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Emerging
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compounds in the
watercycle

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Aanpak: methoden-ontwikkeld voor polaire en semi polaire PFAS

De zoektocht naar analysemethoden voor het zeer polaire F₃-MSA en het semi-polaire HFPO-DA is begonnen met een literatuurstudie, waarna voor beide verbindingen aparte methoden ontwikkeld zijn. Deze methoden zijn ingezet in een meetcampagne in Nederland en België, waarbij monsters uit verschillende drinkwaterbronnen en uit drinkwater zijn onderzocht.

Resultaten: methodeontwikkeling, meetcampagne, toxicologische evaluatie en suspect screening.

Methodeontwikkeling

Uit de literatuurstudie bleek dat het met één methode niet mogelijk is om zowel het zeer polaire F₃-MSA als het semi-polaire HFPO-DA te meten: daarvoor loopt de polariteit te ver uiteen. Gekozen is voor twee methoden, die voorafgegaan worden door dezelfde monstervoorbewerking met vaste-fase-extractie. De analysemethode voor F₃-MSA is gebaseerd op *mixed-mode* chromatografie, waarbij het kolommateriaal bestaat uit een hydrofoob en een anionwisselaar-deel. Dit geeft een goede retentie voor het zeer polaire F₃-MSA. De analysemethode voor HFPO-DA is gebaseerd op *reversed phase* chromatografie en geeft ook een goede retentie. Voor de detectie van F₃-MSA en HFPO-DA is gekozen voor de Orbitrap Fusion hoge resolutie massaspectrometer, vanwege de goede gevoeligheid en de mogelijkheid om retrospectief naar nieuwe (perfluor-)verbindingen te screenen. Beide ontwikkelde methoden zijn reproduceerbaar en hebben goede aantoonbaarheids- en rapportagegrenzen. De rapportagegrenzen voor F₃-MSA en HFPO-DA zijn respectievelijk vastgesteld op 1 en 0,2 ng/L. UvA-IBED heeft een bestaande analysemethode voor neutrale en zure PFAS verder ontwikkeld voor FOSA, die is gebaseerd op *reversed phase* LC-tandem-massaspectrometrie, die een goede reproduceerbaarheid, aantoonbaarheidsgrens en rapportagegrens geeft. De rapportagegrens voor FOSA is vastgesteld op 0,25 ng/L.

Meetcampagne

In september 2017 is met de ontwikkelde analysemethoden een meetcampagne uitgevoerd in Nederland en België, waarbij 53 monsters zijn genomen van 11 drinkwaterbedrijven. FOSA is niet aangetroffen in ruw en drinkwater. HFPO-DA was aanwezig in bijna de helft van alle genomen drinkwatermonsters, met concentraties tussen 0,2 en

28 ng/L (gemiddeld 2,9 ng/L). HFPO-DA is niet aangetroffen in grondwater, maar was wel aanwezig in de meerderheid van alle oppervlaktewater-, oeverfiltraat- en duinfiltraat-monsters. F₃-MSA is aangetroffen in de meerderheid van de drinkwatermonsters, in concentraties van 1 tot 150 ng/L (gemiddeld 24 ng/L). F₃-MSA is niet teruggevonden in grondwater, maar was wel aanwezig in alle bemonsterde oppervlaktewater-, oeverfiltraat- en duinfiltraatmonsters. Verder is aangetoond dat HFPO-DA en F₃-MSA niet of onvolledig verwijderd worden door de toegepaste drinkwaterzuiveringen, met uitzondering van *reverse osmose*. Dit houdt in dat het merendeel van de toegepaste zuiveringstechnieken geen robuuste barrière vormt tegen HFPO-DA en F₃-MSA.

Toxicologische evaluatie

Voor de drie PFAS is een toxicologische evaluatie uitgevoerd, en zijn voorlopige richtwaarden voor drinkwater afgeleid voor F₃-MSA en FOSA van respectievelijk 11,9 mg/L en 0,01 µg/L. Het RIVM had de richtwaarde voor HFPO-DA al eerder op 0,15 µg/L gesteld. Alle aangetroffen concentraties voor F₃-MSA en HFPO-DA in drinkwater liggen beneden de afgeleide richtwaarden voor drinkwater, alhoewel de *margin of exposure* voor HFPO-DA relatief gering is.

Screening

Tijdens een suspect screening zijn ook vijf andere gehalogeneerde methaansulfonzuren (Cl₂-MSA, Cl₃-MSA, Br-MSA, Br₂-MSA en BrCl-MSA) waargenomen in de waterketen, in concentraties van 1 tot 148 ng/L (F₃-MSA equivalenten). Gehalogeneerde methaansulfonzuren komen dus voor in de Nederlandse en Belgische waterketen, wat verder onderzoek naar deze stoffen raadzaam maakt.

Implementatie: ontwikkelde analysemethoden PFAS toepassen voor monitoring

De ontwikkelde analysemethoden zijn succesvol toegepast voor de meetcampagnes van HFPO-DA, FOSA en F₃-MSA. Alle drie de methoden kunnen worden ingezet voor periodieke waterkwaliteitsmonitoring en kunnen op verzoek worden geïmplementeerd bij een drinkwaterlaboratorium.

Rapport

Dit onderzoek is beschreven in het rapport *Emerging (per)fluorinated compounds in the watercycle* (BTO 2018.061).

Meer informatie

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Summary

Perfluoroalkyl substances (PFAS) have recently gained interest of the water companies because of their persistence in the environment and their occurrence in sources of drinking water. Both perfluoro octanoic acid (PFOA) and perfluoro octane sulfonic acid (PFOS) are notorious because of their occurrence, and are poorly removed from the drinking water production chain by conventional purification processes. Recent studies have shown that substitutes of PFOA, such as HFPO-DA (FRD-903, constituent of GenX) have been observed in locations where PFOA has been previously reported to be present in surface water. FOSA is another PFAS frequently reported to be present in surface waters, which is not included in regular monitoring programs in the Netherlands. A third relatively poorly known PFAS is the polar trifluoromethanesulfonic acid (F_3 -MSA), which has been recently observed in several locations in Europe up to 1 $\mu\text{g/L}$. The occurrence of these relatively new PFAS in the watercycle initiated the present study.

In the present study analytical methodologies were developed for F_3 -MSA, HFPO-DA, and FOSA in order to determine the occurrence of these PFAS in the watercycle, by means of a sampling campaign. A reversed phase C18 LC-MS/MS method was developed for FOSA by UVA-IBED, yielding satisfactory LOD and LOQ results. For the polar F_3 -MSA and HFPO a mixed-mode and a reversed phase C18 method were developed, respectively, using a high resolution Orbitrap Fusion mass spectrometer for detection, yielding satisfactory LOD and LOQ results for both F_3 -MSA and HFPO-DA.

A sampling campaign for F_3 -MSA, HFPO-DA and FOSA was conducted in September 2017 for The Netherlands and Belgium. A total of 53 samples were collected from 11 drinking water companies. FOSA was not observed in raw and drinking water. HFPO-DA was present in almost half of the drinking water stations that were sampled, with concentrations ranging from 0.2 to 28 ng/L, and an average concentration of 2.9 ng/L. HFPO-DA was not observed in groundwater, but was present in the majority the surface waters and river bank filtrate/dune filtrates. F_3 -MSA was present in the majority of the drinking water samples collected, with concentrations ranging from 1 to 150 ng/L, and an average concentration of 24 ng/L. In none of the sampled groundwaters F_3 -MSA was present, but it was observed in all surface waters and river bank filtrate/ dune filtrates sampled, showing that it is abundantly present in raw water. Furthermore, it was shown that HFPO-DA and F_3 -MSA are not or incompletely removed by the majority of drinking water purification processes applied, with the exception of reverse osmosis. Most of the applied drinking water processes do not constitute a robust barrier for HFPO-DA and F_3 -MSA.

A toxicological evaluation was performed in which provisional health-based drinking water guidelines were derived for F_3 -MSA and FOSA of 11.9 mg/L, and 0.01 $\mu\text{g/L}$, respectively. The provisional guideline value for HFPO-DA was previously derived by RIVM at 0.15 $\mu\text{g/L}$. All concentrations observed for F_3 -MSA, HFPO-DA in drinking water are lower than the derived provisional guideline values. The concentrations observed currently give no cause of concern for negative health effects due to drinking tap water, although the margin of exposure to HFPO-DA is relatively small (approximately a factor of five).

By means of a suspect screening five other halogenated methanesulfonic acids (Cl_2 -MSA, Cl_3 -MSA, Br-MSA, Br_2 -MSA and BrCl-MSA) were observed in concentrations ranging from 1 to 148 ng/L (F_3 -MSA eq.), demonstrating the presence of these other halogenated methanesulfonic acids in the watercycle.

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1 Introduction

Perfluoroalkyl substances (PFAS) have recently gained interest of the water companies. Both perfluoro octanoic acid (PFOA) and perfluoro octane sulfonic acid (PFOS) are notorious because of their persistence in the environment and their occurrence in sources of drinking water. PFOA and PFOS are poorly removed from the drinking water production chain by conventional purification processes, but can be removed with active carbon or by reverse osmosis. Major producers in Europe and the United States have reduced production and emissions of PFOA and PFOS either on a voluntary basis or by regulation (ban on PFOS) and, as a consequence, switched to short-chain or alternative PFAS. PFOS and PFOA continue to be produced in some other countries, e.g., China. For some of the short-chain perfluoro alkanic and alkyl sulfonic acids such as PFBA (PF butanoic acid) and PFBS (PF butane sulfonic acid) removal by active carbon is less efficient and incomplete [1].

Recent studies have shown that substitutes of PFOA, including a fluorinated ether (heptafluoro propoxypropanoic acid, HFPO-DA, also known as FRD-903, which is one of the constituents of GenX) have been observed in locations where PFOA has been previously reported to be present, among others in surface waters collected in the river Rhine delta [2-4]. FOSA (1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-1-octanesulfonamide) is another PFAS frequently reported to be present in surface waters [5-7]. Trifluoromethanesulfonic acid (F₃-MSA) is a third relatively poorly known PFAS that has been observed recently in several locations in Europe. F₃-MSA is a member of the group of highly persistent halogenated methanesulfonic acids that have been reported to occur in groundwater, surface waters and drinking water [8]. Until now, none of these three perfluorinated compounds (see table 1-1) are included in regular monitoring programs in The Netherlands.

HFPO-DA is a polar persistent compound with an estimated log K_{ow} value of 0.1, an aqueous solubility of 7.1 g/L and a half-life in water of 17280 h. According to REACH dossiers HFPO-DA is produced annually in volumes between 10 and 100 tons. In 2016 in river water downstream of a production location in Dordrecht, HFPO-DA was observed in concentrations up to 800 ng/L [4]. In the same study drinking water samples were analysed and a maximum concentration of 11 ng/L was reported [4]. In 2017 in a collaborative study by the Dutch water suppliers HFPO-DA was found in drinking water prepared from river bank filtrate originating from the river Beneden-Merwede, and levels amounted up to 30 ng/L in drinking water [2].

