were derived from water quality standards pursuant to 40 CFR 122.44 (d)(1)(vii)(A). The calculation of WQBELs are as follows:

Daily Max = 8.2 ug/L

Monthly Ave. = 4.7 ug/L (based on sampling 2x/month).

Reasonable Potential Conclusions for total zinc

Reasonable potential to cause or contribute to the excursion above a water quality standard has been indicated for total zinc for the Class 2B Minnesota WQS . The effluent limits were derived from water quality standards pursuant to 40 CFR 122.44 (d)(1)(vii)(A). The calculation of WQBELs are as follows:

Daily Max = 288 ug/L

Monthly Ave. = 167 ug/L (based on sampling 2x/month).

Reasonable Potential Conclusions for total mercury

Monitoring results of the effluent include 45 data points at a calculated a default CV of 0.6. The default statistics were used because several of the mercury data points were below the reporting level. Projected effluent quality (PEQ) is derived as an upper bound value from the highest value measured (120 ng/l), and the determined variability (CV = 0.6) and number of data points (45). The preliminary effluent limit (PEL) calculation assumes that the background mercury concentration is at the water quality standard (6.9 ng/l) when no local river water column analytical data exist. To assure that the discharge does not cause or contribute to a water quality standards excursion for mercury impaired waters, the numeric water quality standard (6.9) is applied at the point of discharge for the mass balance equation for the subsequent preliminary effluent limit calculations. Where PEQ exceeds the PEL, there is reasonable potential to cause or contribute to a water quality standards excursion. Since PEQ exceeds the PEL in this case, reasonable potential to cause or contribute to an excursion above water quality standards is indicated. A water quality-based effluent limit (WQBEL) is needed. Reasonable potential to cause or contribute to the excursion above a water quality standard has been indicated for total mercury. The effluent limits were derived from water quality standards pursuant to 40 CFR 122.44 (d)(1)(vii)(A). The calculation of WQBELs are as follows:

Daily Max = 16.8 ng/L

Monthly Ave. = 9.7 ng/L (based on sampling 2x/month).

Monitoring for Non-PFAS Chemicals

Hexavalent Chromium

A reasonable potential analysis for hexavalent chromium was not able to be performed because total chromium was analyzed not hexavalent chromium. The federal requirements in the priority pollutant scan require chromium to be sampled as total chromium. However, the class 2B WQS for chromium is hexavalent chromium. When this facility performs priority pollutant scans for this outfall, they will sample for hexavalent chromium as well as total chromium. This will provide data that matches the hexavalent chromium WQS. The reporting limit for hexavalent chromium will be 11 ug/L.

Cyanide Sampling

A reasonable potential analysis for cyanide was not able to be performed because total cyanide was analyzed not free cyanide. The federal requirements in the priority pollutant scan require cyanide to be sampled as total cyanide. However, the class 2B WQS for cyanide is free cyanide.

This facility will need to monitor for total cyanide and free cyanide (or amendable cyanide method since free cyanide chemistry is rarely available. The reporting limits for total cyanide and amendable cyanide as close to the chronic WQS of 5.2 ug/L as possible.

Total Lithium

Because of the lithium salts associated with the PFAS in the effluent, this outfall will monitor quarterly for total lithium. Please use Standard Method 3111 B with a reporting limit of 2 ug/L.

Reporting Limits for Metals

The reporting limits for total cadmium, total lead, total copper, total nickel, total zinc, and total antimony will be no greater than 5 ug/L.

Salty Monitoring

The permittee has not reported any data for salty parameters such as total dissolved solids, chloride, sulfate or specific conductance. Due to the low stream dilution ratio, this outfall must sample quarterly for the following salty parameters in Table 7.

Table 7. Salty Parameter Monitoring.

Total Chloride (mg/L)
Total Dissolved Salts (meas. as Total Dissolved Solids) (mg/L)
Total Sulfate (mg/L)
Specific Conductivity (in umhos/cm) and
Total Hardness (Mg +Ca as CaCO3 in mg/L).

Approved Additives

Illustrated below in Table 8 are the additives which are approved for use for the entire 3M Cottage Grove facility.

Table 8. Approved additives for 3M Cottage Grove

Table 6. Approved Additives for 3M Cottage Grove.			
Additive Brand Name	Location-Notes	Max Dose	Max Units
Evonik TMT 15	Evonik TMT 15 additive -	16.72	gal/day
Nalco 3D Trasar 3DT401	3M Chemolite Cottage Grove	6.4	gal/day
Water Safe	well additive- 1 time use for 3-4 weeks	185	gal/day
BoreSaver IKL Pro	well additive- 1 time use for 3-4 weeks	20	gal/day
Muric acid	well additive- 1 time use for 3-4 weeks	185	gal/day
NW-310	NW-310 is a polymer . 1 time use for 3-4 weeks	9	gal/day
Nalco Rustphree 73924	3M Chemolite Cottage Grove	100	gal/day
Nalco 9005 microbiocide	Nalco 9005 microbiocide, 2x/week	5	gal/day
MEM-3900	is a pre approved type addiive	9.37	gal/day
sodium hydroxide 50% diaphragm	System B , Location UF- pre approved chemical-	408	gal/year
Azone 15	System B , Location Feed- pre approved chemical-bleach	45,168	gal/year
Citric Acid 50% FG	System B , Location UF- pre approved chemical-	439	gal/year
Sodium Bisulfite (SBS)	System B , Location UF- pre approved chemical-	972	gal/year
Azone 15	System C , Location Feed- pre approved chemical-Bleach	11,552	gal/year
Nalco PP01-3911	3M Cottage Grove, June 2020 updated defoamer request. Increased usage	4.9	gal/day
Sodium bisulfite (SBS)	System B , Location RO- pre approved chemical-	8424	gal/year
Azone 15	System A , Location Feed- pre approved chemical- bleach	74,977	gal/year
Kemira PIX-312	System A , Location Feed- pre approved chemical -ferric sulfate	3566	gal/year
Azone 15	System A , Location UF- pre approved chemical -bleach	11,193	gal/year
Kemira PIX-312	System C , Location Feed- pre approved chemical- ferric sulfate	4187	gal/year
Sulfuric Acid 66'	System B , Location Feed- pre approved chemical	18,091	gal/year
MEM-2930	is a pre approved type addiive	9.86	gal/day
Kemira PIX-312	System B , Location Feed- pre approved chemical-Ferric sulfate	27,124	gal/year
Evoqua Alumafloc 1	Alumafloc 1	0.67	gal/day
Azone 15	System B , Location UF- pre approved chemical-bleach	4210	gal/year
Nalco PP01 3911	3M Cottage Grove----- Nalco PP01 3911	1.47	gal/day
Sodium Bisulfite (SBS)	System A , Location UF- pre approved chemical	2583	gal/year
Chemtreat CL-5643	part of the permit renewal process	0.45	gal/day
Sodium Bisulfite (SBS)	System C , Location UF- pre approved chemical-	715	gal/year
Citric acid 50% FG	System C , Location UF- pre approved chemical-	715	gal/year

Please see the link below for the latest updated additive list:

Effluent Limits - Additive list: Chemical additives - Tableau Server (state.mn.us)

River monitoring associated with the Remediation activities

Any river monitoring of fish, water, or sediment associated with any remedial activities should also be submitted with the NPDES reporting requirements.

Priority Pollutants: **The permittee must send in the entire priority pollutant report, including the QC section each time the priority pollutant scan is performed. The permittee must send four priority pollutant scans each year for the life of the permit. DEHP sampling cannot encounter any kind of plastic, especially soft plastic. Plastic commonly leaches out DEHP and thereby contaminant the sampling. If the 24-hr. composite sampler has any kind of plastic or plastic tubing, then DEHP sampling must be taken as a grab sample using non-plastic material.**

Interim Limits

On March 26th, 2024 the discharger requested a compliance schedule for the parameters in the table below. The following interim limits are recommended to be included during the duration of the compliance schedule.

Table 9. Recommended interim limits for SD001 to be applicable during the duration of the compliance schedule.

Compound	Value	Interim Limit Type	Unit	Method
PFBA	288125	Monthly Max	ng/L	99th percentile value of reported data assuming 2 samples per month
PFBS	20782	Monthly Max	ng/L	99th percentile value of reported data assuming 2 samples per month
PFHxA	1720	Monthly Max	ng/L	99th percentile value of reported data assuming 2 samples per month
PFHxS	1615	Monthly Max	ng/L	99th percentile value of reported data assuming 2 samples per month
PFOA	1798	Monthly Max	ng/L	99th percentile value of reported data assuming 2 samples per month
PFOS	14	Monthly Max	ng/L	Jan 21, 2021 non-public enforcement action
PFOS	7	Monthly Average	ng/L	Jan 21, 2021 non-public enforcement action
Antimony	1044	Monthly Max	ug/L	99th percentile value of reported data assuming 2 samples per month
DEHP	73.1	Monthly Max	ug/L	99th percentile value of reported data assuming 2 samples per month
Mercury	11.8	Monthly Max	ng/L	99th percentile value of reported data assuming 2 samples per month
Selenium	29.6	Monthly Max	ug/L	99th percentile value of reported data assuming 2 samples per month
Cadmium	11.8	Monthly Max	ug/L	99th percentile value of reported data assuming 2 samples per month

References

1991. Guidance Manual For the Preparation of NPDES Permit Applications For Storm-water Discharges Associated With Industrial Activity. EPA-505/8-**91**-002.

2021. PFAS treatability Alternatives Identification Plan. 3M Cottage Grove Facility. Submitted to the MPCA.

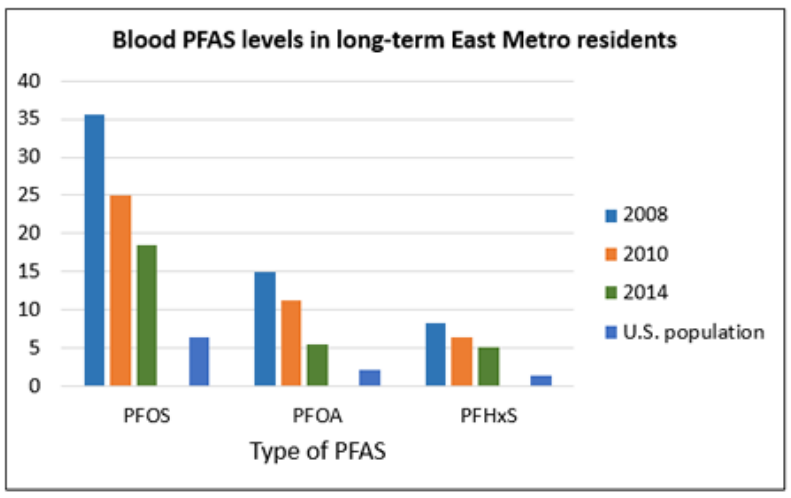
DEPARTMENT OF HEALTH

PFAS Biomonitoring in the East Metro

Per- and Polyfluoroalkyl substances (PFAS) are also referred to as perfluorochemicals (PFCs).

Per- and polyfluoroalkyl substances or PFAS are chemicals that have been used for many years to make products that resist stains, grease, water and heat. In the early 2000s, some drinking water sources in the East Metro – a suburban area east of St. Paul– were found to be polluted with PFAS. A public health intervention in 2006, including installing filtration systems for polluted public and private wells, reduced PFCs in drinking water.

Since 2008, MDH has done three projects testing blood levels of PFAS in people who live in the East Metro as directed by the Minnesota Legislature. The most recent results are from MDH's third PFAS biomonitoring project, which tested blood levels of PFAS in over 300 residents of Oakdale, Lake Elmo and Cottage Grove, Minnesota in 2014.



Results from these three studies found:

- PFAS blood levels are going down in long-term residents who were exposed to PFAS in drinking water before the public health interventions in 2006 (see graph above). Blood levels in these residents are still higher than in the background U.S. population.
- PFAS levels in newer residents are similar to levels seen elsewhere in the U.S. These people moved to Oakdale after the intervention.

For more information

- Report to the Community 2015, (PDF) [LINK http://www.health.state.mn.us/communities/environment/biomonitoring/docs/pfc2015communityreport.pdf] with results from third biomonitoring project
- Report to the Community 2011, (PDF) [LINK http://www.health.state.mn.us/communities/environment/biomonitoring/docs/pfccommunitypresent2011.pdf] with results from second biomonitoring project: results from blood testing (PDF) [LINK http://www.health.state.mn.us/communities/environment/biomonitoring/docs/pfcfollowuprpt2011.pdf] and survey responses 2013, (PDF) [LINK http://www.health.state.mn.us/communities/environment/biomonitoring/docs/communityreportmay2013.pdf]
- Reports to the Community 2009, (PDF) [LINK http://www.health.state.mn.us/communities/environment/biomonitoring/docs/pfccomrpttocomm2009.pdf] with results from first biomonitoring project: results from blood testing and blood v. water levels 2010, (PDF) [LINK http://www.health.state.mn.us/communities/environment/biomonitoring/docs/pfcwatertoblood.pdf] .
- Updated report on cancer incidence in Dakota and Washington Counties 2015, (PDF)
- More on PFAS in Minnesota
  - Per- and Polyfluoroalkyl Substances (PFAS) - Learn more [LINK http://www.health.state.mn.us/communities/environment/hazardous/topics/pfcs.html]
  - Cancer incidence in Dakota and Washington Counties, 2015 (PDF) [LINK http://www.health.state.mn.us/data/mcrs/docs/rptuwashdakota.pdf]

These results have also been published in a scientific journal. The full article requires a subscription to read – contact us for access: health.biomonitoring@state.mn.us or 651-201-5900.

Related links

About Biomonitoring [LINK http://www.health.state.mn.us/communities/environment/biomonitoring/about/index.html]

Biomonitoring Reports [LINK http://www.health.state.mn.us/communities/environment/biomonitoring/reports/index.html]

Minnesota Environmental Public Health Tracking [LINK http://www.health.state.mn.us/communities/environment/tracking/index.html]

Last Updated: 07/30/2024

DEPARTMENT OF HEALTH

Update March 2024

Waterbody Specific Safe-Eating Guidelines—Mississippi River Pools 2, 3, and 4, including all of the Minnesota lakes and backwaters

MDH is issuing updated fish consumption guidance for Mississippi River Pools 2, 3, and 4 and all of the Minnesota lakes and backwaters. This area includes Ford Dam Parkway in Saint Paul to Wabasha, including Lake Rebecca. Cities associated with these pools include Saint Paul, Saint Paul Park, Inver Grove Heights, Hastings, Red Wing, Lake City, and Wabasha.

This updated fish consumption guidance uses more stringent waterbody-specific guidelines and data for PFAS in fish and provides additional protections for fish consumers. While this guidance primarily looks at PFAS data, it is protective for other contaminants (mercury, PCBs).

MDH recommends the following guidance for all fish species in Mississippi River Pools 2-4 and all of the Minnesota lakes and backwaters:

MDH recommends not eating fish obtained from Mississippi River Pools 2-4 for sensitive populations, including people who are or may become pregnant, people who are breastfeeding or plan to breastfeed, and children under age 15.

MDH recommends limiting fish consumption from Mississippi River Pools 2-4 to one serving a month for the general population of people not planning to become pregnant, men and boys over age 15.

We continue to learn more about PFAS and update our fish consumption guidance when needed. As we learn more about PFAS and gather more data on PFAS in fish, Minnesota fish consumption guidance will continue to be updated.

More information on reducing exposures to PFAS can be found here:
Reducing Exposures: Per- and Polyfluoroalkyl substances (PFAS) (PDF) [LINK http://www.health.state.mn.us/communities/environment/hazardous/docs/pfas/pfasreducingexp.pdf]

More information on PFAS and health can be found here:
PFAS and Health [LINK http://www.health.state.mn.us/communities/environment/hazardous/topics/pfashealth.html]

More information about Mississippi River Pools 2, 3, and 4 and can be found here:

U.S. Lock & Dam #2 Pool (19000500) [LINK https://www.dnr.state.mn.us/lakefind/lake.html?id=19000500]
These updated guidelines for the sensitive and general populations also apply to Lake Rebecca in Hastings and other backwaters of Pool 2.

U.S. Lock & Dam #3 Pool (25001700 [LINK https://www.dnr.state.mn.us/lakefind/lake.html?id=25001700])

U.S. Lock & Dam #4 Pool (79000500) [LINK https://www.dnr.state.mn.us/lakefind/lake.html?id=79000500]

Update July 2023 for waterbody specific safe-eating guidelines - Lus Hmoob / Español

Expand All

Lus Hmoob: Qee leej neeg yuav tsum tsis txhob noj cov ntses ntawm tus dej no.

Xeev Minnesota tau muaj kev ntxiv ntaub ntawv tshiab rau cov lus taw qhia kev noj ntses los mus taw qhia tias qee leej neeg tsis tsim nyog noj cov ntses uas nyob hauv ob tug dej ntawm cheeb tsam Twin Cities. Ob tug dej no yog Mississippi River txij ntawm yav dej tauv Ford Dam hauv St Paul mus txog rau yav dej tauv Hastings Dam (muaj ib lub npe hu ua Pool 2 thiab) thiab lub pas dej Rebecca Lake nyob ze rau Hastings. Cov tib neeg uas tsis tsim nyog noj cov ntses ntawm cov cheeb tsam no yog xws li tej menyuam yaus uas hnub nyoog yaus dua 15 xyoos, cov neeg lub cev tab tom xeeb menyuam lossis npaj yuav xeeb menyuam, thiab cov neeg uas tab tom pub niam mis rau menyuam los yog npaj yuav pub niam mis rau menyuam.

Español: Algunas personas no deben comer pescado de esta masa de agua.

El Estado de Minnesota ha actualizado la guía de consumo de pescado para recomendar que ciertas personas no coman pescado de dos cuerpos de agua en el área metropolitana de las Ciudades Gemelas (Twin Cities). Esos cuerpos de agua son el Río Mississippi - desde la represa llamada Ford Dam en St. Paul hasta la represa llamada Hastings Dam (conocida como Pool 2) - y el Lago Rebecca cerca de Hastings. Las personas que deben evitar comer pescado de estos lugares incluyen: niños menores de 15 años, personas que están embarazadas o podrían quedar embarazadas y aquellas personas que están amamantando o planean amamantar.