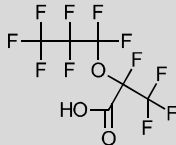
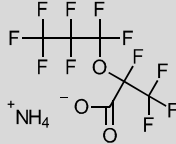
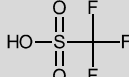
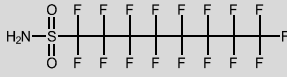
FOSA is a persistent compound (estimated half-life in water >4320 h) with a relatively high log K_{ow} value (>7). FOSA production by the 3M company has been terminated in the first decade of this millennium, but production continues in China.

F₃-MSA is registered in REACH with a production volume of more than 100 t/y. It is a highly persistent compound with an estimated log K_{ow} value of -0.49. In both groundwater and surface waters from Europe levels of more than 1 µg/L have been reported to be present, and concentrations of F₃-MSA in drinking water have been observed between 10 and 1000 ng/L [8].

These findings spurred the development and operationalisation of analytical methodologies for the three substances mentioned above. In addition a sampling campaign was carried out to assess the occurrence of the substances in relevant raw waters (including groundwater, surface waters, river bank and dune filtrates) and in drinking water. The study was completed

by a desk literature search on human toxicity data. The present report describes the methodologies developed, presents the results of the field survey and summarises the toxicological information available.

TABLE 1-1 TECHNICAL NAMES, STRUCTURES, CAS NUMBERS AND FULL MOLECULAR NAMES OF EMERGING FLUORINATED COMPOUNDS

Technical name	Structure	CAS	Full names	Note
HFPO-DA		13252-13-6	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid / heptafluoropropoxypropanoic acid / perfluoro[2-(n-propoxy)propanoic acid	synonym: FRD-903; precursor of FRD-902
FRD-902		62037-80-3	ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate	
F ₃ -MSA		1493-13-6	trifluoromethanesulfonic acid	
FOSA		754-91-6	perfluorooctanesulfonamide perfluorooctylsulfonamide heptadecafluorooctanesulphonamide	synonym: PFOSA

2 Development and validation of analytical methods for the determination of (per)fluorinated compounds

2.1 Introduction method development

In order to perform a sampling campaign for F₃-MSA, FOSA and HFPO-DA in the watercycle, first analytical methodologies have to be developed. For the method development and analysis of emerging perfluorinated substances in the watercycle, a collaboration was started with the Institute for Biodiversity and Ecosystem Dynamics (IBED, University of Amsterdam). IBED has extensive expertise in analysing perfluorinated substances in environmental samples [1, 9]. Because IBED already conducted initial research on the apolar FOSA, it was agreed that IBED would adapt their current methodology and optimise and validate it for FOSA analysis. KWR on the other hand would develop and validate analytical method(s) for the analysis of the polar F₃-MSA and HFPO-DA in drinking water, groundwater, and surface water.

2.2 Method development FOSA

The method development and optimisation of FOSA was performed by UVA-IBED. The analytical method developed is based on reversed phase C18 liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). For the sample pre-treatment a solid phase extraction (SPE) method was developed using weak anionic exchange (WAX) SPE Columns. The final analytical method for analysing FOSA in water is described in attachment I.

2.3 Method development F₃-MSA and HFPO-DA

A literature study was performed for analytical methodologies for the determination of F₃-MSA and HFPO-DA in water. This resulted in that multiple analytical methods were found for F₃-MSA [8, 10] and HFPO-DA [3, 4, 11]. These analytical methods were used as a starting point for method development.

One of the objectives for method development is to obtain low LOQs (< 1 ng/L) for F₃-MSA and HFPO-DA in drinking water, groundwater and surface water, in order to detect relevant concentrations during the sampling campaign. Furthermore it is preferred that only one analytical method is developed for both F₃-MSA and HFPO-DA, in order to keep analysis time and costs as low as possible.

2.3.1 F₃-MSA and HFPO-DA liquid chromatography method development

The high performance liquid chromatography method development for F₃-MSA and HFPO-DA was expected to be challenging, due to the difference in hydrophobicity between F₃-MSA (log D = -3.88) and HFPO-DA (log D = 1.34). Because F₃-MSA is highly polar, it is not possible to analyse this compound quantitatively using C18 reversed phase chromatography, due to a lack of retention. However, both compounds are relatively strong acids, meaning that they are always negatively charged (i.e. independent of the pH), which is a property that can be used for chromatographic separation. Therefore only analytical columns were considered for method development which have anion exchange as primary or secondary interaction for

chromatographic separation. The following three columns were selected for method development:

- Macherey-Nagel Nucleodor HILIC, 2 x 150 mm, 1.8 μm
- Dionex Acclaim Mixed-mode WAX-1, 2.1 x 150 mm, 3 μm
- SIELC Obelisc N, 2.1 x 150 mm, 5 μm

First the Nucleodor HILIC column was tested, which is the same column that was used by Zahn et al. for the identification and quantification of $\text{F}_3\text{-MSA}$ [8]. The Nucleodor HILIC column is a zwitterionic column which has positive charged ammonium ligands and also negative charged sulfonic acid ligands, which should be suited for the separation of hydrophilic and ionic analytes. Initially, for method development the chromatographic conditions were used as described in Zahn et al [8] (starting condition: 95% acetonitrile + 5mM ammonium formate at pH 3.0). This resulted in an almost unretained peak for $\text{F}_3\text{-MSA}$, which cannot be used for quantification purposes. Other mobile phase conditions were also tested, including adjusting the ammonium formate concentration (higher and lower) and starting percentage of acetonitrile, but no improvement in retention was made. Furthermore the column also showed severe column bleeding which resulted in a high background during mass spectrometry analysis. In the end the Nucleodor HILIC column was found suboptimal for $\text{F}_3\text{-MSA}$ analysis.

The second column that was tested was the Dionex Acclaim Mixed-mode WAX column. This column consists of hydrophobic alkyl chains to which an ionisable terminus is attached that provides weak anion exchange properties, which should be suited for retaining both $\text{F}_3\text{-MSA}$ and HFPO-DA. With low buffer concentrations (i.e. 5mM ammonium acetate) both compounds were retained strongly, resulting in long retention times and broad peaks. When the buffer concentration was increased to above 20 mM, reasonable retention was obtained, but unsatisfactory peak shape was obtained for $\text{F}_3\text{-MSA}$. This column also showed substantially bleeding during analysis. Therefore the decision was made to stop further testing of this column.

The third column that was tested was the SIELC Obelisc N column. Obelisc N is a zwitterionic column which has positive and negative charged functional groups attached to hydrophobic alkyl chains. The positively charged groups are placed near the terminus of the alkyl chain which provides the anionic exchange property of the column, while negatively charged groups are placed near the silica surface. This column was tested extensively using different organic modifiers such as acetonitrile and methanol, and varying ammonium acetate buffer concentrations and the column was tested in both the reversed phase and HILIC mode. The best results for $\text{F}_3\text{-MSA}$ were obtained by using the column in reversed phase mode and using methanol as organic modifier with ammonium acetate as buffer and 0.05% formic acid. However, for HFPO-DA unsatisfactory results were obtained under these conditions. The chromatographic retention and peak shape were sufficient for HFPO-DA, but the sensitivity decreased substantially (> 10x) due to the presence of formic acid. When no formic acid was added, $\text{F}_3\text{-MSA}$ could not be analysed. Therefore it was decided that two separate methods were needed for analysing both $\text{F}_3\text{-MSA}$ and HFPO-DA. The current Obelisc N method was further optimised for $\text{F}_3\text{-MSA}$ only, and a new method was developed for HFPO-DA using a C18 column (see 2.3.2).

Because no isotopically labeled internal standard was available of $\text{F}_3\text{-MSA}$, PFBA- $^{13}\text{C}_3$ was used as internal standard for quantification. The final mobile phase composition for mobile phase A was; ultrapure water with 10 mM ammonium acetate plus 0.05 v/v% formic acid. Mobile phase B consisted of methanol with 10 mM ammonium acetate plus 0.05 v/v% formic acid.

The applied gradient (0.3 mL/min) started at 20% B and is increased to 90% B in 12 minutes, and was subsequently held at 90% B for 7 minutes. Then returned to initial conditions in 1 min and was held for 6 min. The final methodology for the analysis of F₃-MSA is described in Attachment II.

In the end, satisfactory chromatographic separation and peak shape was obtained for F₃-MSA using the optimised Obelisc N method (see figure 2-1).

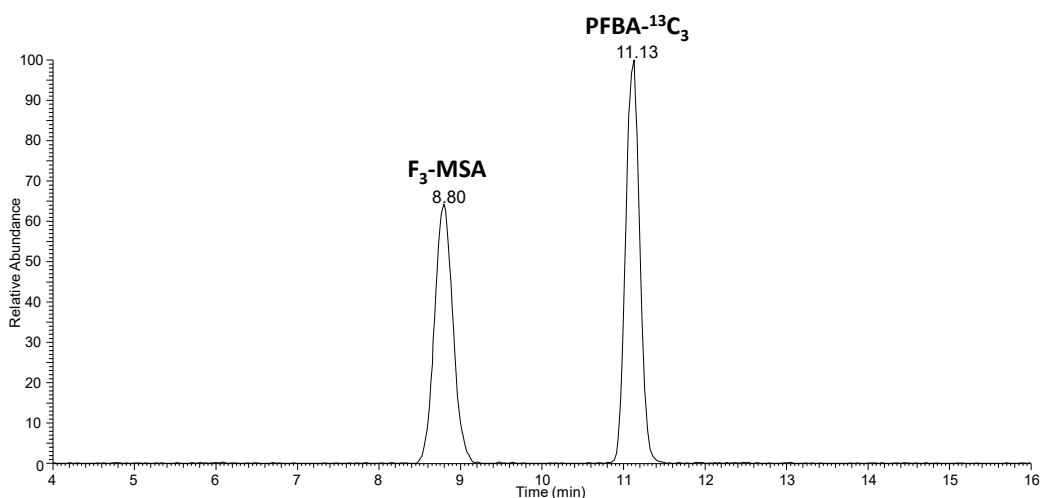


Figure 2-1: Extracted ion chromatogram of F₃-MSA and PFBA-¹³C₃ reference standard (2.5 µg/L)

2.3.2 HFPO-DA liquid chromatography method development

For the analysis of HFPO-DA in water, a new analytical method was developed using reversed phase C18 liquid chromatography. The chromatographic method development was straight forward. From the literature it was known that HFPO-DA is primarily analysed with a regular C18 column with methanol as organic modifier and ammonium acetate as buffer [3, 4, 11]. Therefore an XBridge BEH C18 XP column was chosen for method development using the aforementioned mobile phase. For method development different concentrations of ammonium acetate were tested, for which an optimal concentration of 5 mM was determined. As internal standard the isotopically labeled HFPO-DA-¹³C₃ was used.

The final mobile phase composition for mobile phase A was; ultrapure water with 5 mM ammonium acetate and for mobile phase B; methanol with 5 mM ammonium acetate. The applied gradient (0.25 mL/min) started at 25% B and is increased to 100% B in 10 minutes, and was subsequently held at 100% B for 4 minutes. Then returned to initial conditions in 0.5 min and was held for 3.5 min. The final methodology for the analysis of HFPO-DA is described in Attachment II.

A satisfactory chromatographic method was developed for HFPO-DA using the Xbridge C18 column (see figure 2-2).

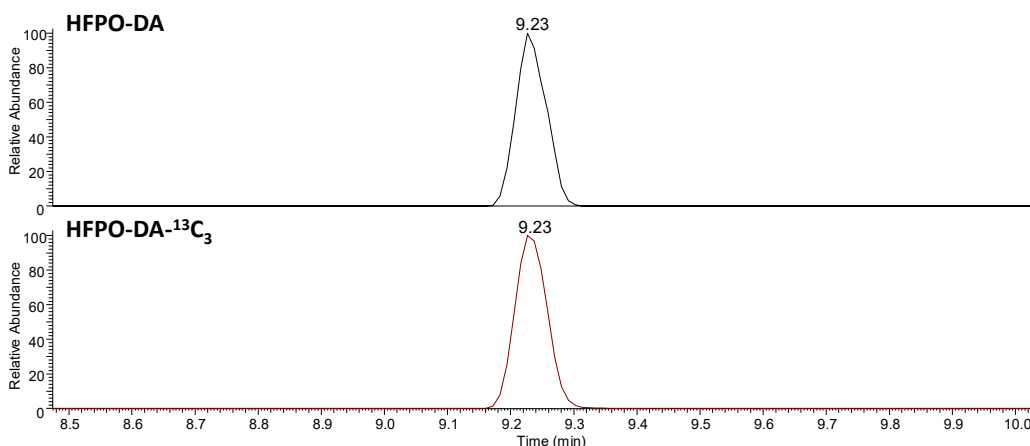


Figure 2-2: Extracted ion chromatogram of HFPO-DA (0.1 µg/L) and HFPO-DA-¹³C₃ reference standard (25 µg/L)

2.3.3 Mass spectrometry method development

For the detection of F₃-MSA and HFPO-DA a high resolution Orbitrap Fusion mass spectrometer was used. The Orbitrap Fusion was chosen because of its non-target screening capabilities, which were needed in order to perform a suspect screening for other (emerging) (per)fluorinated compounds in samples from the sampling campaign. Two separate mass spectrometry methods were developed for F₃-MSA and HFPO-Da because of the two different liquid chromatography methods employed for each analyte.

F₃MSA

For F₃MSA not much improvement in sensitivity was made by optimisation of the source parameters (i.e. gas and temperature settings). The acquisition method consisted of a full-scan with a scan range of 120–500 m/z in the negative ionisation mode at a resolution of 120 000 FWHM, which is used for the quantification of F₃-MSA and suspect screening. The quantification of F₃-MSA is performed on the accurate mass of the deprotonated molecular ion (m/z 148.9526), with a mass accuracy of 5 ppm. For the unambiguous confirmation of F₃MSA a MS/MS spectrum of product ion m/z 149.95 at a high collision dissociation energy (HCD) of 50%, was continuously recorded at a resolution of 15 000 FWHM. For non-target screening purposes also data dependent MS/MS scans were triggered of the highest detected ions of each full scan cycle at a resolution of 15 000 FWHM.

HFPO-DA

By optimisation of the source parameters a substantial improvement in sensitivity was made for HFPO-DA. By using low temperatures for the ion transfer tube (250 °C) and vaporizer temperature (200 °C), a fivefold increase in sensitivity was achieved. With the applied heated electrospray source considerable in-source fragmentation was observed in negative ionisation mode, causing a low intensity for the deprotonated molecular ion. Therefore the quantification of HFPO-DA was performed on a specific fragment [C₅HOF₁₁-H]⁻ detected at m/z 284.97790, with a mass accuracy of 5 ppm. The acquisition method consisted of a full-scan with a scan range of 150–500 m/z in the negative ionisation mode at a resolution of 120 000 FWHM, which is used for the quantification of HFPO-DA and suspect screening. For the unambiguous confirmation of HFPO-DA a MS/MS spectrum of product ion m/z 284.98 at a HCD of 30% was continuously recorded at a resolution of 15 000 FWHM. For non-target screening purposes also data dependent MS/MS scans were triggered of the highest detected ions of each full scan cycle at a resolution of 15 000 FWHM.

The final mass spectrometry settings for F₃-MSA and HFPO-DA are described in attachment II.

2.4 F₃-MSA and HFPO-DA sample pre-treatment

In order to achieve low LOQs for F₃MSA and HFPO-DA, sample pre-treatment using solid phase extraction (SPE) is needed. Literature has shown that both HFPO-DA and F₃MSA can be extracted from water using weak anionic exchange (WAX) SPE cartridges [3, 4, 8, 11]. Both HFPO-DA and F₃MSA are strong acids, meaning that they are always negatively charged independent of the pH. Therefore extraction using a WAX SPE column is a valid strategy and the WAX cartridge was selected for the sample pre-treatment method development. For the sample pre-treatment a sample volume of 500 mL was selected in order to achieve a sufficient concentration factor (500x) in order to reach the required LOQs. For eluting HFPO-DA and F₃MSA from the SPE cartridge, a final volume of 10 mL of methanol containing 0.25% ammonium hydroxide was chosen. The eluent was further concentrated by using heated nitrogen until a volume of 250 µL was reached and was then reconstituted to 1 mL of ultrapure water:methanol 75:25 (v/v). In the end good recoveries for HFPO-DA and F₃MSA were obtained with the developed sample pre-treatment method (see section 2.5). The final sample pre-treatment for F₃-MSA and HFPO-DA is described in attachment II.

During the sample pre-treatment extra precautions were taken in order to avoid PTFE (Teflon) materials. Therefore for sample handling only glass and high quality plastics such as polypropylene and nylon were used.

2.5 Absolute recovery

The absolute recovery was determined for F₃-MSA and HFPO-DA in spiked surface water, yielding a recovery of 93.5% (50 ng/L; n=2) and 89.16% (20 ng/L; n=2), respectively. No major loss of analytes was observed, showing that the sample pre-treatment method developed is satisfactory. Subsequently the matrix effects in surface water were determined. No matrix effects were observed for HFPO-DA, but moderate ion suppression (circa 25%) was observed for F₃-MSA in surface water. Because no isotope labeled internal standard is available for F₃-MSA, for which now PFBA-¹³C₃ is used as a surrogate internal standard, it is currently not possible to correct the results obtained for the observed ion suppression.

2.6 Sample stability

In order to determine the maximum holding time that a water sample can be stored before analysis, a stability study was performed. Perfluorinated compounds (PFCs) are known to be persistent, therefore it is unlikely that degradation would occur during the storage time study. However it is also known that PFCs can adsorb to surfaces and therefore also possibly adsorb to sampling bottles. In order to determine if adsorption really is an issue for F₃-MSA and HFPO-DA, a stability study was performed in polypropylene sampling bottles. To this end samples of drinking water and surface water were spiked with 200 ng/L of HFPO-DA and 1 µg/L of F₃-MSA, and stored at 1-5°C during 21 days. In addition, a number of blank samples were prepared, in order to demonstrate that the sampling bottles do not contain any PFCs. The storage time was determined by analysing the "0 day" and "21 day" old samples using the analytical methods described in attachment II. The results of the storage time study are shown in table 2-1.

TABLE 2-1 STORAGE TIME STUDY RESULTS FOR F₃-MSA AND HFPO-DA IN DRINKING AND SURFACE WATER AFTER 21 DAYS (N=7)

	Drinking water					Surface water				
	0 days		21 days		difference	0 days		21 days		difference
	conc (µg/L)	RSD (%)	conc (µg/L)	RSD (%)		conc (µg/L)	RSD (%)	conc (µg/L)	RSD (%)	
F ₃ -MSA	1.12	3.5	1.16	2.0	3.2	0.967	4.1	0.978	4.3	1.2
HFPO-DA	0.189	3.6	0.190	2.7	0.7	0.187	2.6	0.190	2.1	1.1

No degradation or adsorption was observed for F₃-MSA and HFPO-DA in drinking- and surface water after 21 days. This shows that the samples can be safely stored for 21 days at 1-5°C before sample analysis. Furthermore, no PFCs were detected in the blank samples, showing that the polypropylene sample bottles are suitable for use in the sampling campaign.

2.7 Method validation

The method development and validation for FOSA was performed by UvA-IBED. A low LOQ of 0.25 ng/L was obtained for FOSA, and good recoveries for drinking- and surface water were obtained (95-102%). The validation characteristics for FOSA are described in attachment III. The validation results for FOSA in drinking- and surface water show that the method developed can be applied for the sampling campaign of FOSA in the Dutch and Belgian water samples.

2.7.1 Method validation F₃-MSA and HFPO-DA

The two methods developed for F₃-MSA and HFPO-DA were validated for drinking and surface water. First the instrumental repeatability was determined using a reference standard of 20 ng/L of HFPO-DA and 50 ng/L F₃-MSA, for which an instrumental repeatability (n=8) was found of 0.4% and 2.0%, respectively. The limit of detection (LOD), limit of quantification (LOQ), repeatability (RSD) and SPE recovery were determined in drinking water and surface water. The validation results are shown in table 2-2 and 2-3 for F₃-MSA and HFPO-DA, respectively.

TABLE 2-2 VALIDATION RESULTS OF F₃-MSA IN DRINKING- AND SURFACE WATER (N=8)

Matrix	LOD	LOQ	Repeatability (%)		SPE recovery (10 ng/L)
	ng/L	ng/L	1 ng/L	50 ng/L	(%)
Drinking water	0.24	1.0	6.0	7.1	118
Surface water	*	1.0	4.8**	6.9	75.8

* = Because there was no surface water available in which low concentration of F₃-MSA (< 2 ng/L) were present, it was not possible to determine the LOD in surface water. The LOD of drinking water is therefore used as reference

** = Determined at 10 ng/L.

TABLE 2-3 VALIDATION RESULTS OF HFPO-DA IN DRINKING- AND SURFACE WATER (N=8)

Matrix	LOD	LOQ	Repeatability (%)		SPE Recovery (0.2 ng/L)
	ng/L	ng/L	0.2 ng/L	20 ng/L	(%)
Drinking water	0.01	0.20	1.8	1.9	103
Surface water	0.05	0.20	6.5	1.0	99.2

Satisfactory LOD and LOQ results were obtained for F₃-MSA and HFPO-DA in drinking- and surface water. For F₃-MSA the LOD could not be determined in surface water, due to the presence of low concentrations of this compound, which was present in every surface water that was tested. Therefore the LOD of drinking water was used as a reference. The LOQ (i.e. $\geq 3 \times$ LOD) was determined at 1.0 and 0.2 ng/L for F₃-MSA and HFPO-DA, respectively. Recoveries in drinking- and surface water are between 75 and 120% and are satisfactory. The recovery of 75.8% obtained for F₃-MSA is not due to the loss of analyte, but because of matrix effects (ion suppression, see 2.5). The repeatability for both compounds is lower than 7% and is satisfactory.