Last Updated: 07/30/2024

Minnesota Center for Environmental Advocacy
26 East Exchange Street • Suite 206 • Saint Paul, MN 55101-1667 • 651.223.5969

February 3, 2011

Scott Knowles
MPCA – Duluth Office
Municipal Division
525 Lake Avenue South, Suite 400
Duluth, Minnesota 55802

Re: Draft NPDES Permit No. MN0001449, 3M Cottage Grove Center
Comments of Minnesota Center for Environmental Advocacy

Thank you for the opportunity to submit these comments on behalf of the Minnesota Center for Environmental Advocacy on the draft permit for the 3M Cottage Grove Center facility.

MCEA is a Minnesota non-profit environmental organization whose mission is to use law, science and research to preserve and protect Minnesota's wildlife, natural resources and the health of its people. MCEA has statewide membership. MCEA has been concerned about impacts on Minnesota's waters from wastewater discharges for a number of years, has made wastewater pollution a significant component of its work, and has participated in a number of related policy and legal matters.

MCEA objects to issuance of the 3M Cottage Grove Center permit because it does not state a deadline to meet the water quality-based effluent limit calculated to achieve water quality standards for Perfluorooctane Sulfanate (PFOS). The draft permit authorizes the facility to continue discharging PFOS, a type of PCB, at current rates into an unnamed creek (07010206-517) flowing into the Minnesota River (07010206-502). The receiving stretch of the Mississippi River was listed on the MPCA's 303(d) list of impaired waters as impaired for aquatic consumption by PCBs in fish tissue in 1998. No TMDL has been completed for the receiving water.

The compliance schedule in the permit provides a lengthy timeline for facility planning, but no date by which the WQBEL must be met. Under EPA guidance, "Any compliance schedule contained in an NPDES permit must include an enforceable final effluent limitation and a date for its achievement."[1] The draft permit contains the WQBEL of 7 ng/L without a date to achieve that limit. Instead, the permit sets out the requirement to "commence work" to achieve the WQBEL through the compliance schedule "as soon as possible."

[1] James Hanlon, "Compliance Schedules for Water Quality-based Effluent Limits in NPDES Permits," May 10, 2007, at 2.

The fact sheet also states that the permit contains interim limits for SD001 and SD002.[2] The permit does not provide these limits; it contains only a mass limit for the combined discharges at point SD003.[3]

MCEA urges the MPCA to provide a date in the permit by which the water quality-based effluent limit for PFOS that would protect the Mississippi River will be met, as the federal regulations require. Please feel free to contact us should you have any questions with respect to MCEA's comments. MCEA looks forward to working with MPCA to achieve a sound decision in this matter. Thank you for the opportunity to comment.

Sincerely,

/s/ Kris Sigford

Kris Sigford
Water Quality Director

/s/ Mike Schmidt

Mike Schmidt
Water Quality Associate

[2] Fact Sheet at 22, 24.
[3] Permit at 23.

To: Minnesota Center for Environmental Advocacy (MCEA)
From: Courtney Ahlers-Nelson, Industrial Division Director, Minnesota Pollution Control Agency (MPCA)
Date: 07/19/2024; 7/29/2024

RE: Questions on the Draft NPDES/SDS Permit Reissuance for the 3M Cottage Grove Center

1. Table 7 established PFAS discharge limits only for PFBS, PFBA, PFHxS, PFHxA, PFOS, and PFOA. Why are these the only PFAS with discharge limits? How were the limits derived?

Response: The PFAS limits were derived to protect the six Mississippi river PFAS site-specific water quality criteria (SSC). No dilution was allowed when calculating the PFAS limits because there was evidence that 1) the discharge was increasing PFAS concentrations in Unnamed Creek 2) there was evidence that PFAS concentrations were elevated at the confluence of unnamed creek and the Mississippi River and 3) the SSC apply not as an average concentration across the entire waterbody but as a not to be exceed value anywhere in the waterbody. MPCA used the standard statistical Reasonable Potential (RP) calculation recommended by U.S. Environmental Protection Agency (EPA) to calculate the PFAS limits; this statistical process is the same as used for the heavy metals and other pollutants. This involved evaluating past discharge concentrations, future PFAS treatment performance and analyzing the variability of the PFAS concentrations over time. All of the detailed math is in the fact sheet and is also available upon request.

The Water Quality Based Effluent Limits (WQBELs) for PFOS, PFOA and PFHxS were so low that they were below the reporting level of current analytical capabilities. Since these limits were so low that they cannot be reliably measured, the MPCA established compliance limits that reflects current analytical capabilities. In order to comply with their compliance limits for PFOS, PFOA and PFHxS, 3M cannot have a reportable PFAS value above 2 ng/L.

2. Given the presence of HFPO-DA and PFNA compounds in the DMR reports for the various discharge locations, as well as the recently finalized federal MCLs for these two compounds, why are there no numeric limits for these compounds in the permit? While we don't have site specific WQ criteria for these compounds, we do have narrative WQ standards and the federal MCLs for these compounds have, as of June 25, been adopted as statewide Class 1 WQS.

Response: The data to develop site-specific water quality criteria were not as robust for these two chemicals. To calculate a bioaccumulation factor (BAF), both water and fish tissue data need to be available in sufficient amounts. For these two chemicals, they were detected infrequently in surface water and/or fish tissue, making calculation of a BAF statistically challenging, due to being unable to determine the accurate quantity of the PFAS in the sample. For example, HFPO-DA was only detected in 5% of fish tissue samples, and 13% of surface water samples. Determining an accurate amount of HFPO-DA in those samples would be challenging when 95% of fish samples are unquantifiable (less than reporting limit). Having sufficient paired data for water and fish tissue is imperative to calculating a BAF, which is needed to calculate a SSC. While the chemical may actually be present in more samples than that, at concentrations lower than the reporting limit, we are limited by the data we have, and our ability to be able to calculate reliable values.

The new federal PFAS MCLs are applicable in Minnesota, yes, but only on Class 1 waters. This is not a Class 1 water, so applying them in this case is not appropriate. The narrative standard would need to be applied using some sort of narrative translator, that would translate the narrative standard into a numeric value to assess the discharge against. Because this is a Class 2 water, the translator would need to utilize Class 2 methodology, which, as discussed above, is challenging, due to the high number of non-detect values obtained for these two chemicals.

We also considered the fact that the treatment to remove the longer chain PFAS like PFOS and PFOA will also remove PFNA, so in setting limits for those, PFNA would also be reduced, even though we could not develop a reliable SSC.

3. Can you explain why the permit writers did not find a reasonable potential for PFHxA to cause or contribute to an exceedance of the site specific WQ criteria for this compound in Pool 2?

Response: Both SD001 and SD002 have Reasonable Potential for PFHxA using the pool-2 SSC and have numeric PFHxA water quality based effluent limitations (WQBELs) included in the permit.

4. Why are the site-specific water quality criteria for Class 2B waters immediately upstream of Pool 2 significantly lower for PFBS, PFBA, and PFHxA? For example, from the Ford Dam to Mississippi River Mile 820 the site specific WQ criteria for PFBS is a 30-day average of 350 ng/L, but in Pool 2 where the Cottage Grove facility discharges it is a 30-day average of 3,000 ng/L for Class 2B waters.

Response: The site-specific water quality criteria (SSC) for River Miles 820-812 were recently developed, using updated EPA toxicological values, and recently collected site data to develop site-specific bioaccumulation factors (BAFs). The SSC for Ford Dam to Mile 820 were previously developed and had previously included the Cottage Grove discharge area. MPCA utilized the most recent data to develop a SSC for the site because localized data were available. The SSC for the upstream portion uses a different dataset, which calculates higher BAFs, and thus lower SSC. The data for Miles 820-812 is more representative of the site, since it includes data collected there.

5. Given the strict numeric limits at the threshold of detection levels for PFOA, PFOS, and PFHxS, can you explain why the WQBEL limits are so much higher for PFBS and PFBA? Unlike legacy compounds of PFOA and PFOS, these shorter chain compounds are still in production at 3M and the instream PFAS characterization study and DMRs provide consistent evidence of discharges/contamination. What is the toxicological evidence that these are less toxic to humans and wildlife?

Response: The currently available Reference Doses (RfD) for PFBS and PFBA are significantly higher than those for PFOA, PFOS and PFHxS. The RfD is the toxicological value used in the calculation of the site-specific water quality criteria (SSC), which is used along with the bioaccumulation factor (BAF), and intake rates. The RfDs for PFBS and PFBA are 300 and 1000 ng/kg/d, respectively, compared to those for PFOA, PFOS and PFHxS, which are 0.03, 0.1, and 0.0002 ng/kg/d, respectively. The differences in these values greatly impact the calculated SSC. Additionally, for PFOS, the BAF at the Cottage Grove site is significantly higher than the other compounds, which also reduces the SSC. For PFOA, it is a carcinogen, and its cancer slope factor is very high, which drives down the calculation of the SSC for that chemical.

6. Why is there not PFBA discharge limit established for SD002?

Response: The PFBA concentrations at SD002 weren't high enough to demonstrate the reasonable potential to cause or contribute to an exceedance of the Pool 2 SSC for PFBA.

7. Does MPCA really anticipate that the multi-stage treatment train described in the permit will not be able to remove PFBS, PFBA, and PFHxA below 241.8, 861.6, and 354.5 kg/day, respectively? (The combined discharge limits for SD001 and SD002). For the 5-year permit period, these discharge limits would allow for the release of up to 441,285 kg of PFBS, 1,577,420 kg of PFBA (or higher, if there really isn't a discharge limit for SD002), and 646,960 kg of PFHxA.

Response: We believe MCEA may have exchanged grams/day for kg/day - please follow up with us to walk through the calculation.

8. What is the technological capacity of the current or planned filtration systems at the facility to remove shorter-chained PFAS compounds like HFPO-DA, PFNA, PFHxA, PFBS, and PFBA in comparison to legacy compounds like PFOS and PFOA?

Response: The technological capacity to remove HFPO-DA, PFNA, PFHxA, PFBS and PFBA is excellent. The MPCA expects that all of those compounds to be removed to less than single digit PPT to non-detect levels with the proposed treatment system.

9. On page 3 the draft permit notes that polymers will be added to the Phase 3 wastewater system, presumably to aid in flocculation and precipitation. What polymers will be used? If polyacrylamide or polydiallyldimethylammonium chloride (polyDADMAC), will there be any monitoring for the monomers of these polymers (acrylamide and DADMAC) in the discharge from this treatment train?

Response: A polyacrilamide polymer will be used to treat upstream of the PFAS treatment system as a coagulant. The MPCA is not recommending any monitoring of acrylamide monomers in the discharge because the treatment system will likely remove monomers to a high level.

10. On page 4 there are descriptions of the various ponds and basins in which wastewater is detained prior to treatment. Are monitoring wells located around these basins? If so, what are the monitoring requirements for those wells?

Response: There are a number of monitoring wells installed under the direction of the MPCA's Remediation program that are located near the ponds, including MW-108, MW-116, and MW-117 near Pond 1, MW-10 near Pond 2, and MW-15, MW-110, and MW-119 near Pond 3. While these wells were neither installed for nor designed to monitor leaks or seepage from the ponds as this is not common practice for industrial wastewater ponds, a number of the wells (MW-108, MW-110, MW-116, MW-117, and MW-119) are sampled on a quarterly basis for PFAS as part of the groundwater monitoring network required by the MPCA's Superfund program.

11. In Table 5, internal waste stream monitoring stations WS001 & WS002 (Bldg 151 just upstream of SD001 and SD002), WS005 (Bldg 185 lead vessel), WS006 & WS007 (Bldg 92 potable and non-potable lag), and several of the industrial stormwater sectors have intervention limits and response action requirements. Why are there no intervention limits and response action requirements for the other WS monitoring locations?

Response: These are the critical waste stream stations because they best capture the PFAS being treated and discharged.

12. Table 5 sets a quarterly monitoring frequency for priority pollutants at SD001 and SD002. How was this frequency determined? It does not appear to be specified in Minn. R. 7001. If it is not established in rule, does the MPCA have the authority to require more frequent monitoring, such as monthly or bi-monthly the first year of the permit before reducing the frequency to quarterly?

Response: For SD 002, priority pollutant scans were increased from 2 times per year to 4 times per year. SD 001 is the same as the last permit (4 times per year). This frequency of PP scans is the greatest of any discharger in the state. Also, in order to meet low-level PFAS limits, the treatment system will pass through three unit operations in series (RO, GAC, IX) that will also remove metals and organic contaminants to very low levels. There is a very low likelihood of high levels of any pollutant in the discharges and the proposed monitoring frequency will allow the MCPA to verify removal of PP pollutants with certainty and include any future limits as needed.

13. For monitoring stations where priority pollutant monitoring is required (SD001, SD002), Table 5 states: "Reporting limits for all priority pollutant analyses shall be *as close as analytically possible* to the Class 2B chronic water quality standards" [emphasis added]. How will this requirement be evaluated? Who determines what is "analytically possible"?

Response: This is a function of both the analytic method and the sample matrix. Wastewater and stormwater complex matrices and there can be interferences effects from some of the contaminants, suspended sediments can affect the ability to measure, there might be dilution required depending on the concentrations, and others.

14. Table 5, section 5.68.62 (regarding PFOS, PFOA, and PFHxS) final effluent limitations states the limits must be met by 12/31/2026, unless the Permittee requests a modification of the compliance schedule or other appropriate provisions of the permit if the Permittee determines that the limits are not consistently attainable with the advanced wastewater treatment system. Does this mean the Permittee can seek more time to meet the effluent limitations (presumably by expanding the treatment train), or could this also mean the Permittee could seek (and receive) permission to discharge PFOS, PFOA, and/or PFHxS at concentrations or volumes higher than the final effluent limitations?

Response: Yes, after permit issuance all permittees are always allowed to request more time to comply with effluent limits in a compliance schedule or to request a higher limit.

In order to extend the length of a compliance schedule or relax an effluent limitation, the permit would have to be public noticed using a major modification. The Clean Water Act and Minnesota Rules do not allow for a "easy" relaxing of compliance schedule date or effluent limitations. In order to justify such relaxation of permit conditions, an exceptionally strong legal argument would need to be made to the MPCA and EPA.

15. Table 5, section 5.69.76 (related to PFAS monitoring) says that "process control sampling" does not have to meet the reporting limits required for other samples. What is the reason for that?

Response: It is a standard practice for all wastewater permittees to perform their own internal sampling for process control reasons. This allows the permittee to optimize their treatment system and monitor removal efficiencies with less lag time and greater data resolution. Typically, internal process sampling prioritizes getting data quickly over absolute accuracy and this allows the permittee to diagnose treatment performance in closer to real time. Internal process sampling is always supplemental to required permit sampling.

3M is planning to perform their own internal process sampling that is outside of required sampling in the permit. This sampling will allow 3M to get PFAS data back within days (3M internal lab) versus months (external lab). Without timely data on PFAS removal efficiencies, 3M simply cannot operate their proposed system. MPCA has the authority to review all internal process data upon request and collecting this internal data does not replace requirements to sample as required in the permit.

16. Table 5, section 5.69.76 (related to PFAS monitoring) says that non-targeted PFAS analysis will be conducted at least once during the 5-year period of the permit. Why is this not required at the very beginning of the permit period so the results may be used to determine the location and frequency of any needed future sampling?

Response: Pursuant to the ongoing non-public investigation subject to Minn. Stat. 13.39, MPCA cannot disclose the reason as to why the NTA analysis is not required at the beginning of the permit cycle.

17. Table 5, section 5.69.68 establishes what appear to be rather long maximum timelines for simply assessing underground pipeline integrity. Three years to simply assess “high priority” pipelines seems very long. How were these maximum timeframes established?

Response: These timelines were established in consideration of multiple factors, including but not limited to:

- A desire to get the pipelines assessed as timely as reasonably possible.
- Operational constraints (e.g. safety, planned shutdown events at the facility vs. facility operational with flows in pipes).
- Existing televising performed prior to the issuance of this permit.

18. Table 5, section 5.69.68 also sets a timeline of 1 year to restore the integrity of any pipeline that is found to be leaking, but there does not appear to be any requirement to investigate the magnitude and extent of any release that may have occurred, and if necessary undertake remedial actions. Is it just assumed that such an investigation/response will occur (based on “Discovery of a Release” requirements in section 5.79.403), or should this be stated here?

Response: Section 5.79.403 applies to all types of releases including any potential releases from pipes. Minnesota Rule 7060.0600 Subp. 2 also applies to releases of this nature. Section 5.79.403 discusses the duty to notify as required by Minn. Stat. 115.061. The investigation/response will be required based on the duty officer notification and the follow-up by the appropriate program, which could require those remedial actions under either the compliance and enforcement programs or as part of the on-going Superfund work. That chain of events is a standard part of the duty to notify process. Section 6.60.25 requires that an annual

underground piping report be submitted that summarizes the actions taken responsive to the Underground Piping Integrity Plan.

19. Table 5, section 5.69.90 requires a work plan regarding instream PFAS characterization of "surface water, sediments, and fish tissue". Past sampling has shown high concentrations of PFAS in pore-water in the nearshore zone of the river adjacent to the 3M property and it is critical information that needs to be considered in evaluating the overall discharge of the 3M facility/property to the river. Was pore water sampling meant to be included in this work plan? If not, why not?

Response: Yes, sediment pore water is meant to be included in the work plan.

20. Table 5, section 5.69.111 lists treatment performance standards for 8 PFAS. How were those specific PFAS selected?
Response: MPCA selected the list of 8 PFAS based on reasons that are currently non-public due to an ongoing investigation.

21. Table 5, section 5.69.115 requires submission of any river monitoring of fish, water, or sediment related to remedial activities be submitted with the required NPDES reports. Does "water" refer only to surface water or also pore-water in the nearshore section of the river? (see question 18)

Response: water refers to surface water, surface water micro-layer and sediment pore water.

22. Table 5, section 5.79.392 states that results below the reporting limit (RL) will simply be reported as "<" the value of the RL. Shouldn't any results below the RL but above the Method Detection Limit (MDL) be reported, but flagged to indicate that they are estimated values?

Response: This permit requirement relates to how a value is reported on the DMRs. Reporting zeros or any other narrative is not allowed. Section 5.69.80 also establishes requirements for the reporting of data below reporting limits and this language is considered standard language amongst MPCA-issued wastewater permits.