2.8 Summary/conclusion

Two LC-Orbitrap-MS methods were developed for the analysis of F₃-MSA and HFPO-DA in drinking water and surface water. The analytical method for F₃-MSA is based on mixed mode chromatography (i.e. C18 and ion exchange) and the method for HFPO-DA used reversed phase C18 chromatography. For both methods the same solid phase pre-treatment was developed based on weak anionic exchange (WAX) SPE cartridges. Satisfactory LOD and LOQ results were obtained for both drinking and surface water. The validation results for F₃-MSA and HFPO-DA in drinking and surface water show that the methods developed are applicable for the sampling campaign in the Dutch and Belgian waters.

3 Sampling campaign

3.1 Sampling campaign

The sampling campaign for FOSA, F₃-MSA and HFPO-DA in Dutch and Belgian (The Watergroep) waters was conducted in September 2017. In total 53 samples (see table 3-1) were collected from 11 drinking water companies including the following water types: drinking water (DW), surface water (SW), river bank filtrate (RBF), dune filtrate (DF) and process water (PW). Furthermore, two drinking water treatment processes (reverse osmosis and UV/H₂O₂) were studied at various stages of the drinking water treatment process. More detailed information about the samples collected can be found in attachment IV.

TABLE 3-1 OVERVIEW OF THE NUMBER OF SAMPLES PER WATERCOMPANY AND SAMPLE TYPE

Water company	Number of samples
Evides	6
WML	6
Brabant water	4
Dunea	4
PWN	7
Waternet	5
Vitens	7
Oasen	5
WBG	2
WMD	2
De Watergroep	4
Other	1
Sample type	
Drinking water	22
Groundwater	5
Surface water	13
River bank/ dune filtrate	7
Process water	6

For the sampling campaign, polypropylene bottles were sent to the drinking water companies and drinking water laboratories for sample collection. The samples were collected in fourfold per sampling point. After sample collection the samples were transported to KWR from which two samples per sampling point were sent to UvA-IBED for the analysis of FOSA. These were stored at -20 °C. The remaining samples were stored at 1-5 °C at KWR where F₃-MSA and HFPO-DA analysis was performed within three weeks after sample collection.

3.2 Results sampling campaign

An overview of results of the sampling campaign for F₃-MSA, HFPO-DA per water type is presented in table 3-2. The results of the process water samples of RO and UV/H₂O₂ water treatment are not included in this overview. The results per sample for FOSA, F₃-MSA, HFPO-DA are shown in attachment V.

TABLE 3-2 FREQUENCY OF PRESENCE OF F₃-MSA AND HFPO-DA ABOVE LOD IN VARIOUS WATER TYPES

	Drinking water		Groundwater		Surface water		River bank filtrate/ dune filtrate	
	F ₃ -MSA	HFPO-DA	F ₃ -MSA	HFPO-DA	F ₃ -MSA	HFPO-DA	F ₃ -MSA	HFPO-DA
Number of samples (n)	22	22	5	5	13	13	7	7
Detected (n)	15	10	-	-	13	10	7	6
Detected (n) > 1 (ng/L)	11*	5	-	-	10*	3	6*	3
Detected (%)	68.2	45.5	-	-	100	76.9	100	85.7
Average conc. (ng/L)	24	2.9	-	-	42	2.2	78	10.2
Highest conc. (ng/L)	165	28	-	-	150	10.2	230	59

* > 10 ng/L

FOSA

FOSA was not observed (< 0.25 ng/L) in any of the samples collected from source and drinking waters in The Netherlands and Belgium. This result demonstrates that FOSA is currently not relevant for the Dutch and the Belgian watercycle in so far as represented in the sampling campaign.

HFPO-DA

In 45.5% of the 22 drinking water stations that were sampled HFPO-DA was observed (≥ 0.2 ng/L). HFPO-DA was not present in groundwaters, but was observed in 76.9% and 85.7% of the surface waters and river bank filtrate/dune filtrate waters. The average concentration of HFPO-DA in drinking water is rather low (2.9) ng/L and it is only detected in concentrations above 4 ng/L in drinking water from Oasen, Evides and Dunea. These three drinking water companies abstract their raw surface water from locations close to manufacturing or processing sites of PTFE [2]. The highest concentration of HFPO-DA was observed in RBF from Lekkerkerk with a concentration of 59 ng/L in river bank filtrate and 28 ng/L in the corresponding drinking water. Furthermore, the results of the sampling campaign suggest that HFPO-DA is only partly removed by drinking water treatment (see section 3.2.1).

F₃-MSA

F₃-MSA was observed (≥ 1.0 ng/L) in 68.2% of the 22 drinking water stations that were sampled. In none of the sampled groundwaters F₃-MSA was observed, but it was present in all samples from surface waters and river bank / dune filtrates. The average concentration of F₃-MSA in drinking waters, surface waters, and riverbank/dune infiltrates was 24, 42 and 78 ng/L, respectively. The highest concentrations for F₃-MSA were observed at Heel (RBF 230 ng/L and SW 150 ng/L), which are substantially higher than other source waters that were analysed. This could indicate that there is a local emission of F₃-MSA in the vicinity of Heel.

The results of the sampling campaign show that relatively high concentrations of this newly emerging compound are detected in various matrices (except groundwater), which is in agreement with previous reports [8]. The results also show that F₃-MSA is only partly removed by drinking water treatment (see section 3.2.1).

3.2.1 Raw water versus drinking water

The sampling campaign showed that F₃-MSA and HFPO-DA were observed multiple times in drinking water at various concentrations, which indicates that F₃-MSA and HFPO-DA are incompletely removed by the drinking water treatment. Therefore a comparison was made between the concentrations of F₃-MSA and HFPO-DA observed in raw water with those of the corresponding drinking water. The results are shown in figures 3-1 and 3-2.

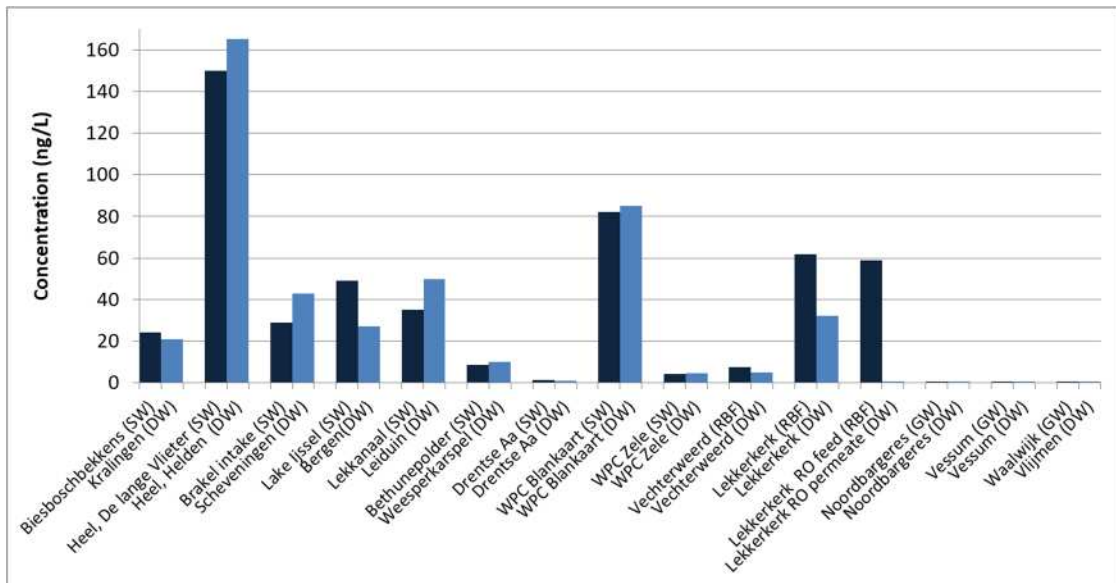


Figure 3-1: concentration of F₃-MSA observed in various types of water. Raw water is dark blue, drinking water light blue

The raw versus drinking water comparison shown in figure 3.1 reveals that F₃-MSA is not or incompletely removed by the majority of drinking water purification processes applied. The only exception is the reverse osmosis process. Samples collected at RO facility at Lekkerkerk showed almost complete removal of F₃-MSA in permeate water. This is a confirmation of results obtained by UvA (Albergamo et al, submitted) where both compounds were shown to be removed by RO. These results demonstrate that the applied drinking water processes (with the exception of RO) do not constitute a robust barrier for the polar F₃-MSA.

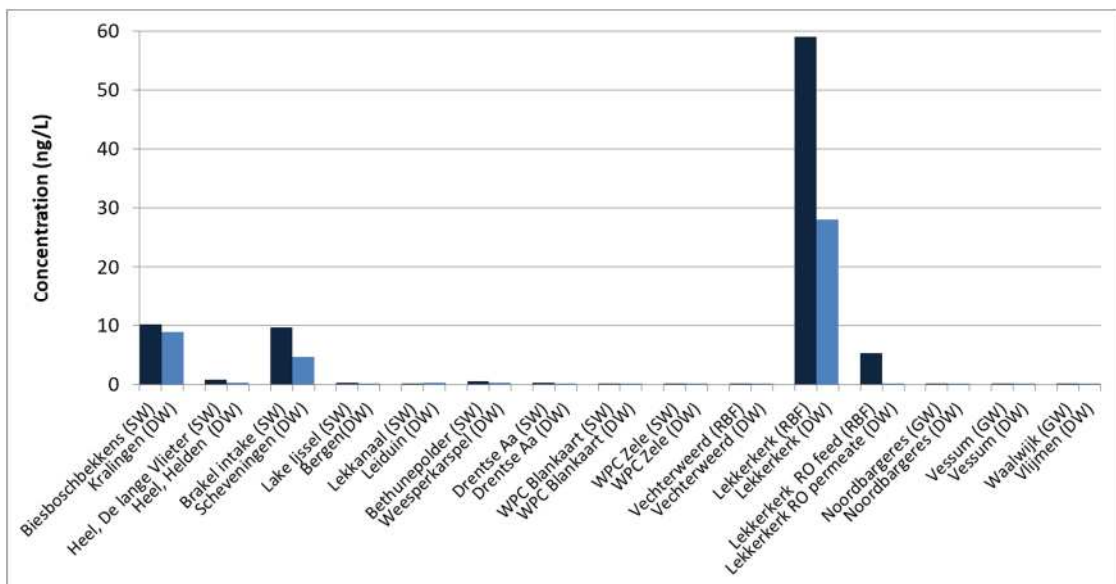


Figure 3-2: Concentration of HFPO-DA observed in various types of water. Raw water is dark blue, drinking water light blue

The raw versus drinking water comparison (figure 3.2) shows that HFPO-DA is incompletely removed by the majority of drinking water purification processes applied. Once again, only reversed osmosis achieves an almost complete removal of HFPO-DA in permeate water. The incomplete removal of the semi-polar HFPO-DA implies that relatively high concentrations of

HFPO-DA can be present in drinking water when high concentrations are present in source water.