23. Table 5, section 5.79.420 discusses how TMDL impacts may be factored into the permit requirements. Will TMDL evaluations include consideration of groundwater discharges of PFAS to the Mississippi river from the 3M property and from the groundwater plumes and contaminated surface waters (e.g. Raleigh Creek and Battle Creek) related to the known 3M waste disposal sites in Washington County? Will this also include consideration of the groundwater and surface water discharges of PFAS to the St. Croix River, which enters the Mississippi River downstream of the 3M facility?

Response: The MPCA has no PFAS TMDL anywhere in Minnesota. Limits, site-specific criteria, and permit conditions in this permit were developed in the absence of an approved final TMDL.

24. Table 7 establishes a long list of intervention limits for SD009 (Basin 3U overflow) – arsenic, BOD, cadmium, chromium, COD, cyanide, lead, ammonia, pH, selenium, silver, TSS, and zinc. Other basins (SD010 Basin 2AA-1, SD011 Basin AD, SD012 Basin 3Z, SD025 Basin 1E) have significantly shorter lists of intervention limits. What is the reason for this difference and how were the intervention limit values determined?

Response: These stormwater stations are sector specific. The intervention limits vary depending on the specific sector. This is consistent with the ISW general permit. See page 128 of the fact sheet.

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October 2, 2023

VIA EMAIL

Karie Blomquist, P.E.
Remediation Senior Manager, Global EHS
3M Environment, Health, Safety and Product Stewardship
3M Center Building 225-1N-22
St. Paul, MN 55144

RE: Request for additional investigation by 3M near Hastings

Dear Karie Blomquist,

The Minnesota Pollution Control Agency (MPCA) has been investigating the occurrence and sources of per- and polyfluoroalkyl substance (PFAS) impacts observed in the municipal wells for the city of Hastings. Additional data collected recently in southern Washington and northern Dakota Counties indicate that releases from the 3M Company (3M) Cottage Grove facility (Facility) are likely contributing to the observed drinking water impacts in the Hastings municipal wells. The MPCA is requesting that 3M perform additional investigation in northern Dakota County to determine if the Cottage Grove Facility is impacting the Hasting's municipal wells or any other drinking water receptors in northern Dakota County.

The MPCA requested 3M to fully delineate the extent and magnitude of all contaminants in all media related to the Facility in a letter dated May 9, 2023, and develop a conceptual site model (CSM) documenting this information. As stated in the MPCA's May 9, 2023 letter, MPCA staff recognize that 3M has undertaken a significant number of environmental investigations at the Facility under the oversight of several regulatory programs. MPCA staff also recognize that 3M has operated at the Facility since the late 1940s where production facilities, waste disposal methods, and types of material being produced have changed throughout the years.

Additional data collected by 3M in and near the Mississippi River was recently submitted to the MPCA in the Instream PFAS Characterization Study Final Report, Mississippi River, Cottage Grove, Minnesota (ICS Report) dated June 2023. This submittal provides significant information related to the migration of contaminants from the Facility into the Mississippi River and potentially downstream. The data in the ICS Report, along with additional analytical data recently collected by the MPCA in the city of Hastings, provide an updated understanding of the distribution of PFAS in this area of Washington and Dakota Counties. The new analytical data combined with updated geologic mapping completed by the Minnesota Geological Survey indicate more significant interaction between the Mississippi River and bedrock aquifers on both banks of the river in this area than has previously been understood.

The results from the ICS Report showed that lithium bis-trifluoromethanesulfonimid (HQ-115) is being discharged from the Facility based on surface water samples collected from the Facility and the Mississippi River near and downstream of the Facility. MPCA's Site Assessment program recently completed sampling of the Hastings municipal wells that included analyzing for HQ-115. HQ-115 was detected in Hastings Well 5, which is located near a mapped fault in the updated MGS geologic mapping.

This fault provides a potential preferential pathway from the Mississippi River, where HQ-115 impacts from the Facility are observed, to Hastings Well 5. The MPCA provided 3M with this information in a phone call on August 24, 2023. In addition to the HQ-115 detection at Hastings Well 5, other analytical data collected as part of the MPCA's investigation indicate a potential connection between releases from the Facility and PFAS observed in the municipal wells for Hastings. Specifically, the same PFAS compounds present in Hastings's municipal wells are also detected in releases from the Facility. In addition, modeling efforts in the area suggest the cones of depression from municipal well pumping intersect, thus leading to potential mixing and spreading of contamination between the municipal wells.

The MPCA is requesting 3M complete additional investigation to determine if the PFAS observed in the Hastings municipal wells originated from releases from the Facility and if releases from the Facility are impacting any other drinking water in the northern portion of Dakota County. The MPCA expects that this additional investigation will include installation of monitoring wells, collection of additional groundwater samples for PFAS analysis, and the completion of modeling to evaluate groundwater and groundwater contaminant movement in this area. The PFAS analyte list shall include, at minimum, the PFAS identified in the December 14, 2022, Administrative Order along with any other PFAS that are or have been present at the Facility. All PFAS samples shall be analyzed to the minimum reporting levels available.

As this work compliments the requirement to delineate extent and magnitude of impacts in all media in the CSM, the MPCA expects this additional work will be completed concurrently with the CSM development and will be submitted as part of or as an addendum to the CSM that will be submitted to the MPCA by the end of January 2024. Please provide a response to this request by October 16, 2023, and provide a work plan outlining 3M's proposed investigations to the MPCA by October 30, 2023.

MPCA staff are available to meet to discuss this letter. If you have any questions regarding this letter, please contact me at 651-757-2436 or tom.higgins@state.mn.us.

Sincerely,
Tom Higgins

This document has been electronically signed.

Tom Higgins M.S. | Manager
Superfund Section
Remediation Division
Minnesota Pollution Control Agency

CC: James Kotsmith, 3M
Shane Waterman, 3M
Pam Anderson, MPCA
Liz Kaufenberg, MPCA
Andri Dahlmeier, MPCA
Michael Ginsbach, MPCA

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August 9, 2024

VIA EMAIL

Karie Blomquist, P.E.
Remediation Senior Manager, Global EHS
3M Environment, Health, Safety and Product Stewardship
3M Center Building 225-1N-22
St. Paul, MN 55144

RE: Hastings Municipal Well 5
Cooperative Responsible Party Invitation
MPCA Site IDs: SR0000033, SA0010066

Dear Karie Blomquist,

The Minnesota Pollution Control Agency (MPCA) has been investigating the occurrence and sources of per- and polyfluoroalkyl substance (PFAS) impacts observed in the municipal wells for the city of Hastings.

As stated in the MPCA's October 2, 2023, letter to the 3M Company (3M), additional data collected recently in southern Washington and northern Dakota Counties indicated that releases from the 3M Cottage Grove facility (Facility) are likely contributing to the observed drinking water impacts in the Hastings municipal wells. In that letter, the MPCA requested 3M conduct additional investigation in northern Dakota County to determine if releases from the Facility are impacting the Hasting's municipal wells or any other drinking water receptors in northern Dakota County.

In a letter dated May 9, 2023, the MPCA requested that 3M fully delineate the extent and magnitude of all contaminants in all media related to the Facility and develop a conceptual site model (CSM) documenting this information.

MPCA staff recognize that 3M has undertaken a significant number of environmental investigations at the Facility under the oversight of several regulatory programs. MPCA staff also recognize that 3M has operated at the Facility since the late 1940s where production facilities, waste disposal methods, and types of material being produced have changed throughout the years. MPCA acknowledges that 3M has been working with the MPCA on this ongoing investigation and appreciates the cooperation.

Remedial investigations at the Facility began in the early 1980s and continue to this day, with sampling of environmental media for PFAS at and near the Facility beginning in the early 2000s. An extensive investigation at the Facility and the nearby portion of the Mississippi River for PFAS occurred in 2021 and is documented in the *Instream PFAS Characterization Study Final Report, Mississippi River, Cottage Grove* dated June 29, 2023, prepared for 3M by Weston Solutions (IPCS Report).

One PFAS analyte evaluated in the IPCS Report is lithium bis(trifluoromethylsulfonyl)imide. Per the IPCS Report, 3M Global EHS Laboratory updated its laboratory information management system in November

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2022 to change the reporting of lithium bis(trifluoromethylsulfonyl)imide from the 3M trade name HQ-115 to reporting the anion that is measured by the analytical method, bis(trifluoromethylsulfonyl)imide with the acronym TFSI. Previous communications from the MPCA to 3M have referenced this compound by the trade name of HQ-115 but this letter will refer to it by the acronym TFSI in line with 3M's updated reporting.

The results from the ICS Report showed that TFSI is being discharged from the Facility based on surface water samples collected from the Facility and the Mississippi River near and downstream of the Facility. MPCA's Site Assessment program completed sampling of the Hastings municipal wells that included analyzing for TFSI. TFSI was detected in Hastings' municipal Well 5 (Well 5), which is located near a mapped fault in the updated Dakota County Geologic Atlas published by the Minnesota Geological Survey. This fault provides a potential preferential pathway from the Mississippi River, where TFSI and other PFAS impacts from the Facility are observed, to Well 5. The MPCA provided 3M with this information in a phone call on August 24, 2023. The same PFAS compounds present in Hastings's municipal wells are also detected in releases from the Facility.

Additional information collected by the MPCA, the City of Hastings, and 3M since the October 2, 2023, letter, has improved the understanding of the impacts from the release in this area.

3M collected surface water and groundwater samples in December 2023 and analyzed these samples for water quality parameters and PFAS. The results of these analyses were shared with the MPCA in a presentation given by Integral at the request of 3M on March 27, 2024. The summary of preliminary findings in the presentation discussed that seepage from under the Hastings dam may discharge into Lake Rebecca, which provides a pathway for surface water from the Mississippi River to Lake Rebecca. The presentation also stated that flow from the drift beneath Lake Rebecca towards Well 5 is possible under pumping conditions, providing a transport mechanism for releases from the 3M Cottage Grove facility to migrate to Well 5. In addition, the presentation stated that based on the water quality parameters, the sample collected from Well 5 appeared to be more similar to samples collected from Lake Rebecca than from the samples collected from the Mississippi River or from the baseline Prairie du Chien results from the United States Geological Survey. The MPCA's Site Assessment program collected samples for stable isotope analysis in 2024. These data also identified that water from Lake Rebecca is more similar to samples collected from Well 5 than the other municipal wells. This similarity provided additional corroboration for the connection between Lake Rebecca and Well 5.

The current data, including the likely connection between the Cottage Grove Facility and Well 5, indicates that 3M is a Potentially Responsible Party (PRP) under the Minnesota Environmental Response and Liability Act (MERLA). Specifically, the information gathered to date indicates that releases from the 3M Cottage Grove Facility, located at 10746 Innovation Road, Cottage Grove, Minnesota, are a source of the PFAS detected in Well 5.

The MPCA requests that 3M enroll in the MPCA Superfund Program as a Cooperative Responsible Party (CRP) to complete the ongoing investigation and conduct remedial actions for the identified release. Once the investigation and necessary remedial actions for the identified release are completed, the CRP

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may be eligible for a determination from the MPCA that no further response actions are necessary for the identified release observed at the Site.

As described in the MPCA's October 2, 2023 letter, additional investigation in northern Dakota County, which includes Mississippi River Pool 2, Lake Rebecca, and the City of Hastings, is required to evaluate the extent and magnitude of PFAS releases from the 3M Cottage Grove facility impacting the Hastings municipal wells in all environmental media, including but not limited to surface water, shallow groundwater, deep groundwater, sediment, soil, surface microlayer, and porewater.

Subsequent to the October 2, 2023, letter being sent, the Environmental Protection Agency (EPA) announced the final National Primary Drinking Water Regulation for six PFAS. In this announcement on April 10, 2024, EPA established legally enforceable levels, called Maximum Contaminant Levels (MCLs), for six PFAS in drinking water: PFOA, PFOS, PFHxS, PFNA, and HFPO-DA as contaminants with individual MCLs, and PFAS mixtures containing at least two or more of PFHxS, PFNA, HFPO-DA, and PFBS using a Hazard Index MCL to account for the combined and co-occurring levels of these PFAS in drinking water. EPA also finalized health-based, non-enforceable Maximum Contaminant Level Goals (MCLGs) for these PFAS.

As the concentrations of PFAS in Well 5 exceed the EPA's MCLs for PFAS, additional treatment of the drinking water is required to ensure that the city's community water system is in compliance with drinking water standards. 3M must work with the City of Hastings to design and install a treatment system and work with all appropriate state and federal agencies to ensure the concentrations of PFAS in treated drinking water from Well 5 are in compliance with risk-based values established for drinking water for PFAS.

If 3M chooses not to enroll as a CRP to investigate and remediate the identified release at the Site, the MPCA may consider:

- Listing the Site on the state's Permanent List of Priorities (PLP) (i.e., the State Superfund list),
- Undertaking any necessary investigation and remedial action and seeking recovery of expenses from the Responsible Parties for the identified release (see Minn. Stat. 115B.04 and 115B.17), and
- Pursuing enforcement or other formal processes for compelling and overseeing the responsible parties' investigation and response actions.

This cooperative approach is often a more efficient and timely way to carry out the MPCA-approved investigations and cleanups and does not trigger the formal enforcement provisions of the state Superfund laws (i.e., MERLA).

For more information about the enrollment application and to enroll as a CRP using the MPCA's online services, please visit the MPCA's website at https://www.pca.state.mn.us/about-mpca/online-services. For information about the state's Superfund Program, please visit the MPCA's website at https://www.pca.state.mn.us/air-water-land-climate/cleanup-initiatives.

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The MPCA requests that 3M enroll as a CRP for the identified release or send a written response regarding 3M's intent to enroll as a CRP within **seven to ten** business days of receiving this letter. If you feel that you are not the Responsible Party for the identified release or if you have any questions regarding this letter, please contact Andri Dahlmeier (651-757-2718 or andri.dahlmeier@state.mn.us) or Michael Ginsbach (651-757-2329 or michael.ginsbach@state.mn.us). Your continued cooperation is appreciated.

Sincerely,

Elizabeth Kaufenberg

This document has been electronically signed.

Liz Kaufenberg | Manager
Superfund Site Assessment Section
Remediation Division
Minnesota Pollution Control Agency

AD:mg

cc: Jim Kotsmith, 3M (electronic)
Shane Waterman, 3M (electronic)
Kirk Koudelka, MPCA (electronic)
Pam Anderson, MPCA (electronic)
Andri Dahlmeier, MPCA (electronic)
Michael Ginsbach, MPCA (electronic)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF WATER

December 5, 2022

MEMORANDUM

SUBJECT: Addressing PFAS Discharges in NPDES Permits and Through the Pretreatment Program and Monitoring Programs

FROM: Radhika Fox
Assistant Administrator

TO: EPA Regional Water Division Directors, Regions 1-10

The National Pollutant Discharge Elimination System (NPDES) program is an important tool established by the Clean Water Act (CWA) to help address water pollution by regulating point sources that discharge pollutants to waters of the United States. Collectively, the U.S. Environmental Protection Agency (EPA) and states issue thousands of permits annually, establishing important monitoring and pollution reduction requirements for Publicly Owned Treatment Works (POTWs), industrial facilities, and stormwater discharges nationwide. The NPDES program interfaces with many pathways by which per-and polyfluoroalkyl substances (PFAS) travel and are released into the environment, and ultimately impact water quality and the health of people and ecosystems. Consistent with the Agency's commitments in the October 2021 *PFAS Strategic Roadmap: EPA's Commitments to Action 2021-2024 (PFAS Strategic Roadmap)*, EPA will work in cooperation with our state-authorized permitting authorities to leverage the NPDES program to restrict the discharge of PFAS at their sources. In addition to reducing PFAS discharges, this program will enable EPA and the states to obtain comprehensive information on the sources and quantities of PFAS discharges, which can be used to inform appropriate next steps to limit the discharges of PFAS.

This memorandum provides EPA's guidance to states and updates the April 28, 2022 guidance[1] to EPA Regions for addressing PFAS discharges when they are authorized to administer the NPDES permitting program and/or pretreatment program. These recommendations reflect the Agency's commitments in the PFAS Strategic Roadmap, which directs the Office of Water to leverage NPDES permits to reduce PFAS discharges to waterways "*at the source and obtain more comprehensive information through monitoring on the sources of PFAS and quantity of PFAS discharged by these sources.*" While the Office of Water works to revise Effluent Limitation Guidelines (ELGs) and develop water quality criteria to support technology-based and water quality-based effluent limits for PFAS in NPDES permits, this memorandum describes steps permit writers can implement under existing authorities to reduce the discharge of PFAS.

[1] Addressing PFAS Discharges in EPA-Issued NPDES Permits and Expectations Where EPA is the Pretreatment Control Authority, https://www.epa.gov/system/files/documents/2022-04/npdes_pfas-memo.pdf.

This memorandum also provides EPA's guidance for addressing sewage sludge PFAS contamination more rapidly than possible with monitoring based solely on NPDES permit renewals. States may choose to monitor the levels of PFAS in sewage sludge across POTWs and then consider mechanisms under pretreatment program authorities to prevent the introduction of PFAS to POTWs based on the monitoring results.

EPA recommends that the following array of NPDES and pretreatment provisions and monitoring programs be implemented by authorized states and POTWs, as appropriate, to the fullest extent available under state and local law. NPDES and pretreatment provisions may be included when issuing a permit or by modifying an existing permit pursuant to 40 CFR 122.62.

A. Recommendations for Applicable Industrial Direct Dischargers

1. **Applicability:** Industry categories known or suspected to discharge PFAS as identified on page 14 of the PFAS Strategic Roadmap include: organic chemicals, plastics & synthetic fibers (OCPSF); metal finishing; electroplating; electric and electronic components; landfills; pulp, paper & paperboard; leather tanning & finishing; plastics molding & forming; textile mills; paint formulating, and airports. This is not an exhaustive list and additional industries may also discharge PFAS. For example, Centralized Waste Treatment (CWT) facilities may receive wastes from the aforementioned industries and should be considered for monitoring. There may also be categories of dischargers that do not meet the applicability criteria of any existing ELG; for instance, remediation sites, chemical manufacturing not covered by OCPSF, and military bases.

   EPA notes that no permit may be issued to the owner or operator of a facility unless the owner or operator submits a complete permit application in accordance with applicable regulations, and applicants must provide any additional information that the permitting authority may reasonably require to assess the discharges of the facility (40 CFR 122.21(e), (g)(13)).[2] The applicant may be required to submit additional information under CWA Section 308 or under a similar provision of state law.

2. **Effluent-and wastewater residuals monitoring:** In the absence of a final 40 CFR Part 136 method, EPA recommends using CWA wastewater draft analytical method 1633 (*see* 40 CFR 122.21(e)(3)(ii) and 40 CFR 122.44(i)(1)(iv)(B)). EPA also recommends that monitoring include each of the 40 PFAS parameters detectable by draft method 1633 and be conducted at least quarterly to ensure that there are adequate data to assess the presence and concentration of PFAS in discharges. All PFAS monitoring data must be reported on Discharge Monitoring Reports (DMRs) (*see* 40 CFR 122.41(l)(4)(i)). The draft Adsorbable Organic Fluorine CWA wastewater method 1621 can be used in conjunction with draft method 1633, if appropriate. Certain industrial processes may generate PFAS-contaminated solid waste or air emissions not covered by NPDES permitting and permitting agencies should coordinate with appropriate state authorities on proper containment and disposal to avoid cross-media contamination. EPA's draft analytical method 1633 may be appropriate to assess the amount and types of PFAS for some of these wastestreams.[3]

[2] For more, see NPDES Permit Writer's Manual Section 4.5.1.
[3] See https://www.epa.gov/water-research/pfas-analytical-methods-development-and-sampling-research for a list of EPA-approved methods for other media.

3. **Best Management Practices (BMPs) for discharges of PFAS, including product substitution, reduction, or elimination of PFAS, as detected by draft method 1633:** Pursuant to 40 CFR 122.44(k)(4), EPA recommends that NPDES permits for facilities incorporate the following conditions when the practices are "reasonably necessary to achieve effluent limitations and standards or to carry out the purposes and intent of the CWA."[4]
   a. BMP conditions based on pollution prevention/source reduction opportunities, which may include:
      i. Product elimination or substitution when a reasonable alternative to using PFAS is available in the industrial process.
      ii. Accidental discharge minimization by optimizing operations and good housekeeping practices.
      iii. Equipment decontamination or replacement (such as in metal finishing facilities) where PFAS products have historically been used to prevent discharge of legacy PFAS following the implementation of product substitution.
   b. Example BMP permit special condition language:
      i. *PFAS pollution prevention/source reduction evaluation*: Within 6 months of the effective date of the permit, the facility shall provide an evaluation of whether the facility uses or has historically used any products containing PFAS, whether use of those products or legacy contamination reasonably can be reduced or eliminated, and a plan to implement those steps.
      ii. *Reduction or Elimination*: Within 12 months of the effective date of the permit, the facility shall implement the plan in accordance with the PFAS pollution prevention/source reduction evaluation.
      iii. *Annual Report*: An annual status report shall be developed which includes a list of potential PFAS sources, summary of actions taken to reduce or eliminate PFAS, any applicable source monitoring results, any applicable effluent results for the previous year, and any relevant adjustments to the plan, based on the findings.
      iv. *Reporting:* When EPA's electronic reporting tool for DMRs (called "NetDMR") allows for the permittee to submit the pollution prevention/source reduction evaluation and the annual report, the example permit language can read, "The pollution prevention/source reduction evaluation and annual report shall be submitted to EPA via EPA's electronic reporting tool for DMRs (called "NetDMR").

4. **BMPs to address PFAS-containing firefighting foams for stormwater permits:** Pursuant to 122.44(k)(2), where appropriate, EPA recommends that NPDES stormwater permits include BMPs to address Aqueous Film Forming Foam (AFFF) used for firefighting, such as the following:[5]
   a. Prohibiting the use of AFFFs other than for actual firefighting.
   b. Eliminating PFOS and PFOA -containing AFFFs.
   c. Requiring immediate clean-up in all situations where AFFFs have been used, including diversions and other measures that prevent discharges via storm sewer systems.

5. **Permit Limits:** As specified in 40 CFR 125.3, technology-based treatment requirements under CWA Section 301(b) represent the minimum level of control that must be imposed in NPDES permits. Site-specific technology-based effluent limits (TBELs) for PFAS discharges developed on a best professional judgment (BPJ) basis may be appropriate for facilities for which there are no applicable effluent guidelines (*see* 40 CFR 122.44(a), 125.3). Also, NPDES permits must include water quality-based effluent limits (WQBELs) as derived from state water quality standards, in

[4] For more on BMPs, see NPDES Permit Writer's Manual Section 9.1 and EPA Guidance Manual for Developing Best Management Practices.
[5] Naval Air Station Whidbey Island MS4 permit incorporates these provisions.

addition to TBELs developed on a BPJ basis, if necessary to achieve water quality standards, including state narrative criteria for water quality (CWA Section 301(b)(1)(C); 40 CFR 122.44(d)). If a state has established a numeric criterion or a numeric translation of an existing narrative water quality standard for PFAS parameters, the permit writer should apply that numeric criterion or narrative interpretation in permitting decisions, pursuant to 40 CFR 122.44(d)(1)(iii) and 122.44(d)(1)(vi)(A), respectively.

B. Recommendations for Publicly Owned Treatment Works

1. **Applicability:** All POTWs, including POTWs that do not receive industrial discharges, and industrial users (IUs) in the industrial categories above.

2. **Effluent, influent, and biosolids monitoring:** In the absence of a final 40 CFR Part 136 method, EPA recommends using CWA wastewater draft analytical method 1633 (*see* 40 CFR 122.21(e)(3)(ii) and 40 CFR 122.44(i)(1)(iv)(B)). EPA also recommends that monitoring include each of the 40 PFAS parameters detectable by draft method 1633 and be conducted at least quarterly to ensure that there are adequate data to assess the presence and concentration of PFAS in discharges. All PFAS monitoring data must be reported on DMRs (*see* 40 CFR 122.41(l)(4)(i)). The draft Adsorbable Organic Fluorine CWA wastewater method 1621 can be used in conjunction with draft method 1633, if appropriate.

3. **Pretreatment program activities:**
   a. Update IU Inventory: Permits to POTWs should contain requirements to identify and locate all possible IUs that might be subject to the pretreatment program and identify the character and volume of pollutants contributed to the POTW by the IUs (*see* 40 CFR 403.8(f)(2)). As EPA regulations require, this information shall be provided to the pretreatment control authority (*see* 40 CFR 122.44(j) and 40 CFR 403.8(f)(6)) within one year. The IU inventory should be revised, as necessary, to include all IUs in industry categories expected or suspected of PFAS discharges listed above (*see* 40 CFR 403.12(i)).[6]
   b. Utilize BMPs and pollution prevention to address PFAS discharges to POTWs. EPA recommends that POTWs:
      i. Update IU permits/control mechanisms to require quarterly monitoring. These IUs should be input into the Integrated Compliance Information System (ICIS) with appropriate linkage to their respective receiving POTWs. POTWs and states may also use their available authorities to conduct quarterly monitoring of the IUs (*see* 40 CFR 403.8(f)(2), 403.10(e) and (f)(2)).
      ii. Where authority exists, develop IU BMPs or local limits. 40 CFR 403.5(c)(4) authorizes POTWs to develop local limits in the form of BMPs. Such BMPs could be like those for industrial direct discharges described in A.3 above.
      iii. In the absence of local limits and POTW legal authority to issue IU control mechanisms, state pretreatment coordinators are encouraged to work with the POTWs to encourage pollution prevention, product substitution, and good housekeeping practices to make meaningful reductions in PFAS introduced to POTWs.

[6] ELG categories of **airport deicing, landfills, textile mills, and plastics molding and forming do not have categorical pretreatment standards**, and therefore small-volume indirect dischargers in those categories would not ordinarily be considered Significant Industrial Users (SIUs) and may not be captured on an existing IU inventory. IUs under the Paint Formulating category are only subject to Pretreatment Standards for New Sources (PSNS), and existing sources may need to be inventoried.

C. Recommended Biosolids Assessment

1. Where appropriate, states may work with their POTWs to reduce the amount of PFAS chemicals in biosolids, in addition to the NPDES recommendations in Section B above, following these general steps:[7]

a. EPA recommends using draft method 1633 to analyze biosolids at POTWs for the presence of 40 PFAS chemicals.[8]

b. Where monitoring and IU inventory per section B.2 and B.3.a above indicate the presence of PFAS in biosolids from industrial sources, EPA recommends actions in B.3.b to reduce PFAS discharges from IUs.

c. EPA recommends validating PFAS reductions with regular monitoring of biosolids. States may also use their available authorities to conduct quarterly monitoring of the POTWs (*see* 40 CFR 403.10(f)(2)).

D. Recommended Public Notice for Draft Permits with PFAS-Specific Conditions

1. In addition to the requirements for public notice described in 40 CFR 124.10, EPA recommends that NPDES permitting authorities provide notification to potentially affected downstream public water systems (PWS) of draft permits with PFAS-specific monitoring, BMPs, or other conditions:

a. Public notice of the draft permit would be provided to potentially affected PWS with intakes located downstream of the NPDES discharge.

b. NPDES permit writers are encouraged to collaborate with their drinking water program counterparts to determine on a site-specific basis which PWS to notify.

   i. EPA's Drinking Water Mapping Application to Protect Source Waters (DWMAPS) tool may be helpful as a screening tool to identify potentially affected PWS to notify.

c. EPA will provide instructions on how to search for facility-specific discharge monitoring data in EPA's publicly available search tools.

[7] EPA is currently evaluating the potential risk of PFOA and PFOS in biosolids and supporting studies and activities to evaluate the presence of PFOA and PFOS in biosolids. This recommendation is not meant to supersede the PFOA and PFOS risk assessment or supporting activities. The conclusions of the risk assessment and supporting studies may indicate that regulatory actions or more stringent requirements are necessary to protect human health and the environment.

[8] While water quality monitoring activities (including monitoring of PFAS associated with NPDES permit or pretreatment requirements) at POTWs are generally not eligible for Clean Water State Revolving Fund (CWSRF), monitoring for the specific purpose of project development (planning, design, and construction) is eligible. Monitoring in this capacity, and within a reasonable timeframe, can be integral to the identification of the best solutions (through an alternatives analysis) for addressing emerging contaminants and characterizing discharge and point of disposal (e.g., land application of biosolids). Though ideally the planning and monitoring for project development would result in a CWSRF-eligible capital project, in some instances, the planning could lead to outcomes other than capital projects to address the emerging contaminants.

NOVEMBER 2023

MCEA
MINNESOTA CENTER FOR ENVIRONMENTAL ADVOCACY

Forever Chemicals in our Wastewater

How Minnesota can build on the PFAS source reduction laws passed in 2023

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Introduction

This report outlines why wastewater streams are critical to the broader per- and polyfluoroalkyl substances ("PFAS") contamination crisis, what legal tools are available to help address this problem, and proactive steps other states have taken to better prevent PFAS contamination from wastewater streams. Minnesota can learn from these approaches and implement a regulatory framework that better protects Minnesota's waters, land, and wildlife from PFAS contamination, and helps secure Minnesota's communities from further damage caused by the toxic effects of PFAS on human health.

MCEA recommendations include:

• Add PFAS as a pollutant under the Minnesota Sewage Sludge Management Rule;

• Require wastewater treatment plants to monitor influent, effluent, and land applied biosolids for PFAS so we can better understand the scope of contamination;

• Use pretreatment programs to require industrial dischargers to use best management practices and treatment options to reduce and remove PFAS from industrial wastewater before it reaches municipal wastewater treatment plants;

• Label Class A EQ biosolids sold for public distribution as potential sources of PFAS;

• Investigate sensitive sites (based on soil type/hydrology) where biosolids have been land applied for decades for legacy soil and groundwater contamination;

• Require PFAS data in the environmental review (Minnesota Environmental Policy Act) process, such as the Met Council wastewater treatment plant's proposed addition of a fourth incinerator;

• Monitor ambient groundwater for PFAS contamination from landfill leachate and land applied biosolids;

• Develop strong statewide Class 1 Water Quality Standards that mirror the proposed federal Maximum Contaminant Levels (MCLs) for 6 PFAS compounds.

PFAS are the emergent contaminants of our time. Known colloquially as "forever chemicals," PFAS are a family of over 1,000 synthetic chemicals that have been used for decades to make products that resist heat, oil, stains, grease, and water. One of the largest corporate manufacturers of these chemicals, 3M, is based here in Minnesota, and since the 1950s, 3M has been at the epicenter of the production and global circulation of these substances.[1]

Today, PFAS are ubiquitous in our environment and have been detected at dangerous levels in water, soils, and wildlife across the world. PFAS dissolve in water and bioaccumulate, which means that they build up in humans, fish, and animals over time.[2] Elevated levels of PFAS have been correlated with human health impacts such as adverse birth outcomes, thyroid disease, various forms of cancer, and more. In a recent proposed rule, the EPA determined that two of the most common PFAS compounds, PFOA and PFOS, are "likely to be carcinogenic" to humans, with safe levels measured in shockingly small amounts of parts per trillion.[3]

Minnesota took a decisive step forward on PFAS contamination in the 2023 legislative session, when the state passed some of the strongest source reduction laws in the country.

Pictured: MCEA Legislative Director Andrea Lovoll speaking at a press conference after the passage of Amara's law, which banned the non-essential use of PFAS and required reporting of the use of PFAS.

The laws passed in 2023 will "turn off the tap" on intentionally added PFAS in common consumer products such as carpets/rugs, cookware, cosmetics, dental floss, and juvenile products.[4] They will also require manufacturers to disclose to the Minnesota Pollution Control Agency when PFAS has been intentionally added to their products.[5]

PFAS is incredibly difficult to remove once it's in the environment, so Minnesota's source reduction laws are a critical step forward in our statewide approach to PFAS contamination. However, more work remains to be done. The next frontier is to use our bedrock environmental laws, such as the Clean Water Act, to regulate PFAS pollution from wastewater streams and remediate the PFAS that is already in the environment.

Why are wastewater streams so important? We need to better regulate wastewater streams for PFAS pollution because wastewater treatment plants are one of the primary pathways of PFAS into the environment.

There are two main ways this happens:

1) through direct discharge of PFAS-contaminated wastewater to lakes, rivers, and streams; and

2) through soil and groundwater contamination from the land application of sewage sludge, or biosolids, produced in the wastewater treatment process or through landfill leachate.

In a report released in June of 2023, MPCA said that clean-up costs for PFAS contamination in wastewater streams across Minnesota over the next twenty years are likely to range from $14 to 28 billion[6]. We need to ensure that those costs are borne by the responsible parties to the extent possible, through tools like PFAS pollution limits in wastewater permits and pre-treatment programs that require industrial dischargers to treat PFAS contaminated wastewater before it is sent to municipal wastewater treatment plants.

When these sources of contamination are not regulated, the public ends up bearing the costs of contamination. We can see the brunt of these costs in the exorbitant treatment costs that water utilities across the country face to make water safe for human consumption, in the tragic stories of “cancer clusters” at places like Tartan High School in the East Metro region, and in rural communities that have had to deal with the forced closure of farms because of soil and groundwater contamination from biosolids.

Two primary paths for ongoing PFAS pollution

Direct discharge

Influent refers to the raw, untreated wastewater that flows into the wastewater treatment plants, and effluent refers to the treated water that is discharged from the wastewater treatment plants into surface waters like lakes and rivers. In Minnesota, wastewater treatment plants discharge effluent into waterbodies like the Mississippi River and Lake Superior.

TREATED WASTEWATER DISCHARGED INTO WATERBODIES

Land application

In the wastewater treatment process, the liquids are separated from the solids. The solids are either incinerated or chemically treated to produce a nutrient-rich product known as biosolids or sewage sludge. This product is then sold to the public as garden/lawn fertilizer or farmers can apply for a land application permit to apply biosolids in bulk as a crop fertilizer. Landfills are another disposal method for sewage sludge.

BYPRODUCT OF WASTEWATER TREATMENT SPREAD AS FERTILIZER

I. The PFAS Problem

Why these synthetic chemicals have created a public health threat across the state, across the nation, and across the world.

PFAS compounds replace the common carbon-hydrogen bond with a carbon-fluorine bond—one of the strongest bonds in organic chemistry—which makes them resistant to heat, water, and oil.[7] For decades, PFAS have been added to raincoats, cookware, dental floss, carpets, medical devices, mascara, and thousands more consumer products. PFAS has also been a key component of firefighting foams used for fire suppression across the country.

However, the same characteristics that made PFAS a prized chemical in industry also allow them to remain stable in the natural environment. PFAS chemicals do not degrade in the environment, are water soluble, and bioaccumulate in humans, fish, and animals. These compounds have been found in the blood of polar bears, Norwegian arctic ice, and rainfall in Antarctica and the Tibetan Plateau. In addition to their ubiquity, elevated levels of PFAS have been correlated with impacts to human memory,[8] heart development,[9] and myriad other adverse health effects such as thyroid disease,[10] kidney cancer,[11] hypercholesterolemia,[12] and more.[13]

In August of 2023, the U.S. Geological Survey released a study that tested tap water from 716 private wells and public water supplies across the country and found PFAS in at least 45% of the faucets it sampled from.[14] PFAS is a national and a global problem.

PFAS in Minnesota

Here in Minnesota, the most prominent PFAS hotspot is the groundwater near 3M's global headquarters in the East Twin Cities Metro region. This area is now home to one of the country's largest PFAS contamination plumes, caused by waste disposal from four nearby 3M sites in Washington County.[15] In addition, discharges from the 3M wastewater treatment plant in Cottage Grove have polluted the Mississippi River.[16] The human toll from PFAS in Minnesota is evident in places like Tartan High School, which drew its water from the contaminated aquifer, and where a group of high school students who suffered from various forms of cancers called themselves the "cancer cluster." One of those students, Amara Strande, passed away in April of 2023 from a rare form of cancer. She was 20 years old.