3.2.2 Results reverse osmosis and UV/H₂O₂ treatment

Before the start of the sampling campaign two drinking water treatment processes were selected to be studied more in-depth for the removal of F₃-MSA, HFPO-DA and FOSA. The following water treatment techniques were selected: UV/H₂O₂ advanced oxidation (PWN) and reverse osmosis (Oasen). The results of the measurements are presented in table 3-3.

TABLE 3-3 CONCENTRATIONS OF F₃-MSA, HFPO-DA AND FOSA OBSERVED IN VARIOUS STAGES OF UV/H₂O₂ AND REVERSE OSMOSIS WATER TREATMENT. AKF = ACTIVE CARBON FILTRATION

	F ₃ -MSA ng/L	HFPO-DA ng/L	FOSA ng/L
UV/H₂O₂ (PWN)			
Lake IJssel	49	0.28	< 0.25
Effluent waterwinstation Prinses Juliana	46	0.30	< 0.25
Influent UV/H ₂ O ₂ -AKF	39	0.22	< 0.25
Effluent UV/H ₂ O ₂ -AKF	39	0.22	< 0.25
After dune filtration	45	0.22	< 0.25
Drinking water Bergen	27	0.20	< 0.25
RO (Oasen)			
Reverse osmosis feed	59	5.3	< 0.25
Reverse osmosis permeate	< 1.0	< 0.20	< 0.25
Reverse osmosis concentrate	165	28	0.92

UV/H₂O₂ (PWN)

The results obtained for the advanced oxidation water treatment with UV/H₂O₂ show that the UV/H₂O₂ process itself has no or negligible effect on the removal of F₃-MSA and HFPO-DA (Influent UV/H₂O₂-AKF vs. effluent). Also dune infiltration has no effect on the removal of F₃-MSA and HFPO-DA. At the end of the complete treatment chain, both F₃-MSA and HFPO-DA appear to be partly removed by the treatment, but residues remain present in the final drinking water. Overall, the concentration observed for HFPO-DA in drinking water is rather low.

RO (Oasen)

Water treatment using RO shows a complete removal (lower than LOQ) of F₃-MSA and HFPO-DA from feed water and demonstrates that RO is a very effective purification process for the removal of F₃-MSA and HFPO-DA.

FOSA was not detected in source and drinking waters, but was detected in RO concentrate at a relatively low concentration of 0.92 ng/L. This shows that it is likely that FOSA is present in source water used by Oasen, but present at a concentration lower than the LOQ for FOSA (< 0.25 ng/L).

3.3 Suspect screening halogenated methanesulfonic acids

One of the major advantages of using a high resolution mass spectrometer is the possibility to do a suspect screening for relevant compounds other than the target substances. A suspect screening can be performed retrospectively on previous recorded data by searching

for specific accurate masses calculated from the molecular formulas of the compounds that are suspected in the waters sampled.

It was shown by Zahn et al. [8] that besides F₃-MSA other halogenated methanesulfonic acids (HMSAs) can also be present in the watercycle. In total six different chlorinated and brominated methanesulfonic acids (see figure 3-3 for the general structure of HMSAs) were detected in the Zahn study [8].

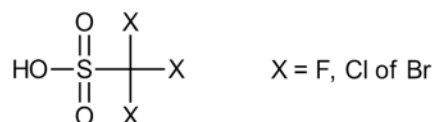


Figure 3-3 General structure of the detected halogenated methanesulfonic acids

Because these chlorinated and brominated methanesulfonic acids had been observed before in the watercycle, it was decided to perform a suspect screening for these six HMSAs (see table 3-4) using the raw data files recorded for the samples from the sampling campaign. The data set recorded using the F₃-MSA analytical method was chosen for the suspect screening, due to similar structural properties of these six HMSAs with that of F₃-MSA.

TABLE 3-4 HMSAs SELECTED FOR SUSPECT SCREENING

Name	Abbreviation	Formula	Accurate mass [M-H]
Chloromethane sulfonic acid	Cl-MSA	CH ₃ ClSO ₃	128.9419
Dichloromethane sulfonic acid	Cl ₂ -MSA	CH ₂ Cl ₂ SO ₃	162.9029
Trichloromethane sulfonic acid	Cl ₃ -MSA	CHCl ₃ SO ₃	196.8639
Bromomethane sulfonic acid	Br-MSA	CH ₃ BrSO ₃	172.8914
Dibromomethane sulfonic acid	Br ₂ -MSA	CH ₂ Br ₂ SO ₃	250.8019
Bromochloromethane sulfonic acid	BrCl-MSA	CH ₂ BrClSO ₃	206.8524

3.3.1 Results suspect screening halogenated methanesulfonic acids

The results of the suspect screening for HMSAs are presented in figure 3-4 (see attachment VI for concentrations and sampling points). The concentrations of HMSAs are calculated using F₃-MSA as calibration standard and are therefore an indication of the actual environmental concentration. F₃-MSA is shown as reference in the presented figure.

All detected HMSAs were confirmed to identification level 3 [12], by annotation of the HR MS² spectrum. Confirmation to level 2a was not possible, because no reference MS² spectra were available. The identity of these HMSAs can only be confirmed unambiguously when reference standards are available.

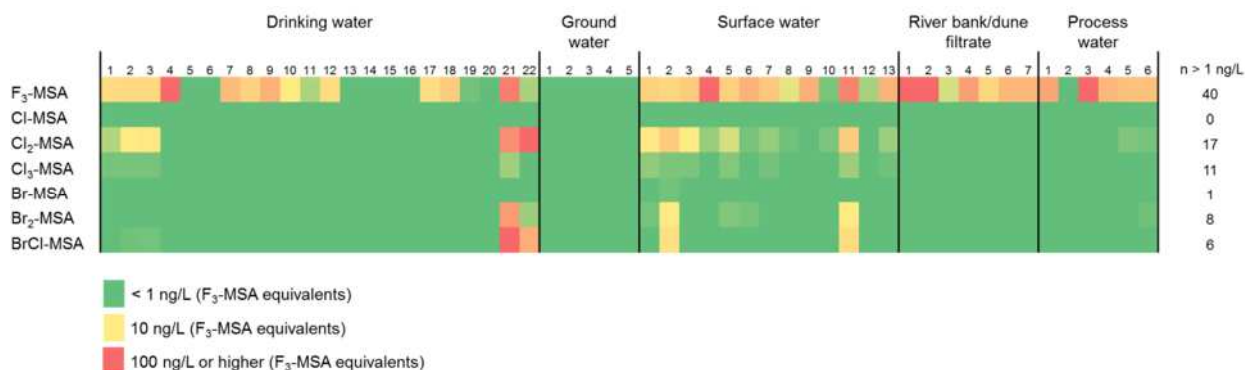


Figure 3-4 Results suspect screening for HMSAs in various water types. All concentrations of HMSAs were calculated using F₃-MSA and are indicative. Results of F₃-MSA were added as reference.

Five of the six halogenated methanesulfonic acids (Cl₂-MSA, Cl₃-MSA, Br-MSA, Br₂-MSA and BrCl-MSA) were observed to be present with the suspect screening, with concentrations ranging from 1- 148 ng/L (F₃-MSA eq.). Only Cl-MSA was not observed with the suspect screening. Cl₂-MSA and Cl₃-MSA were most frequently present: in 17 and 11 samples (from 53 samples total), respectively. HMSAs were present mostly in surface waters, although the highest concentrations for Cl₂-MSA, Br₂-MSA and BrCl-MSA were observed in two drinking water samples (WPC Blankaart, DW 21 and WPC Zele, DW 22). These HMSAs were not present or present at substantially lower concentrations in the corresponding source (surface) water (SW 11 and 12), which could indicate that these HMSAs were formed during drinking water treatment, or are possibly a contaminant from an unknown source.

Of the halogenated methanesulfonic acids, F₃-MSA is present in almost all compartments of the watercycle (except groundwater). The chlorinated and brominated methanesulfonic acids occur less frequently, and appear to occur mostly together, which may indicate a common source. In conclusion, the suspect screening has shown that other HMSAs besides F₃-MSA are also frequently observed in the watercycle at relevant concentrations. Therefore it is recommended to further monitor these emerging contaminants and to assess their emissions, occurrence, fate and environmental and human health risk.

4 Toxicological evaluation

4.1 Introduction

There is ample information on long-chain perfluorinated chemicals such as PFOS and PFOA with regard to toxicity and environmental behaviour. In recent years, also short-chain perfluorinated compounds are being detected in (sources of) drinking water. In this chapter, the available toxicity data for the emerging short-chain perfluorinated chemicals HFPO-DA, F₃-MSA and FOSA are described and provisional health-based drinking water guidelines are derived. Information was retrieved from the following sources:

- Risk assessment reports published by the European Food Safety Authority (EFSA), European Chemicals Agency (ECHA), US Environmental Protection Agency (EPA), and Dutch National Institute for Environment and Health (RIVM);
- Toxicological databases: TOXNET, International Toxicity Estimates for Risk (ITER), International Programme on Chemical Safety (IPCS), and OECD eChemPortal;
- ToxCast database (US-EPA)
- OECD QSAR Toolbox v3.4.0.17 for chemical profiling and available toxicity studies;
- Peer reviewed publications

4.1.1 HFPO-DA

HFPO-DA (FRD-903) is used to manufacture the ammonium salt FRD-902, which is applied to control polymerization in the production of plastics (fluoropolymers). This manufacturing process is referred to as the GenX technology. HFPO-DA is approved by the EFSA to use in the polymerization of fluoropolymers that are processed at or above 265 °C and are for repeated use articles [13]. Under these high temperatures, HFPO-DA completely decarboxylates. No toxicity studies are available for HFPO-DA in scientific literature, REACH registration dossiers [14], ToxCast, or retrieved by the OECD QSAR Toolbox. For the decarboxylation product of HFPO-DA, limited toxicity data are available. Since the decarboxylation product is structurally related to the ammonium salt of HFPO-DA (FRD-902), [13] based its evaluation on toxicity data for FRD-902. Read-across from FRD-902 data is considered justified for HFPO-DA itself as well, since the effects of both substances are caused by the anion 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate and in organisms, absorption and distribution of this anion are expected to be similar after dissolution and dissociation of the acid (HFPO-DA) and the salt (FRD-902) [14].

An overview of toxicity studies for FRD-902 documented in its REACH registration dossier has been included in the RIVM report 'Evaluation of substances used in the GenX technology by Chemours, Dordrecht' [14]. FRD-902 appears not to be mutagenic or genotoxic and adverse effects on reproduction or development are not expected [13, 14]. The OECD QSAR Toolbox does report structural alerts for DNA binding and *in vivo* genotoxicity for both HFPO-DA and FRD-902 (see attachment VII), but retrieves predominantly negative genotoxicity test results for FRD-902 as well. RIVM [14] concludes that FRD-902 and HFPO-DA should be classified as suspected non-genotoxic carcinogens in humans, as carcinogenicity has been observed in experimental animals. In the consulted data sources and scientific literature, no additional toxicological information on HFPO-DA or FRD-902 was found.

A provisional oral Tolerable Daily Intake (TDI) level for FRD-902 was derived by RIVM [15] from the No Observed Adverse Effect Level (NOAEL) from a chronic rat study (submitted by REACH registration applicant): 0.1 mg/kg body weight/day based on an increase in albumin and the albumin/globulin ratio (which indicates possible immunotoxic effects) at higher doses. Using an extrapolation factor for interspecies differences in kinetics (standard value of 4), an additional factor for potential differences in kinetics (a worst-case value of 66 due to lack of data), a factor for differences between species (1.8) and a factor for differences between humans (standard value of 10), a TDI of 21 ng/kg/day was calculated. Additional information on the bioaccumulation of FRD-902 in humans would allow derivation of an improved exposure limit [14, 15]. In addition, potential carcinogenic effects have not been incorporated in this TDI level.

The provisional TDI of 21 ng/kg bw/day was converted to a drinking water guideline value by assuming the WHO default of 20% allocation of the total exposure to drinking-water, an adult body weight of 70 kg and a standard drinking-water consumption of 2L per day. This resulted in a provisional drinking water guideline value for FRD-902 of 0.15 µg/L. This value also applies to HFPO-DA and the anion, and to the sum of the three substances [15].

During the fluoropolymer production process, HFPO-DA is released to air and HFPO-DA and FRD-902 are emitted to wastewater. The estimated concentration of HFPO-DA in air is 20 ng/m³ for the nearest populated areas of Chemours [14]. At a default adult inhalation volume of 20 m³/day, the inhalatory exposure to HFPO-DA would amount to 400 ng/day (5.7 ng/kg bw/day) in this region. Concentrations of HFPO-DA up to 0.02 µg/L have been reported in drinking water produced from surface water downstream from the Chemours plant. Since both substances will be present in water in the anion form, this concentration reflects the emission of both HFPO-DA and FRD-902 to surface water. The summed exposure through drinking water will thus be 40 ng/day (0.6 ng/kg bw/day). No information is currently available regarding levels of HFPO-DA and FRD-902 in food [14]. Exposure of consumers to HFPO-DA via food contact materials, for which quantitative information has not been identified, is expected to be negligible [13]. However, since these substances are persistent, it is likely that they also end up in the food chain. The allocation factor of 20% applied to derive the provisional drinking water guideline value thus seems appropriate.

4.1.2 F₃-MSA

No toxicity studies and health risk assessments for F₃-MSA were retrieved from the consulted authorities and databases. Three negative study results for genotoxicity (Ames mutagenicity test, *in vitro* chromosome aberration, and mammalian gene mutation assay) and no structural alerts for genotoxicity are reported in the OECD QSAR Toolbox. F₃-MSA was inactive in >50 ToxCast *in vitro* assays on various cellular processes [16] and 145 *in vitro* bioassays tests reported by the U.S. EPA Chemistry Dashboard. Literature search did not yield additional toxicological information on F₃-MSA.

The REACH registration dossier for F₃-MSA reports a DNEL (Derived No Effect Level) of 1.7 mg/kg bw/day. This long term exposure threshold was derived from a NOAEL of 1 g/kg bw/day obtained from a subacute oral toxicity study in rats and an overall assessment factor of 600: 6 for extrapolation to chronic exposure, 4 for allometric scaling of interspecies differences and 2.5 for other interspecies differences, and 10 for intraspecies differences [17].

Exposure information on F₃-MSA was not found in the consulted information sources and literature. Although the substance has been predicted not to be a PBT chemical [18, 19], its widespread detection in the aquatic environment [8] suggests human exposure through

other routes than drinking water as well. Applying a default 20% allocation of the total exposure to drinking-water, an adult body weight of 70 kg and a standard drinking-water consumption of 2L per day, a provisional drinking water guideline value of 11.9 mg/L would be derived from the reported DNEL.

4.1.3 FOSA

No results from chronic toxicity and genotoxicity studies or human health risk assessments for FOSA were retrieved from the consulted information sources. Literature search suggests endocrine, developmental, and immunotoxic effects, although the retrieved publications mainly describe (other) perfluorinated compounds and not FOSA specifically. The OECD QSAR Toolbox reports structural alerts for DNA binding, genotoxicity, and nongenotoxic carcinogenicity for FOSA (see Appendix I). Read across was performed to predict toxicity of FOSA based on measured data for structural analogues included in the OECD QSAR Toolbox. Six analogues were identified¹. Only for the Ames test and *in vitro* mammalian chromosome aberration test experimental data for two analogues² (60-90% similarity) were reported. Both substances were negative in both tests, indicating that FOSA may lead to negative results in these genotoxicity assays as well. FOSA was active in 150 ToxCast *in vitro* assays related to development and a wide range of cellular processes including nuclear receptor binding, DNA binding, cell cycle, cell adhesion, and cytokine and (metabolic) enzyme activity [16]. The U.S. EPA Chemistry Dashboard also reports activity of FOSA (in the 1-100 µM range) in 22 different *in vitro* assays related to receptor binding, energy production, and response to DNA damage, heat shock and oxidative stress.

FOSA is not registered under REACH. According to the classification provided to ECHA by producers, FOSA causes skin, eye and possibly respiratory irritation, is toxic if swallowed, and very toxic to aquatic life with long lasting effects. REACH registrants and TOXNET report indications for persistency in the environment and bioaccumulation based on physiochemical properties and QSAR predictions. Biodegradation data in soil or in water are not available [20]. FOSA is known to be widely spread in the environment (including food, drinking water and house dust). One of the sources of FOSA is degradation of the pesticide N-ethyl perfluorooctane sulfonamide (EtFOSA or Sulfluramid) [21-23], the toxicity of which has not been fully characterized [24]. For perfluorooctylsulfonate (PFOS), the other known degradation product of EtFOSA, RIVM [25] reported a provisional drinking water standard of 0.53 µg/L. RIVM later calculated a groundwater concentration representing a negligible health risk when used for drinking water abstraction (assuming no removal during drinking water treatment) of 0.005 µg/L based on this value [26].

Since no acceptable daily intake level or NOAEL from a chronic toxicity study has been reported for FOSA, a substance-specific health based drinking water guideline value cannot be derived. Instead, the generic Threshold of Toxicological Concern (TTC) level of 0.01 µg/L for suspected genotoxic and steroid endocrine chemicals that lack toxicity data could be applied [27].

¹ Category Definition based on Organic Functional groups (nested): 'Perfluorocarbons derivatives' and 'Sulfonamide' yielded the following structural analogues: CAS 4151-50-2, 1691-99-2, 34454-97-2, 30381-98-7, and 67584-55-8.

² CAS 34454-97-2 and 67584-55-8

TABLE 4-1 PROVISIONAL DRINKING WATER GUIDELINE VALUES

Compound	TDI	Allocation factor	Provisional guideline value
HPFO-DA	21 ng/kg/day ¹	20%	0.15 µg/L ^{2,3}
F ₃ -MSA	1.7 mg/kg bw/day ⁴	20%	11.9 mg/L ²
FOSA	N/A	-	0.01 µg/L ⁵

¹ Provisional TDI for FRD-902 [14]

² Based on default values for body weight (70 kg) and drinking water consumption per day (2L)

³ Applies to the sum of HPFO-DA (FRD-903), FRD-902 and their anions

⁴ DNEL reported in REACH dossier

⁵ TTC for genotoxic substances in drinking water [27]

4.2 Observed concentrations in drinking water

The maximum concentration observed for HFPO-DA was 28 ng/L. This leads to a margin of exposure of a factor of five. The maximum concentration observed for F₃-MSA in drinking water amounted to 150 ng/L, which is 79,000 times lower than the provisional guideline value. For FOSA none of the samples contained a level above the LOQ of 0.25 ng/L. Taking the LOQ as the maximum level observed in drinking water, a margin of exposure can be calculated of > 40x. The concentrations observed currently give no cause of concern for negative health effects as a result of drinking tap water. It should be noted, however, that the guidelines mentioned in table 4-1 are provisional and based on limited toxicological and read across information. In particular in the case of HFPO-DA, where the margin of exposure is a factor of five, additional toxicological data and further monitoring are required.

5 Conclusion and recommendations

In this study analytical methodologies were developed for the analysis of F₃-MSA, HFPO-DA and FOSA in water in order to conduct a sampling campaign for the assessment of the occurrence of these substances in relevant raw waters (groundwater, surface waters, river bank and dune filtrates) and in drinking water. A reversed phase C18 LC-MS/MS method was developed for FOSA by UVA-IBED, yielding satisfactory LOD and LOQ results. For the polar F₃-MSA and HFPO a mixed-mode and a reversed phase C18 method was developed using a high resolution Orbitrap Fusion mass spectrometer for detection. Satisfactory LOD and LOQ results were obtained for F₃-MSA and HFPO-DA methods developed.

A sampling campaign for F₃-MSA and HFPO-DA and FOSA was conducted in September 2017 for The Netherlands and Belgium. To this end a total of 53 samples were collected from 11 drinking water companies. FOSA was not found in raw and drinking waters, but was present in a RO concentrate sample at a relatively low concentration of 0.92 ng/L. This shows that FOSA can be present in raw water, but at concentrations below the current LOQ for FOSA.

HFPO-DA was observed in 45.5% of the 22 drinking water samples collected, with concentrations ranging from 0.2 – 28 ng/L. HFPO-DA was not observed in groundwater samples, but was present in 76.9% and 85.7% of the surface waters and river bank filtrate/dune filtrate waters, respectively. The average HFPO-DA concentration observed in drinking water is low, and the substance is only observed in concentrations above >4 ng/L in drinking water from Oasen, Evides and Dunea. These three water companies abstract their raw waters from surface waters and river bank filtrate close to Dordrecht, and from the lower stretches of the rivers Lek and Meuse.

The polar F₃-MSA was present in 68.2% of the 22 drinking water samples collected, with concentrations ranging from 1 – 150 ng/L. In none of the sampled groundwaters F₃-MSA was observed, but F₃-MSA was present invariably in all surface waters and river bank filtrate/dune filtrates sampled, showing that it is abundantly present in raw water. The average concentration of F₃-MSA in the drinking water sampled is 24 ng/L. The highest concentrations for F₃-MSA were observed at Heel (RBF 230 ng/L and SW 150 ng/L) and are substantially higher than other raw waters that were analysed, which could indicate that there is a local emission of F₃-MSA in the vicinity of Heel.

Furthermore, it was shown that HFPO-DA and F₃-MSA are not or incompletely removed by the majority of drinking water purification processes applied. The only exception is reverse osmosis water treatment which shows an almost complete removal of HFPO-DA and F₃-MSA. This finding demonstrates that the applied drinking water processes (with the exception of RO) are not a robust barrier for HFPO-DA and F₃-MSA.