Unfortunately, the impacts of PFAS in Minnesota extend far beyond the Twin Cities Metropolitan Area and affect nearly every corner of the state. PFAS has been detected in groundwater at 100 closed landfill sites across the state. At 62 of those landfills, the detection level exceeded state health standards, while at 10 landfills located from Northeastern to Southern Minnesota PFAS levels were over 10 times the state health standard.[17] Parts of the Mississippi River, Lake Elmo, and dozens more waterways across the state have fish consumption advisories that caution people not to eat fish due to PFAS contamination.[18] PFAS has even reached our region's most pristine water resource, Lake Superior, where fish consumption advisories are in place for smelt due to high concentrations of PFOS, one of the legacy PFAS best understood by the scientific community.

In 2013, MPCA tested nearly 200 wells across the state through its ambient groundwater program and found one or more types of PFAS in 69% of sample sites, with detection clustered in urban areas like the Twin Cities Metropolitan Area, Brainerd, and St. Cloud.[19]

PFAS Drinking Water Regulation

The Minnesota Department of Health ("MDH") has a voluntary program to monitor community water systems across the state for PFAS contamination. Approximately 95% of community water systems chose to participate in this program, which found at least four communities in Greater Minnesota that exceed the state's current Health Risk Index ("HRI") for PFAS: Roosevelt Court, Swanville, Waite Park, and Sauk City.[20] The MDH monitoring program did not include tribal water systems, and new sites of contamination continue to be discovered: in February of 2023, the Leech Lake Band of Ojibwe had to shut down one of its school water systems because of PFAS contamination discovered by the EPA.[21]

In March of 2023, the U.S. Environmental Protection Agency ("EPA") proposed national drinking water standards, called Maximum Contaminant Levels ("MCLs"), for six PFAS compounds at near non-detection levels under the Safe Drinking Water Act. If the rule is adopted as proposed, all public water systems–which serve approximately 90% of Americans–must deliver drinking water that is nearly free from the most toxic and well-studied PFAS. The proposed standards are much lower than Minnesota's current Health Based Values ("HBV"). The proposed MCL for PFOS and PFOA is 4 parts per trillion ("ppt"), and the EPA has recommended a Hazard Index approach to look at the additive risk from mixtures of four additional PFAS: GenX, PFBS, PFNA, and PFHxS.[22] The public health benefits of this proposed rule are enormous.

MDH has since begun to re-evaluate its guidance values for PFOS and PFOA, which means that the number of community water systems above the state Health Risk Index will likely be much higher than the four identified above.

Fish consumption advisories are put in place to notify the public that specific contaminants have been found in a water body and its organisms at levels that are unsafe to eat for certain populations. PFAS, like other contaminants such as mercury, can bioaccumulate in different species. This means that the further up the food chain a species is, the more likely it is to have larger amounts of these contaminants in its system. Because of this, humans increase their exposure rate if they eat fish from contaminated waters. Most recently, in July of 2023, the Minnesota Department of Health released fish consumption guidance for two Twin Cities area waterbodies - Pool 2 of the Mississippi River and Lake Rebecca - because of PFAS contamination.[23]

MINNESOTA STANDARDS

MDH uses three metrics to assess the health risk of contaminants in drinking water: Health Based Values , Health Risk Limits, and a Health Risk Index.

A Health Based Value ("HBV") ") is the concentration of a chemical (or a mixture of chemicals) that is likely to pose little to no human health risk. HBVs are technical guidance values rather than regulatory rules. They are updated as new toxicology data becomes available. MDH has HBVs for PFBS, PFHxS, PFHxA, PFOA, and PFOS.[24]

A Health Risk Limit ("HRL") is a numeric limit adopted as a rule under the Groundwater Protection Act when a contaminant is detected in the groundwater. Like the HBV, it is the concentration of a contaminant that is likely to pose little to no human health risk. MDH has HRLs for PFBS, PFBA, PFOA, and PFOS.[25]

A Health Risk Index ("HRI") is used when more than one contaminant is found in the water to evaluate the combined risk from chemicals that have similar health effects. MDH relies on its Health Based Values and Health Risk Limits for individual substances to base this "additive" numerical assessment.

FEDERAL STANDARDS

EPA sets Maximum Contaminant Levels ("MCLs") and Hazard Indexes to establish standards for the levels of pollutants that can be in drinking water.

In March of 2023, EPA proposed MCLs and a Hazard Index for 6 PFAS compounds: PFOA, PFOS, PFHsX, GenX, PFNA, and PFBS. If these proposed regulations are adopted as rules, they will become enforceable standards for water utilities across the country.

Maximum Contaminant Level Goals ("MCLGs"): Establishes the level at which there are no known or anticipated adverse health effects and includes a margin of safety. The EPA has proposed a MCLG of 0 parts per trillion (ppt) for both PFOS and PFOA.

Maximum Contaminant Levels ("MCLs"): The enforceable standard at which EPA sets drinking water contaminant levels. MCLs are set as technologically and feasibly close to the MCLGs as possible. The EPA has proposed an MCL of 4 ppt for PFOS and PFOA.

Hazard Index: An enforceable standard that uses an additive risk framework to evaluate the health risks from exposure to chemical mixtures. The EPA has proposed a Hazard Index of 1.0 that aggregates numeric limits for PFHsX, GenX, PFNA, and PFBS.

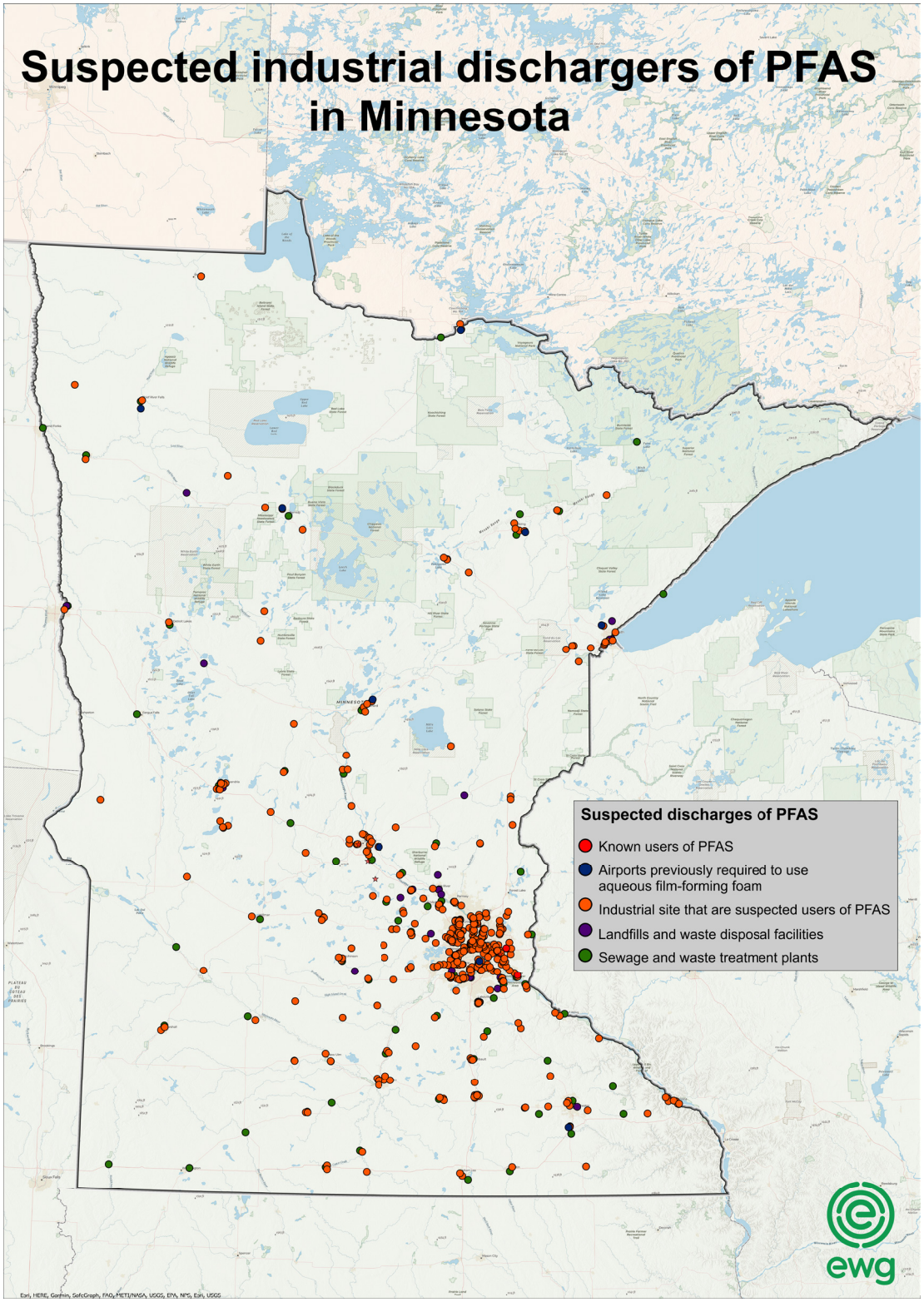
II. PFAS in Wastewater

Wastewater treatment plants are one of the primary pathways for PFAS contamination in our waterways, because they collect and process wastewater from industrial users that are themselves suspected dischargers of PFAS. State and federal regulators have identified 50 classifications of businesses that are "likely to use, emit, or discharge PFAS," including chrome plating facilities, textile mills, paint and varnish manufacturers, and waste treatment facilities.[26] Some of these facilities, like chrome plating facilities and certain manufacturers, are deemed "sources" of PFAS because PFAS is used as a part of the manufacturing or industrial process. Other facilities, such as landfills and wastewater treatment plants, are considered "conduits" of PFAS because they receive PFAS waste from other sources.

The wastewater treatment process was designed to remove pollutants such as heavy metals and pathogens like E. Coli and salmonella from our water. However, traditional treatment technologies are not able to remove PFAS substances because of the strength of their carbon fluorine bond. Even traditional incineration facilities do not generate high enough heat to break apart PFAS' signature bond.

St. Louis Park Chrome Plating Facility Agrees to Pay $1.375 Million for Polluting Minneapolis Chain of Lakes

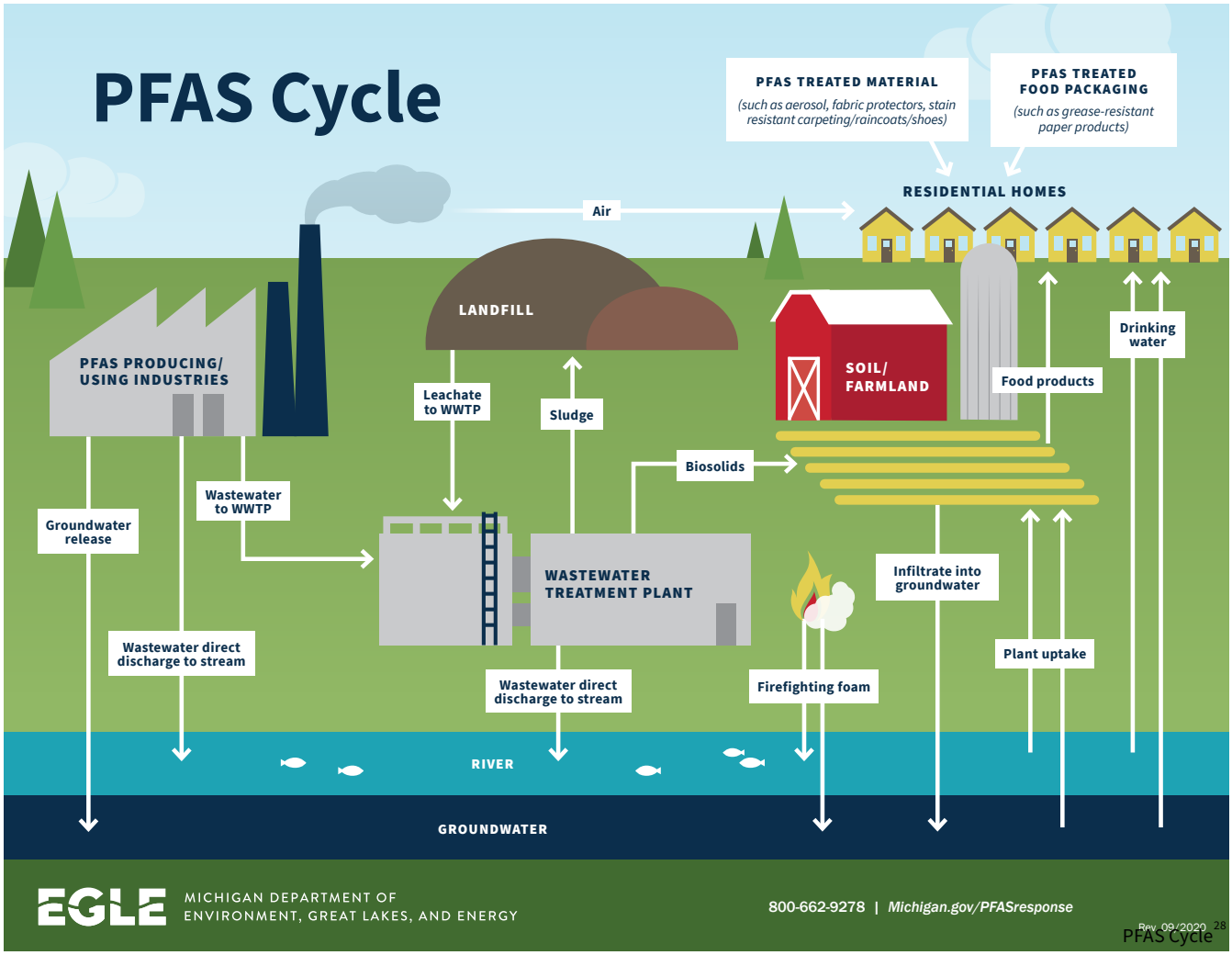
In May of 2023, Douglas Corporation agreed to settle charges that PFAS escaped from its St. Louis Park facility and damaged natural resources, including Bde Maka Ska and Lake Harriet. PFAS are widely used in the metal plating and finishing industries to inhibit corrosion and protect base materials. The investigation began in 2004, where regulators detected elevated levels of PFOS in Bde Maka Ska. Through investigation of nearby stormwater systems, regulators believe that PFOS passed through Douglas Corporation's heating and ventilation system and settled on the roof, where it was eventually brought down to the ground through stormwater and snow melt.[27]


Suspected industrial dischargers of PFAS in Minnesota
Suspected discharges of PFAS
Known users of PFAS
Airports previously required to use aqueous film-forming foam
Industrial site that are suspected users of PFAS
Landfills and waste disposal facilities
Sewage and waste treatment plants
ewg

The Wastewater Treatment Process: Nuts and Bolts

Influent refers to the raw, untreated wastewater that flows into the wastewater treatment plants, and effluent refers to the treated water that is discharged from the wastewater treatment plant into surface waters like lakes and rivers.

In the wastewater treatment process, the liquids are separated from the solids. The solids are either incinerated or chemically treated to produce a nutrient-rich product known as biosolids or sewage sludge. This product is then sold to the public as garden/lawn fertilizer (Class A EQ biosolids) or farmers can apply for a land application permit to apply biosolids in bulk as a crop fertilizer. Landfills are another disposal method for biosolids.



PFAS Cycle [28]

III. PFAS in Biosolids

In Minnesota, many wastewater treatment plants distribute biosolids in bulk to land apply on agricultural fields as a crop fertilizer. In 2018, approximately 44,300 dry tons of biosolids were distributed to the public or land applied as crop fertilizer across the state, with hot spots in areas like St. Cloud. Of that amount, 13,335 dry tons were distributed to the public as Class A EQ biosolids.[29] Overall, about 22% of biosolids in Minnesota are applied to agricultural land as a crop fertilizer, from 171 different wastewater treatment plants.[30]

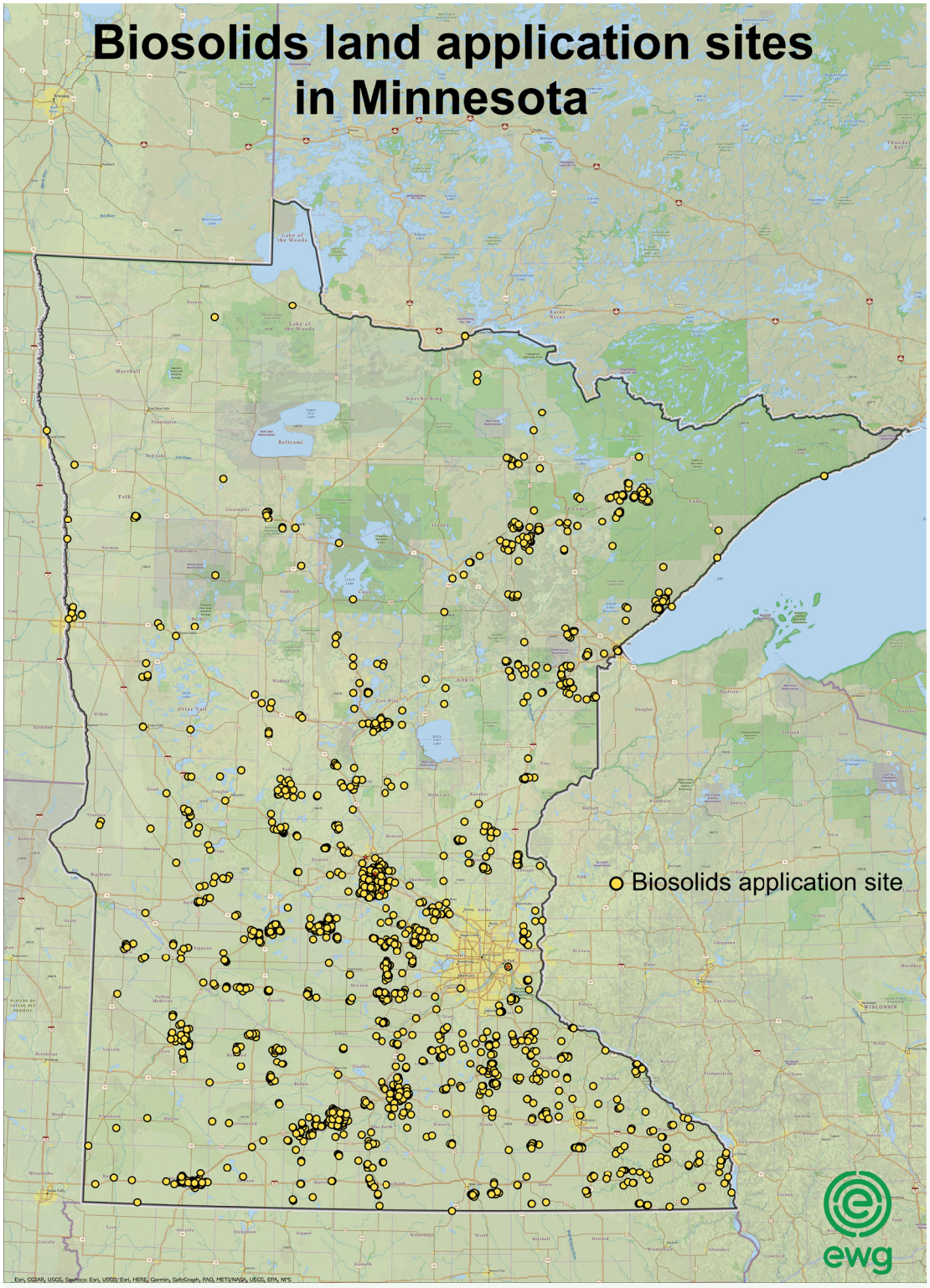
When a wastewater treatment plant's influent is contaminated with PFAS, so are its biosolids. In fact, the concentration of certain PFAS, like PFOS, tend to be higher in biosolids samples than in influent samples, as demonstrated by undated MPCA samples of wastewater influent, effluent, and biosolids at 31 wastewater treatment plants for PFOS concentrations. In these samples, the PFOS concentrations found in the biosolids samples jumped astronomically when compared to the influent samples (the wastewater that comes into the facilities). Across all 31 samples, the median PFOS concentration for influent was 35 ppt, and for sewage sludge it was 25,000 ppt.[31]

None of the other biosolids disposal methods currently used in Minnesota destroy PFAS. In the Twin Cities Metro Region, the majority of biosolids are incinerated, which accounts for 62% of biosolids disposal statewide.[32] However, the incineration process does not currently use high enough temperatures to destroy PFAS, so these compounds are released through incinerator stacks.[33]

To look at the role wastewater streams play as a pathway of PFAS contamination, we worked with public health scientist and University of Minnesota Professor Dr. Matt Simcik to collect water samples at four sites on or near the Mississippi River. These sites targeted two specific wastewater streams: effluent discharge to the Mississippi River, and biosolids land application on fields along tributaries to the Mississippi River.