In this study a toxicological evaluation was performed in which provisional health-based drinking water guidelines were derived for F₃-MSA and FOSA of 11.9 mg/L and 0.01 µg/L, respectively. The provisional guideline value for HFPO-DA was previously derived by RIVM at 0.15 µg/L. All concentrations observed for F₃-MSA, HFPO-DA in drinking water are below the provisional guideline values derived. The concentrations observed currently give no cause of concern for negative health effects due to drinking of tap water, although the margin of exposure to HFPO-DA is relatively small and warrants further precaution.

Additionally a suspect screening was performed for other halogenated methanesulfonic acids (besides F₃-MSA), which resulted in the likely presence of five halogenated methanesulfonic acids (Cl₂-MSA, Cl₃-MSA, Br-MSA, Br₂-MSA and BrCl-MSA) with concentrations ranging from 1-148 ng/L (F₃-MSA eq.). It was shown that F₃-MSA is present in almost all compartments of the watercycle (except groundwater). The chlorinated and brominated methanesulfonic acids occur less frequently and appear to occur mostly together, which may indicate there is a common source for these compounds.

Recommendations:

- To further monitor the emerging contaminant F₃-MSA, and preferably other halogenated methanesulfonic acids in raw and drinking water, and to assess their occurrence, fate and environmental and human health risk.
- To conduct a suspect screening in the recorded C18 method data of the sampling campaign, in search of new emerging semi-polar and apolar PFAS (e.g. NORMAN perfluoroalkylated substance list).

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Attachment I Methodology FOSA

I.1 Chemicals

Ultra-pure water was prepared from an Elga device (Veolia, Ede, the Netherlands). Methanol (UPLC/MS) was obtained from Biosolve (Valkenswaard, the Netherlands). Ammonium acetate (99.999%) and ammonium hydroxide (25-30%) were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands). Acetic acid (glacial, 100%) was obtained from Merck (Darmstadt, Germany).

Stock standard solutions of FOSA and ¹³C8 FOSA (internal standard) were obtained as two separate mixtures with other (labeled) perfluorinated alkyl acids from Wellington laboratories (Ontario, Canada). Stock standards were further diluted to appropriate concentrations with methanol.

I.2 Sample pre-treatment

All materials used for sample storage and handling were from high quality plastics such as polypropylene. Samples were stored at -20 °C until analysis. Drinking water quality control samples were obtained after let the tap running continuously for at least 5 minutes.

Aliquots of 200 ml of sample material were taken by weight and spiked with internal standard and, if necessary, with FOSA (e.g. validation and control samples) and centrifuged (2000 RPM) for at least 20 minutes. Samples were then loaded on a solid phase extraction (SPE) cartridge containing 60 mg Oasis WAX sorbent (Waters Chromatography B.V., Etten-Leur, the Netherlands). The cartridge was subsequently washed with 25 mM ammonium acetate buffer (pH 4) and eluted with 2x 550 µL methanol containing 0.1% ammonium hydroxide. Extracts were filtered (0.20 µm, polypropylene, Filter-Bio, Jiangsu, China), stored at -20 °C, and prior to analysis, diluted twice with an aqueous solution of 0.1% acetic acid.

I.3 Liquid chromatography and mass spectrometry conditions

Quantitative analysis was performed with liquid chromatography (Prominence XR, Shimadzu, Den Bosch, the Netherlands) coupled to a tandem mass spectrometer (4000 Q-TRAP, AB-Sciex (Applied Biosystems, Toronto, Canada). An overview of analytical parameters is shown below.

HPLC settings:

- Column: Kinetex C18 Evo column (100 mm x 2.1 mm ID; 2.6 µm) with C18 Evo guard column (Phenomenex, Utrecht, the Netherlands)
- Eluent A: 2 mM ammonium acetate in ultrapure water
Eluent B: 2 mM ammonium acetate in methanol
- Gradient: linear from 20% to 65% B in 2 min, then increased to 100% B in 7min. Held at 100% B for 1 min. Then returned to initial conditions in 1 min and was held for 5 min.
- Flow: 300 µL/min
- Injection volume: 20 µL (standards) or 50 µL (samples)
- Retention time 3.4

Mass spectrometry settings:

- Source: Electrospray Ionisation (ESI)
- MS polarity: Negative
- MRM time window: 60 sec
- Total dwell time: 0.5 sec
- Transition* (FOSA): 498 -> 78
- Transition* (¹³C₈FOSA): 498 -> 78

** A second transition was also detected for both analytes (498 -> 169 and 506 ->172), but found inadequate due to low sensitivity.*

Attachment II Methodology F₃-MSA and HFPO-DA

II.1 Chemicals

All solvents used were of analytical grade quality. Methanol (ultra gradient HPLC grade) and Ammonium hydroxide were obtained from Avantor Performance Materials B.V. (Deventer, the Netherlands). Formic acid (HPLC quality) and hydrochloric acid 30% suprapur were purchased from Sigma-Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany), respectively. The internal standards HFPO-DA-¹³C₃, PFBA-¹³C₃ and PFPEA-¹³C₃ were obtained from Greyhound Chromatography and Allied Chemicals (Birkenhead, United Kingdom). The reference standards HFPO-DA and trifluoromethanesulfonic acid (F₃-MSA) were obtained from SynQuest Laboratories (Alachua, FL, USA) and Toronto Research Chemicals (Toronto, Canada), respectively. Ultrapure water was obtained by purifying demineralized water in an Elga Purelab Chorus ultrapure water system. (High Wycombe, United Kingdom). Stock solutions of reference and internal standards were prepared in methanol at a concentration of 100 and 5 mg/L, respectively. Stock solutions were stored at -25 °C.

II.2 Sample pre-treatment F₃-MSA and HFPO-DA

All materials used for sample storage and handling were from high quality plastics such as polypropylene, and no PTFE materials were used. Aliquots of 500 mL were acidified to pH 4 using hydrochloric acid, to which 50 ng/L of HFPO-DA-¹³C₃ internal standard was added. Then the samples were loaded onto a SPE cartridge (OASIS WAX, 150 mg, 6 cc) obtained from Waters (Etten-Leur, Netherlands) and subsequently washed with 5 mL ultrapure water (pH 4). The SPE cartridge was dried for 1 hour by air and elution was performed with 10 mL of methanol containing 0.25% ammonium hydroxide. The eluate was evaporated using a Barkey optocontrol (Leopoldshöhe, Germany) with a gentle nitrogen stream at circa 75 °C (block temperature at 300 °C) until a volume of 250 µL was reached. Then 750 µL ultrapure water was added to the extract, containing PFBA-¹³C₃ internal standard at a concentration of 16.67 µg/L. The extracted was filtered using a 0.2 µm Phenomenex Phenex regenerated cellulose filter (Utrecht, Netherlands) and was transferred to a 1.8 mL autosampler vial for LC-Orbitrap-MS analysis.

II.3 Liquid chromatography and mass spectrometry conditions F₃-MSA

Settings UHPLC, autosampler and column oven (Vanquish; Thermo Fisher Scientific, Bremen)

- Column: Obelisc N, 2.1 × 150 mm, 5 µm (SIELC Technologies, IL, USA)
- Mobile phase A: ultrapure water + 10 mM ammonium acetate + 0.05% formic acid
- Mobile phase B: Methanol + 10 mM ammonium acetate + 0.05% formic acid
- Gradient: linear from 20% to 90% B in 7 min. Held at 90% B for 7 min. Then returned to initial conditions in 1 min and held for 6 min.
- Flow: 300 µL/min
- Injection volume: 10 µL
- Column oven: 25 °C

Settings mass spectrometer (Orbitrap Fusion; Thermo Fisher Scientific, Bremen):

- Resolution MS¹: 120 000 FWHM
- Mass range full scan: 120-500 m/z
- Mass accuracy < 2 ppm

- Source: electrospray (ESI)
- Ionisation: negative mode
- Vaporizer: 350 °C
- Ion transfer tube: 300 °C
- Spray voltage: 2500 volt
- Sheat gas: 45 arbitrary units
- Auxiliary gas: 5 arbitrary units
- Sweep gas: 5 arbitrary units
- RF lens: 50%
- Resolution MS²: 15 000 FWHM
- Massrange MS² scan: 50-160 m/z
- Precursor MS²: 148.95
- HCD: 50%
- Data dependent scans per cycle: 8
- Mass range data dependent MS² scan: 120-500 m/z
- HCD data dependent scan: 35%

II.4 Liquid chromatography and mass spectrometry conditions HFPO-DA

Settings UHPLC , autosampler and column oven (Vanquish; Thermo Fisher Scientific, Bremen)

- Column: Xbridge C18 XP, 2.1 x 150 mm, 2.5 µm (Waters, Etten-Leur, Netherlands)
- Mobile phase A: ultrapure water +5 mM ammonium acetate
- Mobile phase B: Methanol + 5 mM ammonium acetate
- Gradient: linear from 25% to 100% B in 10 min. Held at 100% B for 4 min. Then returned to initial conditions in 0.5 min and held for 3.5min.
- Flow: 250 µL/min
- Injection volume: 50 µL
- Column oven: 25 °C

Settings mass spectrometer (Orbitrap Fusion; Thermo Fisher Scientific, Bremen):

- Resolution MS¹: 120 000 FWHM
- Mass range full scan: 150-500 m/z
- Mass accuracy < 2 ppm
- Source: electrospray (ESI)
- Ionisation: negative mode
- Vaporizer: 250 °C
- Ion transfer tube: 200°C
- Spray voltage: 2500 volt
- Sheat gas: 50 arbitrary units
- Auxiliary gas: 10 arbitrary units
- Sweep gas: 5 arbitrary units
- RF lens: 30%
- Resolution MS²: 15 000 FWHM
- Massrange MS² scan: 100-300 m/z
- Precursor MS²: 284.97
- HCD: 30%

Attachment III Validation results

FOSA

VALIDATION RESULTS OF FOSA AS DETERMINED BY THE INSTITUTE FOR BIODIVERSITY AND ECOSYSTEMS (IBED), UNIVERSITY OF AMSTERDAM

III.1 Linearity and measurement range

The linearity (R^2) for all calibration lines was found to be at least 0.998. The linear measurement range was found to be 0.5 -159 pg (injected amount) or 0.1 – 32 ng/L (sample concentration).

III.2 Instrumental repeatability

The instrumental repeatability was found to be 2.41% (see table III-1).

TABLE III-1 INSTRUMENTAL REPEATABILITY

Measurement	Concentration (ng/L) ¹
1	54.5
2	58.0
3	58.5
4	57.5
5	55.5
6	57.0
7	57.5
8	58.0
Average	57.1
Standard deviation	1.37
Repeatability	2.41%

1) Measured/calculated values. Reference concentration is 51.6 ng/L

III.3 Absolute recovery

The absolute recovery found within a set of spiked surface water samples was 97.2% with a relative standard deviation of 6.5 (see table III-2). Consequently, no major losses or matrix effects were suspected.