The sites included:

- The effluent channel of the Metropolitan Wastewater Treatment Plant in Saint Paul and the Mississippi River directly upstream of the effluent channel
- Three tributaries to the Mississippi River in St. Cloud: Johnson Creek, Clearwater River, and Sauk River



Biosolids are regulated by the EPA, and states can adopt more stringent standards. Currently, there are no PFAS regulations for biosolids at the federal level or in Minnesota.

Feature Section: Wastewater Streams and Water Quality in Minnesota

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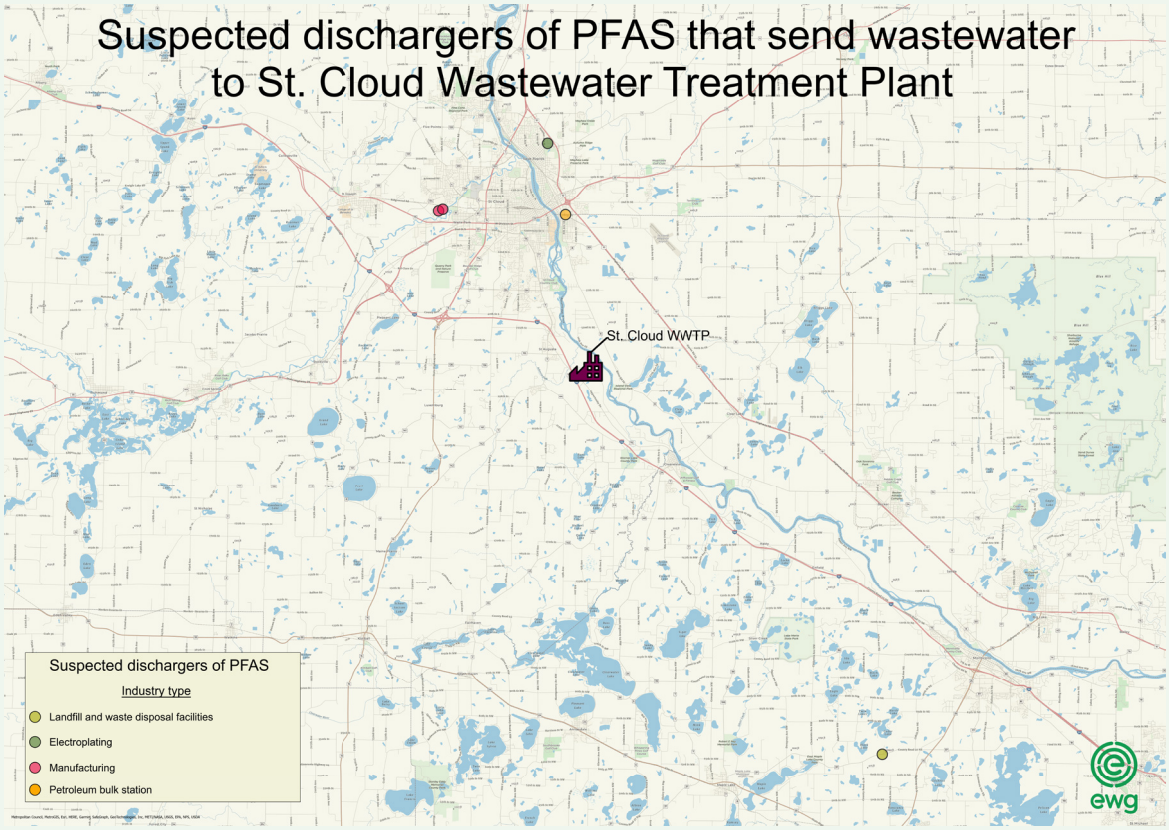
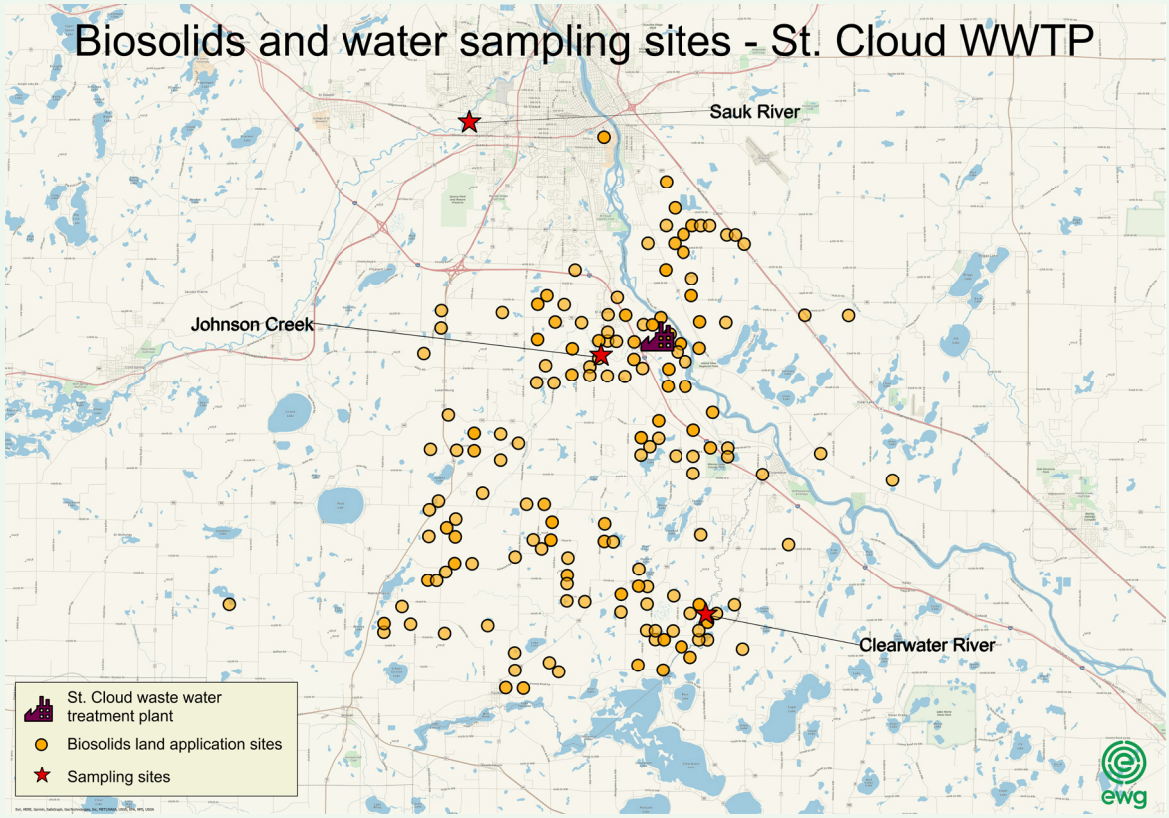
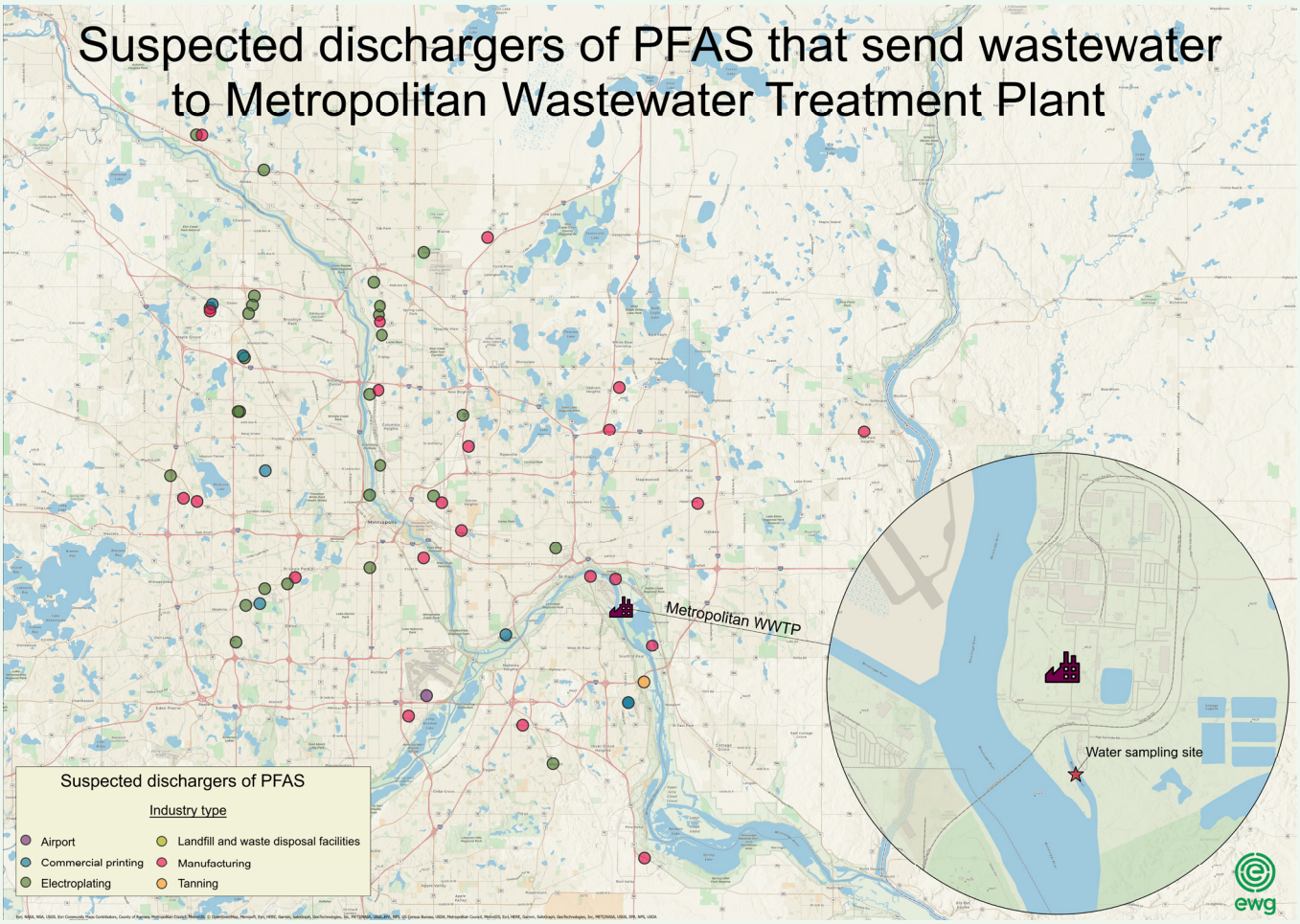
PFAS production in the United States changed earlier this century. Longer chain PFAS (with more than 8 carbon atoms) were replaced by shorter chain PFAS (with fewer than 5 carbon atoms). An example is the replacement of PFOS with PFBS. While short-chain PFAS were also produced earlier, their production increased when used as replacements. Therefore, samples higher in long-chain PFAS represent older source material, whereas samples higher in short-chain PFAS represent newer sources. Some PFAS are considered precursors and converted into others like those found in this study within the wastewater treatment plant.

Wastewater treatment plants are designed to remove three major contaminants: biochemical oxygen demand (BOD), which is essentially organic matter, particles and pathogens. The most common method for removal of these is activated sludge treatment where soil microbes are used in the plant and fed air and waste to chew up the material. This creates its own source of particles through dying microbes and digested organic matter waste. These particles settle out into sludge and are removed from the plant. The sludge can be treated to remove pathogenic organisms (usually through heat and UV by exposure to the sun) transforming it into biosolids.

Municipal wastewater treatment plants are not designed to remove anthropogenic chemicals like PFAS that are present at trace levels. In the plant, PFAS can partition between activated sludge and dissolved phase. Dissolved PFAS leaves the plant to receiving waters through the effluent. PFAS that binds to sludge remains in the biosolids. Many biosolids get applied to soils as a source of organic matter and nitrogen. These soils include agricultural fields, municipal fields, and can even be applied to residential areas as many biosolids are sold in home centers as milorganite, which gets its name from Milwaukee organic matter and nitrogen. The only readily practiced alternative is to burn biosolids. This is usually done as energy recovery, which does not destroy PFAS. No wastewater treatment plant incinerates their biosolids at a temperature high enough to destroy PFAS. A very expensive option is to landfill the biosolids in a lined landfill that would prevent leaching of PFAS into groundwater. Most wastewater treatment plants in the United States land apply their biosolids.


Suspected dischargers of PFAS that send wastewater to St. Cloud Wastewater Treatment Plant
St. Cloud WWTP
Suspected dischargers of PFAS
Industry type
Landfill and waste disposal facilities
Electroplating
Manufacturing
Petroleum bulk station
ewg


Biosolids and water sampling sites - St. Cloud WWTP
Sauk River
Johnson Creek
Clearwater River
St. Cloud waste water treatment plant
Biosolids land application sites
Sampling sites
ewg



Once applied to soils, PFAS can leach from the fields and enter receiving waters (both groundwater and surface water). PFAS can also be discharged directly from wastewater treatment plants into receiving waters through their effluent. However, because MPCA does not require wastewater treatment plants to monitor biosolids or effluent for PFAS, the extent of contamination from these wastewater sources is largely unknown. In order to try and address this question. We sampled the Mississippi River upstream of a major municipal wastewater treatment facility and directly in the channel receiving its effluent. We also sampled three small rivers in Central Minnesota that had varying degrees of biosolids application to their watersheds. Each of these rivers flow into the Mississippi River, upstream of the sampling sites on that river. The dissolved phase of these waters were analyzed from each collection point in triplicate to determine the concentration of 19 PFAS including five of the six for which there is a maximum contaminant level (MCL) proposed by the USEPA: perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), perfluorohexane sulfonate (PFHxS) and perfluorobutane sulfonate (PFBS). The only PFAS on the list not analyzed was Gen-X.

The total PFAS concentration (sum of 19 individual compounds) varied by location. The highest concentration was observed in the channel receiving effluent from the Metro Plant in St. Paul (Figure 1). This concentration was dominated by PFBS (Figure 2), however even if one were to ignore PFBS, this site would still have the highest concentration of PFAS. The second highest concentration of total PFAS was in Clearwater River in Central Minnesota and the Mississippi River upstream of the St. Cloud wastewater treatment plant. Clearwater River receives the greatest number of biosolids applications within its watershed. It is not surprising that the Mississippi River upstream of the wastewater treatment plant would have slightly lower concentrations than Clearwater River because much of the rest of the watershed upstream of that sampling point does not contain biosolids application sites. The lowest concentration of PFAS was found for the Sauk River, a watershed that did not receive biosolids applications.

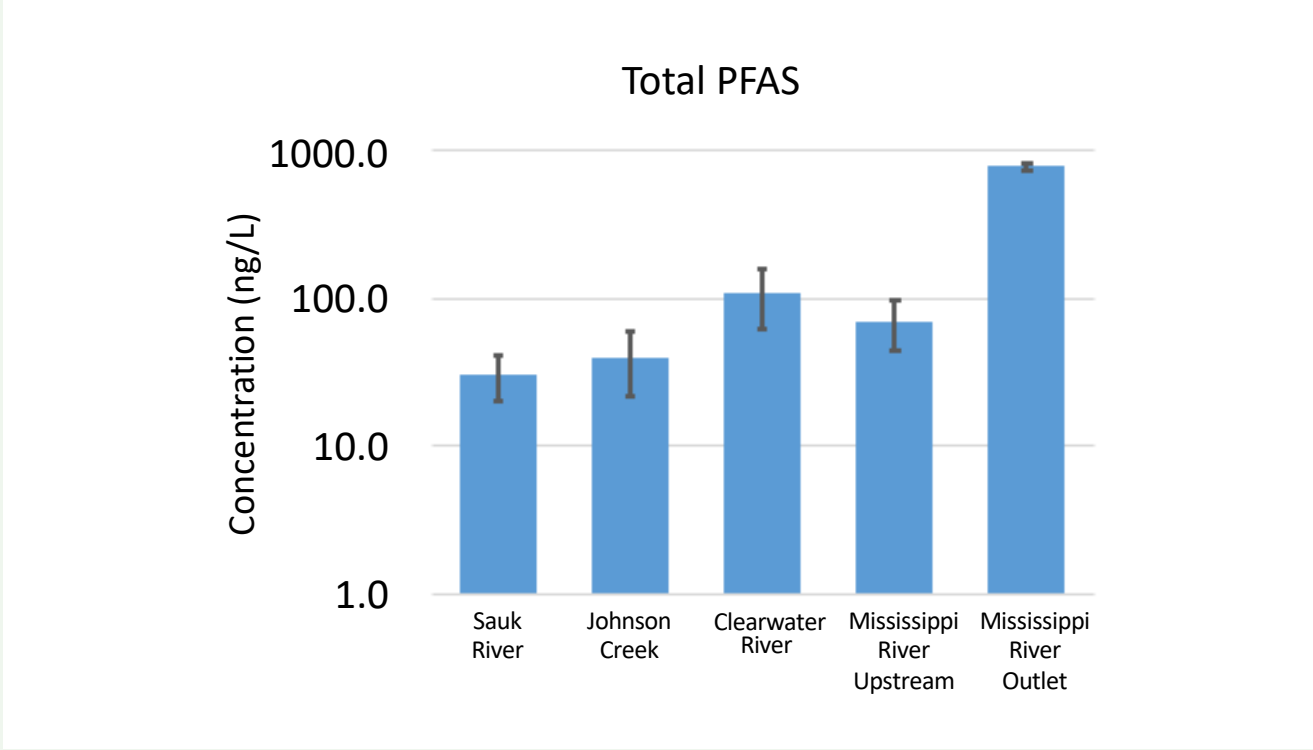


Figure 1. Concentrations (ng/L) of total PFAS (sum of 19 individual compounds) in Rivers in Minnesota.

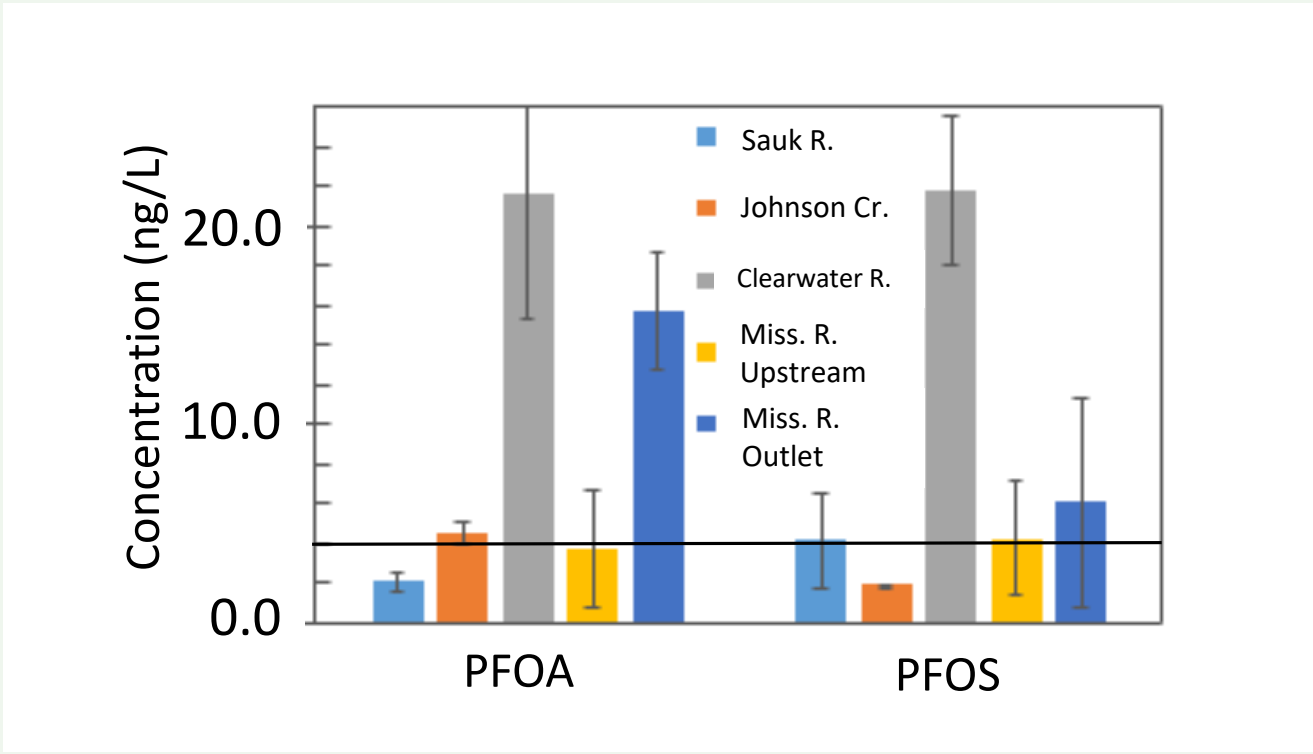


Figure 2. Concentrations (ng/L) of PFOA and PFOS (MCL of 4.0 ng/L indicated by line).

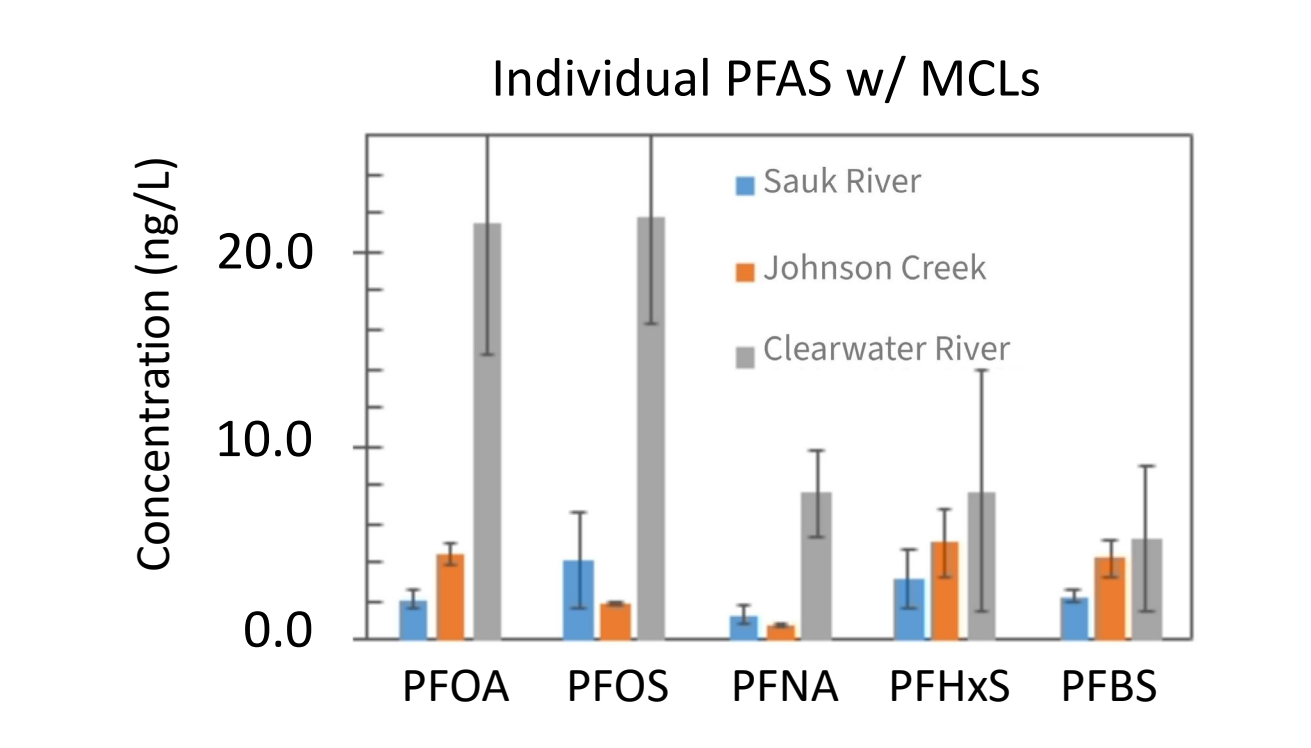


Figure 3. Concentrations (ng/L) of PFAS in St. Cloud waterbodies for which there is a MCL of 4.0 ng/L (indicated by line).

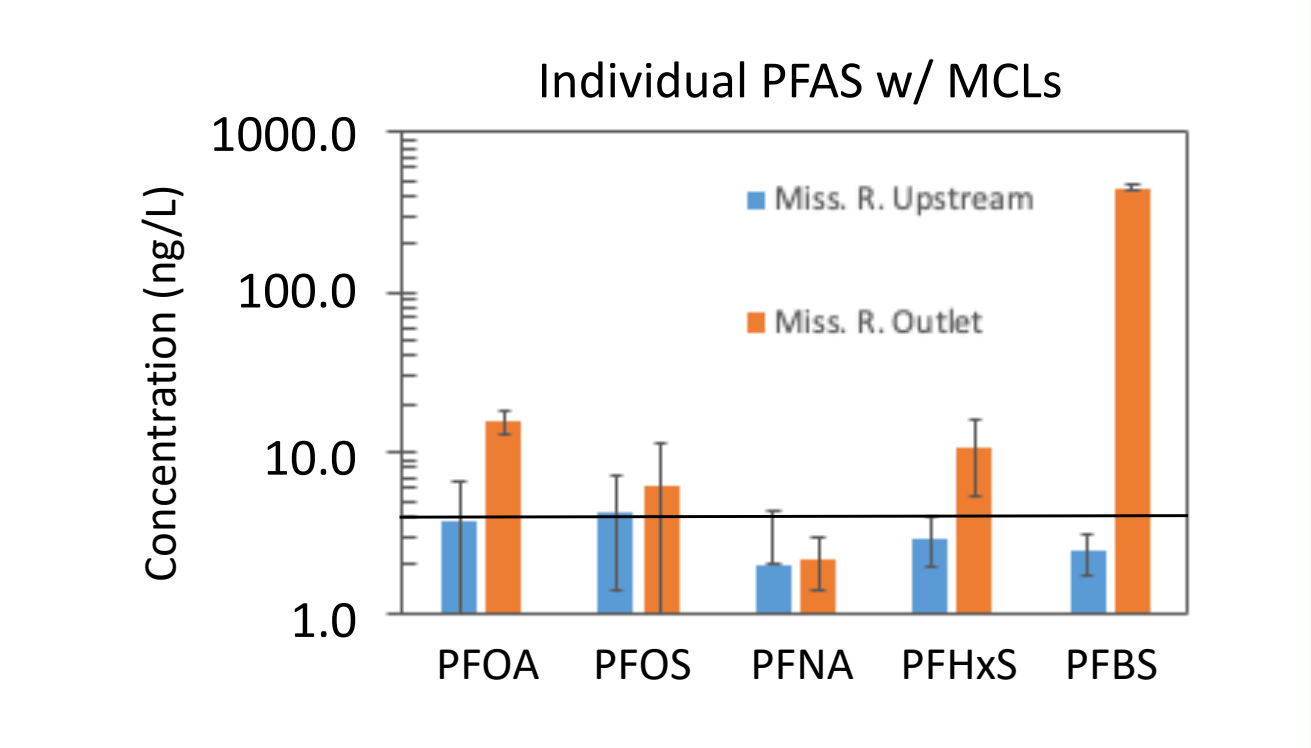


Figure 4. Concentrations (ng/L) of PFAS in the Mississippi River for which there is a MCL of 4.0 ng/L (indicated by line).

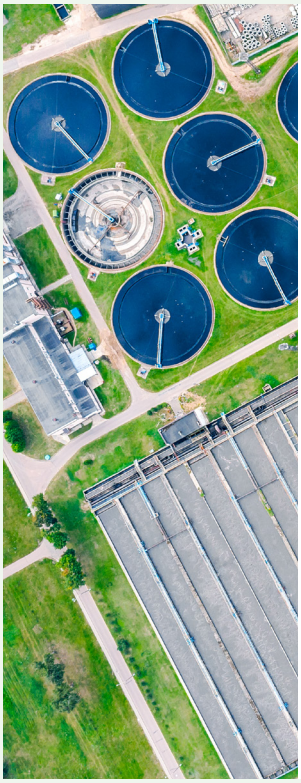
PFAS Production

Two PFAS that are of greatest environmental and toxicological concern are PFOA and PFOS. Clearwater River and the outlet of the St. Paul wastewater treatment plant are above the proposed MCL of 4.0 ng/L, while the other sites are at or below the MCL (Figure 2). In fact, Clearwater River is higher than the outlet of the St. Paul wastewater treatment plant, indicating that historical biosolids application could be a larger source of PFOA and PFOS to a watershed than current effluent from a wastewater treatment plant. The lack of PFOA and PFOS from rivers without high biosolids application in their watersheds indicates a lack of other sources to those rivers.

Many of the five PFAS for which there is a proposed maximum contaminant level (MCL) analyzed in this study were found above the proposed federal limit of 4.0 ng/L (Figure 3). The St. Cloud river with the fewest exceedances of that standard was the Sauk River, which as stated earlier does not have biosolids application in its watershed. The greatest exceedances were for Clearwater River. Again, this is not surprising given the numerous biosolids application sites in the watershed of Clearwater River.

Samples from the Mississippi River indicate a higher concentration of PFAS from the outlet of the St. Paul wastewater treatment plant with most exceeding the MCL (Figure 4). The dominant PFAS is PFBS. As mentioned earlier, PFBS is a short-chained PFAS that has been used as a replacement for PFOS. Therefore, it is not surprising that effluent from a wastewater treatment plant would be high in PFBS as it may still be in use by customers sending their waste to the plant.

It is clear from the data of this study that both wastewater treatment plant effluent and biosolids application to soils are significant sources of PFAS to watersheds in Minnesota. Because PFAS use is not limited to Minnesota, these sources are expected to be relevant across the country. It is imperative that we develop improved wastewater treatment technology to remove trace pollutants such as PFAS without diminishing the ability of the plants to remove BOD, particles and pathogens. In the interim, applying biosolids to agricultural fields where PFAS can be taken up by plants and leach into ground and surface waters should be reconsidered as a disposal technique for these materials. Furthermore, burning of biosolids is not a viable destruction technique for PFAS unless the temperatures are much higher than currently in practice.



EPA Biosolid Regulation

Biosolids have been regulated by the EPA under the Clean Water Act since 1993. Nationwide, biosolids are either land applied as fertilizer and soil amendments, placed in landfills, or incinerated.[34] The EPA regulates biosolids through 40 CFR Part 503, Standards for the Use or Disposal of Sewage Sludge. Under this rule, the EPA has the authority to set pollutant limits for hazardous or toxic components of biosolids that pose harm to human health and the environment. Currently, the EPA only sets pollutant limits for nine heavy metals. In 2018, the EPA Inspector General released a Report on the Biosolids Program that identified 352 unregulated pollutants in biosolids, of which 61 were acutely hazardous, hazardous or priority pollutants in other EPA programs.[35] The EPA conducts a review of pollutants in biosolids every two years, which includes risk assessments, public data on pollutants found in biosolids, and identification of which pollutants exceed EPA's concern levels or pose a risk to human health.[36] This review then informs whether any pollutants should be updated in Part 503. Prompted by this review process, the EPA has announced that it will finalize a risk assessment for PFOS and PFOA in sewage sludge by the winter of 2024. Until this time, there are no federal risk assessments or limits for any PFAS substances found in biosolids. Which means that it is up to states and tribal governments to address PFAS in biosolids.

IV. Soil and Groundwater Contamination from Biosolids

Over the past 10 years, data has shown that the land application of biosolids is directly tied to the PFAS contamination of soil and groundwater. At this point, we can no longer ignore the reality that when you look for PFAS contamination from wastewater streams like biosolids, you will find it.

The discovery of PFAS contamination from land applied biosolids has led to devastating consequences for rural communities across the country. In 2016, a family farm in Maine voluntarily participated in an EPA program that found PFAS contamination on their farm linked to biosolids land application. PFAS was found in their cows and their milk supply, as well as the husband and wife's blood, and they were forced to close their multi-generational farm without any compensation for the chemical contamination.[37] Maine initiated a program to test sewage sludge from different wastewater treatment plants across the state and found at least one PFAS chemical in all 44 samples they collected. The results led to the 2022 passage of a bill that banned the use of PFAS-contaminated biosolids for land application in the state.[38]

When other states have tested their own wastewater streams, the results have been similar. In Michigan, for example, a 2018 study of 42 municipal wastewater treatment plants found PFAS compounds in virtually all samples, which included influent, effluent, and biosolids.[39] Consistently, PFOA and PFOS concentrations in the effluent and biosolids were higher than in the influent, which once again indicates that the wastewater treatment process itself can increase the concentration of PFAS compounds.

Scientific studies have looked at the impact of long-term application of municipal biosolids on agricultural soils in the United States. What they have found is that biosolids from wastewater treatment plants with higher levels of industrial wastewater are connected to exponentially higher concentrations of long-chain PFAS like PFOA and PFOS in the soil.[40] These results emphasize the need to treat industrial discharges and reduce PFAS before it gets to the wastewater treatment plant, which can be done through pretreatment programs that target significant industrial users. MPCA can leverage its authority under the Clean Water Act permitting programs to require pretreatment for industrial users who send their water to wastewater treatment facilities. The objectives of the pretreatment program are to "prevent the introduction of pollutants into [publicly-owned treatment works (POTW)] which will interfere with the operation of a POTW, including interference with its use or disposal of municipal sludge."[41] Pretreatment programs are commonly used to remove the contaminants that the EPA regulates from industrial wastewater, but are not required for PFAS in Minnesota. Other states, like Michigan, have successfully leveraged this authority to address PFAS pollution from industrial sources, and Minnesota can do the same.

Academic research confirms that at sites where biosolids have been land applied for decades, PFAS substances have the ability to leach from the surface, through the soil profile, and into groundwater. In terms of whether PFAS contamination in the soil has the potential to contaminate groundwater, factors like water table depth and soil type are important drivers of risk.[42] Even though they have now largely been phased out of domestic production, legacy PFAS like PFOS and PFOA tend to be found in soil and groundwater in the highest concentrations, because they have been manufactured for the longest. This indicates that historical, long-term use of biosolids to amended soil has a positive correlation with increased levels of PFAS in the soil and in the groundwater below.[43] The research indicates that if we continue to land apply biosolids, we will see more water contamination from newer, short-chain PFAS that have had less time to impact the environment than their legacy counterparts.

PFOS and PFOA are the same two compounds that the EPA found are "likely to be carcinogenic" to humans in the proposed national drinking water regulations. Because Minnesota draws about 80% of its drinking water from groundwater, the inescapable conclusion is that the land application of biosolids can contaminate soil and groundwater with PFAS compounds that endanger public health. This is especially risky for private well owners, who tend to draw their water from shallower aquifers and do not have any of the regulatory protections that people on public water supplies have. Land-applied biosolids also pose significant risks to crops, another pathway for human consumption. Data released by the EPA shows that multiple PFAS substances can transfer into the edible portions of plants when soil is amended with biosolids.[44]

At the state level, agencies and legislatures may enact even stricter regulations for biosolids in land application. Maine and Vermont have revised their state adoption of Part 503 to require measures that address PFAS contamination from biosolids. The approach has been dramatically different in Minnesota. While Minnesota does have state regulations in place for biosolids, none address PFAS. As it currently stands, Minnesota does not consider any PFAS substances to be pollutants under its Sewage Sludge Management Rule, and Minnesota law allows biosolids produced both within and out of state to be applied on agricultural lands with no requirements to test the biosolids or the sites where they are land applied for PFAS contamination.[45] While other states like Maine have launched comprehensive investigations of sites where municipal biosolids were applied to determine the extent of soil and groundwater contamination, Minnesota does not even require municipal wastewater plants to test biosolids for PFAS before they are land applied. In other words, Minnesota is in the dark about the scope of its PFAS problem when it comes to biosolids.

This can be fixed. In its Sewage Sludge Management Rules, MPCA defines a "pollutant" to include any organic or inorganic substance that "after discharge and upon exposure, ingestion, inhalation, or assimilation into an organism either directly from the environment or indirectly by ingestion through the food chain, could, on the basis of information available to the administrator of EPA, cause death, disease, behavioral abnormalities, cancer, genetic mutations, physiological malfunctions . . . or physical deformations. . . ." [46]

Given the concerns identified by the EPA in its proposed MCLs and Hazard Index for six PFAS substances, and its determination that PFOA and PFOS are "likely carcinogenic," these six PFAS compounds fit squarely within MPCA's definition of what should be included as a "pollutant" under the rule.

The data from academic research and other states is clear: until we list PFAS as a pollutant and begin to monitor and test any biosolids that are land applied, we will likely continue to contaminate our soils and groundwater with PFAS. As Minnesota and the federal government work to develop regulations to protect drinking water from PFAS pollution, it is critical that Minnesota take explicit steps available now to address PFAS contamination in biosolids. One of the most immediate and effective ways to do this is to list PFAS as a pollutant under our state Sewage Sludge Management Rules. At a minimum, Minnesota should begin to test municipal biosolids at least annually for PFAS substances that are determined to pose a risk to human health and start to develop more comprehensive data on the risk of PFAS contamination in groundwater from land applied biosolids in different regions of the state.

V. Regulatory Frameworks for Wastewater

Minnesota's Response to PFAS in Wastewater Falls Short of EPA Guidance

At the federal level, EPA has committed to move on multiple fronts to provide regulatory tools to remove PFAS from wastewater streams.[47] One tool, effluent limitation guidelines, will restrict PFAS discharges from industrial sources. Once finalized, industrial sources will be required to institute technology-based pollution limits to remove PFAS from their wastewater discharges. However, it is unclear when EPA will finalize effluent limitation guidelines. In the interim, the agency has encouraged states to use their full authority under the Clean Water Act to control and ultimately reduce the amount of PFAS discharged from permitted facilities.

In December of 2022, EPA released a guidance Memorandum to states as part of its own PFAS Strategic Roadmap. The memo stresses the need for states to use their authority under the Clean Water Act to help wastewater treatment plants reduce PFAS in waste systems. EPA recommends that wastewater treatment plants:

- Monitor influent, effluent, and biosolids for the presence of PFAS at least quarterly;
- Inventory all industrial facilities that are expected or suspected discharges of PFAS. Once the industrial sources are identified, require these industrial sources to monitor their discharges quarterly for the presence of PFAS;
- Use pretreatment program authority to develop local limits, best management practices, or other controls at the industrial facility to control PFAS before it is discharged to the wastewater treatment facility.[48]

States with Clean Water Act authority can **require** wastewater treatment plants to monitor influent, effluent, and biosolids on a quarterly basis – information that will help determine whether pretreatment programs are necessary to reduce and remove PFAS from wastewater influent. Finally, EPA recommends that states with Clean Water Act authority consider site-specific technology-based treatment requirements on a best professional judgment basis and/or water-quality based effluent limits to meet state water quality criteria for PFAS.

The Minnesota Pollution Control Agency is the Clean Water Act Authority in Minnesota

The Clean Water Act is the main federal law governing water pollution, and in Minnesota, the regulatory authority is MPCA. The Clean Water Act functions primarily through a permitting system known as the National Pollutant Discharge Elimination System ("NPDES") permit, which authorizes a facility to discharge pollution into surface water. This permitting system, and the Clean Water Act more broadly, only applies to "point sources," discrete conveyances such as a pipe, ditch, or container. These permits include limits for pollution discharges, monitoring and reporting requirements, and other provisions to ensure the surface water receiving the discharge does not degrade in quality. Minnesota also uses the State Disposal System ("SDS") permitting system to regulate water discharges to protect groundwater, which includes similar limitations, monitoring requirements, and other provisions to ensure groundwater is not adversely impacted from pollution.

In Minnesota, MPCA regulates the design, construction, and operation of industrial and municipal wastewater treatment facilities. Through the NPDES/SDS permit program of the Clean Water Act, MPCA can establish specific limits and requirements to protect Minnesota's surface and groundwater from industrial contamination. This means that MPCA can leverage its NPDES/SDS authority now to ensure that wastewater treatment facilities test and monitor influent, effluent, and biosolids for contaminants like PFAS. MPCA can also require pretreatment programs for industrial users who send their water to wastewater treatment facilities.

Minnesota has declined to follow all of EPA's suggestions. Currently, MPCA does not have any mandatory PFAS pollution control terms in wastewater permits, and MPCA is asking wastewater treatment plants to voluntarily monitor their influent. MPCA's approach is spelled out in a Memorandum of Understanding, where MPCA asks facilities to collect four samples of influent by the end of 2024; inventory industrial users that may be potential contributors of PFAS to the wastewater collection system by the end of 2023; and submit a PFAS Pollutant Management Plan to MPCA by March of 2024 to identify pollution prevention strategies. MPCA is not, however, requiring these facilities to test their effluent or biosolids or requiring any PFAS limits be included directly in the NPDES permit. Additionally, either MPCA or the wastewater treatment facility can terminate the MOU at any time for any reason, eroding what little confidence there is that MPCA is doing everything to tackle this problem.

Despite federal guidance and success stories from places like Michigan (detailed Section VI), Minnesota's approach to controlling PFAS discharges from wastewater treatment plants is inadequate for four main reasons.

First, the wastewater treatment process does not destroy the fluorine-carbon bond that is the hallmark of PFAS' durability. PFAS that enter the wastewater treatment plant, therefore, are either discharged in the effluent–which is frequently discharged directly into surface waters that are sources of drinking water for millions of Minnesotans–or are present in the biosolids that are spread on agricultural fields across the state. Relatedly, certain PFAS transform into "terminal" PFAS, like PFOS or PFOA, as the chemicals proceed through the wastewater treatment process. This means that influent sampling presents an incomplete picture about the PFAS that are being released into the environment. In Michigan, for example, regulators found higher concentrations of certain PFAS in the effluent of the wastewater treatment plant than in the influent.[49]

The second primary issue is when Minnesota is addressing PFAS in wastewater. MPCA currently possesses the regulatory authority to require certain industrial users to pretreat their industrial wastewater before discharging to the wastewater treatment plant. Under the Clean Water Act, wastewater treatment plants are empowered to establish pretreatment programs to help prevent "pass through" discharge of pollutants.

This program works by requiring the industrial user to take steps to remove PFAS from their wastewater before it is discharged to the wastewater treatment plan. Michigan has been requiring PFAS source reduction at locations that knowingly use PFAS to great success, and federal guidance recommends wastewater treatment plants develop best management practices to limit PFAS discharges from industrial sources. Minnesota should do the same, and require industrial facilities known to discharge PFAS to implement pollution management practices on-site before discharging their wastewater to the treatment plant.

Third, MPCA's approach relies solely on voluntary agreements to monitor PFAS. Rather than placing treatment and monitoring requirements in the permits it issues to wastewater dischargers, the MPCA has entered into voluntary "memorandums of understanding" with certain large wastewater treatment systems suspected of processing fluids and solids contaminated with PFAS. Because it has not included limits for PFAS discharges or required PFAS reduction strategies in the permits, MPCA has not exercised its regulatory authority to control PFAS discharges from wastewater treatment plants. The Memorandum of Understanding "can be nullified by either party at any time."[50] MPCA should instead include limits or controls to reduce PFAS discharges directly in the permits it issues to wastewater treatment plants. By placing such requirements in permits, MPCA retains regulatory authority to ensure adequate steps are taken to monitor and reduce PFAS contamination in the effluent and biosolids coming from our state's wastewater treatment plants.

Finally, MPCA's decision not to sample biosolids for PFAS means that agricultural fields, adjacent surface waters, and the crops growing on the fields are at risk of being contaminated with PFAS. By failing to collect this important data, MPCA is ignoring a major PFAS pathway with the potential to contaminate drinking water and our environment. Some states, like neighboring Wisconsin, are sampling effluent and biosolids to better inform the state's pollution reduction strategies.[51] And guidance from the EPA recommends states monitor wastewater effluent and biosolids for the presence of PFAS. Minnesota must start monitoring effluent and biosolids to better understand where PFAS are entering our environment.

Haw River Assembly settlement w/ Burlington Wastewater Treatment Plant

In 2019, the Southern Environmental Law Center ("SELC") and a local group in Burlington, North Carolina filed a "notice of intent to sue" letter with the City for unauthorized PFAS discharges from its wastewater treatment plant into the Haw River. The letter included public data about historical concentrations of PFAS in the wastewater treatment plant's influent and independent sampling from several sites in the Haw River and the wastewater treatment plant's effluent. The testing revealed extreme differences in PFAS contamination in the Haw River upstream and downstream of the facility, with downstream PFAS concentrations nearly 40 times greater. This data, the letter asserted, evidenced that the facility was discharging PFAS directly into the Haw River without a permit in violation of the Clean Water Act.

In August of 2023, the city of Burlington agreed to settle the matter. The agreement documented three likely industrial sources that were discharging PFAS to the wastewater treatment plant and explained steps Burlington would take to ensure the facilities either ceased using PFAS or implemented pretreatment programs to control their discharge.[52] These are tools presently available to wastewater treatment plants under the Clean Water Act and recommended by the EPA. Importantly, the costs of implementing these treatment technologies are levied upon the industrial user, who must limit PFAS in the wastewater it discharges to the wastewater treatment plant.

VI. Models from Other States

- What approaches have other states taken to proactively address PFAS contamination from wastewater streams?

Maine

Effective August 8, 2022, Maine became the first state to place a ban on the land application of biosolids.[53] This ban was in response to an increase in testing and data, finding that biosolids land application was a critical pathway to PFAS exposure leading to contaminated water, milk, and food. While Maine had already passed a PFAS non-essential use ban and PFAS specific water quality standards, the Maine legislature recognized that exposure to PFAS through biosolids still presented a public health threat because biosolids land application was directly linked to soil and groundwater contamination in several rural communities.[54]

Maine's biosolids ban requires that no new licenses be issued for land application of biosolids that are either septic sludge themselves or come from products, such as compost, where septic sludge has been incorporated. The ban additionally prohibits the sale of these biosolids intended for land application. For those who already hold land application licenses, the ban requires that groundwater and drinking water near the land application location be tested for PFAS. If testing finds an exceedance of water quality standards, land application is prohibited. PFAS substances are defined to include any "fluorinated organic chemicals containing at least one fully fluorinated carbon atom" that can reasonably be quantified in a laboratory.

As part of its response to the PFAS crisis, in January of 2023, Maine's legislature also enacted S.P. 92, an emergency order requiring the testing of wastewater effluent for PFAS. The order requires that any entity who is licensed to discharge effluent into groundwater or any waters of the state, must not only test for PFAS, but also pay the cost to test themselves. There are caveats in the order where the cost burden may shift to the State of Maine, but at its crux, this is an example of PFAS producers, rather than taxpayers, bearing the burden of PFAS contamination.

Vermont

In 2019, Vermont passed Act 21, which requires its water providers to test for PFAS. The Act then mandates a continuous testing schedule, dependent on initial results. Vermont also has some of the strictest drinking water standards for PFAS. On March 17, 2020, a revised Vermont Water Supply Rule was issued to limit the concentrations for PFOA, PFOS, PFHxS, PFHpA, and PFNA to not exceed 20 ppt in aggregate. The rule also requires the public to be notified when these limits are exceeded. One of the most progressive actions Vermont has taken to address PFAS from wastewater streams has been through its biosolids regulations. Vermont requires that all Environmental Quality ("EQ") biosolids be labeled as potentially containing PFAS.[55] EQ biosolids are those solids derived from domestic waste or dairy waste that have been screened for pathogens and are intended for sale and land application.

Additionally, any biosolids, septage, or EQ biosolids must be tested at least annually for PFAS substances that are either already regulated or are determined to pose a risk to human health or the health of living organisms.[56] And depending on the facility's certification, soil, groundwater, and plant tissue must also be tested for PFAS at least once per year.[57]

Michigan

Michigan is addressing the PFAS problem on multiple fronts. In 2020, the Michigan Department of Environment, Great Lakes, and Energy (EGLE) promulgated the strictest rules regulating PFAS in drinking water in the nation. After the rules became effective, 3M sued, arguing that the rules should be invalidated because the Department failed to consider the costs for businesses to comply with related groundwater-cleanup standards that automatically resulted from the new drinking water rules, in other words – the costs of compliance.[58] The Michigan Court of Appeals agreed with 3M and invalidated the rules, concluding that the state failed to properly consider costs before finalizing the rules. After the ruling, a spokesperson for the EGLE complained that the lawsuit is evidence of the length that 3M, one of the parties most responsible for PFAS contamination in the world, will go to avoid confronting its responsibility for the PFAS problem.

On the wastewater front, Michigan started confronting the problem in 2018, when it studied 95 wastewater treatment plants that were required by their NPDES permit to implement industrial pretreatment programs (IPP). By 2020, the EGLE concluded that "there is significant evidence to support that utilizing the established authorities under the IPP to identify and control industrial sources of PFAS (specifically PFOS) to wastewater treatment plants is highly effective at reducing the discharge of this pollutant into the environment."[59] After the study was expanded to look at PFAS in biosolids, results showed that six wastewater treatment plants produced biosolids with high levels of PFAS. Land application of biosolids from those facilities was ceased, and implementation of screening technologies upstream from the plants through pretreatment programs dramatically lowered the amount of residual PFAS that ended up in biosolids from those facilities. The study also looked at fields that had received biosolids from wastewater treatment plants and, unsurprisingly, sites that received biosolids from the six plants previously mentioned showed the greatest levels of contamination.

VII. Recommendations

Minnesota has a lot of urgent work to do to build on the PFAS-source reduction laws our state legislature passed in 2023. In June of 2023, MPCA released a report on the exorbitant costs to remove PFAS from wastewater streams across the state, which it estimates will cost $14 - 28 billion over the next 20 years.[60] The report acknowledges that wastewater streams and solid waste management systems are "key routes" for PFAS to enter the environment, and confirms that "[t]o date, none of the biosolids management techniques practiced in Minnesota destroy PFAS."[61] The report recognizes that the cost per mass of PFAS destroyed is lower for higher-concentration waste streams like biosolids, and that treatment is much more cost effective at "upstream" facilities like industrial dischargers, where the contamination is more concentrated, than at municipal wastewater treatment facilities that receive blended influent. [62]

MPCA has stated that PFAS removal and destruction from municipal wastewater will be unaffordable for the foreseeable future, and that pollution prevention and source reduction are the best path forward.[63] MCEA agrees that source reduction through the non-essential use ban is a critical step to "turn off the tap" on PFAS production. However, state agencies must also take steps to remediate the PFAS that is already pervasive in the environment and continues to be discharged from wastewater streams every day. There is also the bottom line of what we need to do to protect public health: as federal and state governments propose new regulations to protect drinking water sources, Minnesota agencies must use the tools available under our bedrock environmental laws to ensure that responsible parties bear the burden of pollution clean up to the extent possible, and that the costs aren't externalized to the public.

In this report, we have identified some of the steps that the MPCA can take now, through its Clean Water Act authority, to better understand the scope of PFAS contamination from wastewater streams and ensure that responsible parties bear the costs of pollution clean-up wherever possible. To protect Minnesota's communities from further damage caused by the toxic effects of PFAS on human health, our recommendations are to:

- Add PFAS as a pollutant under the Minnesota Sewage Sludge Management Rule;
- Require wastewater treatment plants to monitor influent, effluent, and land applied biosolids for PFAS so we can better understand the scope of contamination;
- Use pretreatment programs to require industrial dischargers to use best management practices and treatment options to reduce and remove PFAS from industrial wastewater before it reaches municipal wastewater treatment plants;
- Label Class A EQ biosolids sold for public distribution as potential sources of PFAS;
- Investigate sensitive sites (based on soil type/hydrology) where biosolids have been land applied for decades for legacy soil and groundwater contamination;
- Require PFAS data in the environmental review (Minnesota Environmental Policy Act) process, such as the Met Council wastewater treatment plant's proposed addition of a fourth incinerator;
- Monitor ambient groundwater for PFAS contamination from landfill leachate and land applied biosolids;
- Develop strong statewide Class 1 Water Quality Standards that mirror the proposed federal Maximum Contaminant Levels (MCLs) for 6 PFAS compounds.

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