TABLE III-2 ABSOLUTE RECOVERIES OBTAINED FROM SPIKED SURFACE WATER

Measurement	Absolute recovery ¹ (%)
1	96.7
2	91.1
3	104
Average	97.2
RSD (%)	6.5

¹ Quantified by external calibration

III.4 Repeatability, recovery and detection limits

Average concentrations, standard deviations, the repeatability, recovery and detection limits are summarized in table III-3.

TABLE III-3 PERFORMANCE CHARACTERISTICS IN SAMPLE MATRIX

Addition ng/L	Ultrapure water			Drinking water			Surface water		
	0	0.35	2.5	0	0.35	2.5	0	0.35	2.5
Average ng/L	N.D.	0.340	2.30	N.D.	0.352	2.34	< LOQ	0.428	2.50
Standard deviation (ng/L)	-	0.018	0.160	-	0.023	0.105	-	0.011	0.063
Repeatability (%)	-	5.20	6.96	-	6.54	4.48	-	2.48	2.53
Recovery (%)	-	98.4	95.1	-	102	96.7	-	95.2	99.3
Detection limit (ng/L)	0.08								
Reporting limit (ng/L)	0.25								

For all tested matrices (ultrapure-, drinking and surface water) the repeatability was found to be no more than 7% and recoveries ranged between 95% and 102%. The detection limit and reporting limit were both in the sub ng/L range: 0.08 ng/L and 0.25 ng/L, respectively.

III.5 Concluding remarks

The validation showed that the tested methodology was characterized with sufficiently low detection limit and good repeatability, recovery and linearity. For this reason, we assumed the analytical methodology to be suitable for application for the analysis of water samples.

Attachment IV Sampling campaign sample information

TABLE IV-1 SAMPLE DESCRIPTION SAMPLING CAMPAIGN

Sample description	Sample code	Matrix	Sampling date
Evides			
Keizersveer	Keizersveer	Surface water	12-09-2017
Afgeleverd water Biesboschbekkens	Afgeleverd water	Surface water	12-09-2017
Reinwater Kralingen	HD2 Kralingen	Drinking water	11-09-2017
Ruw grondwater Jeugddorp (Dordrecht)	Jeugddorp	Groundwater	11-09-2017
Reinwater grondwaterzuivering Baanhoek	Reinwater BHK	Drinking water	11-09-2017
Reinwater Baanhoek (dw uit ow en gw)	HD1 + 2 BHK	Drinking water	11-09-2017
WML			
Heel, Innamewerk Lateraalkanaal	0045 RUO 0100	Surface water	12-09-2017
Heel, Spaarbekken De lange Vlieter	0045 BS 10501	Surface water	13-09-2017
Heel, Gezamenlijk ruwwater Galgenberg	0045 RUG 0301	River bank filtrate	12-09-2017
Heel, Gezamenlijk ruwwater De Reut en Langven	0045 RUG 0401	River bank filtrate	12-09-2017
Heel, Reinwaterlevering Helden	0045 RWL 0100	Drinking water	12-09-2017
Brabant Water			
Vessem ruwwater	Ruw Tak 1	Groundwater	12-09-2017
Vessem reinwater	Rein Tak 1	Drinking water	12-09-2017
Waalwijk ruwwater	Waalwijk	Groundwater	12-09-2017
Waalwijk reinwater (vlijmen)	Vlijmen	Drinking water	12-09-2017
Dunea			
Inname Lagedruk pompstation Brakel	1032469	Surface water	11-09-2017
Duinfiltraat Meijendell	1032479	Dune filtrate	12-09-2017
Duinfiltraat Berkheide	1032480	Dune filtrate	12-09-2017
reinwater Scheveningen	1032481	Drinking water	12-09-2017
PWN			
IJsselmeerwater	1032470	Surface water	11-09-2017
Effluent WPJ	1032482	Surface water	12-09-2017
Toevoer UV/H2O2-AKF	1032483	Surface water	12-09-2017
Toevoer duin (na passage UV/H2O2)	1032484	Surface water	12-09-2017
Na duinpassage	1032471	Dune filtrate	11-09-2017
Drinkwater Bergen	1032478	Drinking water	11-09-2017
Grondwater secundair Zuid in Laren	1032472	Groundwater	11-09-2017
Waternet			
Ruw water inlaat WCB (Lekkanaal)	1032473	Surface water	11-09-2017
Bethunepolder	1032474	Surface water	12-09-2017
Ruwwater (duinfiltraat)	1032475	Surface water	11-09-2017
Reinwater Leiduin	1032476	Drinking water	11-09-2017
Reinwater Weesperkarspel	1032477	Drinking water	11-09-2017
Vitens			
Vechterweerd ruw	Pb Vechterweerd Ruw	River bank filtrate	14-09-2017
Vechterweerd rein	Pb Vechterweerd Rein	Drinking water	14-09-2017
Pb. Engelse Werk rein	Pb Eng. werk Rein	Drinking water	13-09-2017
Pb. Buren rein	Pb Buren	Drinking water	12-09-2017
Pb. Doorn rein	Pb Doorn Rein	Drinking water	13-09-2017
Pb. Soestduinen rein	Pb Soestduinen	Drinking water	14-09-2017
Pb. Edese Bos rein	Pb Edese Bos Rein	Drinking water	14-09-2017
Pb. Dinxperlo rein	Pb Dinxperlo	Drinking water	13-09-2017
Oasen			
Lekkerkerk ruw	GLSPE99B	River bank filtrate	14-09-2017

Sample description	Sample code	Matrix	Sampling date
Lekkerkerk rein	PLSLR99C	Drinking water	14-09-2017
RO Feed	GLTPE99C	River bank filtrate	14-09-2017
RO Permeaat	PLKMH022	River bank filtrate	14-09-2017
RO Concentraat	PLKMP02Z	River bank filtrate	15-09-2017
Waterbedrijf Groningen			
Drentse Aa	1746066	Surface water	11-09-2017
Reinwater	1746065	Drinking water	11-09-2017
WMD			
Noordbargeres ruw	1746002	Groundwater	11-09-2017
Noordbargeres rein	1746001	Drinking water	11-09-2017
De Watergroep			
WPC Blankaart ruwwater	WPC Blanckaart ruw	Surface water	13-09-2017
WPC Blankaart reinwater	WPC Blanckaart rein	Drinking water	13-09-2017
WPC Zele ruwwater	Ruw	Surface water	12-09-2017
WPC Zele reinwater	Rein	Drinking water	12-09-2017
Overige			
Lobith	Lobith *Rijn	Surface water	14-09-2017

Attachment V Results study

TABLE V-1 RESULTS SAMPLING CAMPAIGN: CONCENTRATION OF FOSA, F₃-MSA AND HFPO-DA

Sample description	Matrix	Concentration		
		FOSA ng/L	F ₃ -MSA ng/L	HFPO-DA ng/L
Evides				
Keizersveer	Surface water	< 0.25	28	5.8
Afgeleverd water Biesboschbekkens	Surface water	< 0.25	24	10
Reinwater Kralingen	Drinking water	< 0.25	21	8.9
Ruw grondwater Jeugdorp (Dordrecht)	Groundwater	< 0.25	< 1.0	< 0.20
Reinwater grondwaterzuivering Baanhoek	Drinking water	< 0.25	22	10
Reinwater Baanhoek (dw uit ow en gw)	Drinking water	< 0.25	22	9.8
WML				
Heel, Innamewerk Lateraalkanaal	Surface water	< 0.25	32	0.60
Heel, Spaarbekken De lange Vlieter	Surface water	< 0.25	150	0.84
Heel, Gezamenlijk ruwwater Galgenberg	River bank filtrate	< 0.25	135	0.40
Heel, Gezamenlijk ruwwater De Reut en Langven	River bank filtrate	< 0.25	230	0.22
Heel, Reinwaterlevering Helden	Drinking water	< 0.25	165	0.34
Brabant Water				
Vessem ruwwater	Groundwater	< 0.25	< 1.0	< 0.20
Vessem reinwater	Drinking water	< 0.25	< 1.0	< 0.20
Waalwijk ruwwater	Groundwater	< 0.25	< 1.0	< 0.20
Waalwijk reinwater (vlijmen)	Drinking water	< 0.25	< 1.0	< 0.20
Dunea				
Inname Lagedruk pompstation Brakel	Surface water	< 0.25	29	9.7
Duinfiltraat Meijendell	Dune filtrate	< 0.25	24	6.7
Duinfiltraat Berkheide	Dune filtrate	< 0.25	45	5.0
reinwater Scheveningen	Drinking water	< 0.25	43	4.6
PWN				
IJsselmeerwater	Surface water	< 0.25	49	0.28
Effluent WPJ	Surface water	< 0.25	46	0.30
Toevoer UV/H ₂ O ₂ -AKF	Surface water	< 0.25	39	0.22
Toevoer duin (na passage UV/H ₂ O ₂)	Surface water	< 0.25	39	0.22
Na duinpassage	Dune filtrate	< 0.25	45	0.22
Drinkwater Bergen	Drinking water	< 0.25	27	0.20
Grondwater secundair Zuid in Laren	Groundwater	< 0.25	< 1.0	< 0.20
Waternet				
Ruw water inlaat WCB (Lekkanaal)	Surface water	< 0.25	35	< 0.20
Bethunepolder	Surface water	< 0.25	8.5	0.53
Ruwwater (duinfiltraat)	Surface water	< 0.25	52	0.20
Reinwater Leiduin	Drinking water	< 0.25	50	0.28
Reinwater Weesperkarspel	Drinking water	< 0.25	10	0.28
Vitens				
Vechterweerd ruw	River bank filtrate	< 0.25	7.4	< 0.20
Vechterweerd rein	Drinking water	< 0.25	4.9	< 0.20
Pb. Engelse Werk rein	Drinking water	< 0.25	21	< 0.20
Pb. Buren rein	Drinking water	< 0.25	< 1.0	< 0.20
Pb. Doorn rein	Drinking water	< 0.25	< 1.0	< 0.20
Pb. Soestduinen rein	Drinking water	< 0.25	< 1.0	< 0.20
Pb. Edese Bos rein	Drinking water	< 0.25	< 1.0	< 0.20
Pb. Dinxperlo rein	Drinking water	< 0.25	20	< 0.20
Oasen				

Sample description	Matrix	Concentration		
		FOSA ng/L	F ₃ -MSA ng/L	HFPO-DA ng/L
Lekkerkerk ruw	River bank filtrate	< 0.25	62	59
Lekkerkerk rein	Drinking water	< 0.25	32	28
RO Feed	River bank filtrate	< 0.25	59	5.3
RO Permeaat	River bank filtrate	< 0.25	< 1.0	< 0.20
RO Concentraat	River bank filtrate	0.92	165	28
Waterbedrijf Groningen				
Drentse Aa	Surface water	< 0.25	1.5	0.28
reinwater	Drinking water	< 0.25	1.1	< 0.20
WMD				
Noordbargeres ruw	Groundwater	< 0.25	< 1.0	< 0.20
Noordbargeres rein	Drinking water	< 0.25	< 1.0	< 0.20
De Watergroep				
WPC Blankaart ruwwater	Surface water	< 0.25	82	0.20
WPC Blankaart reinwater	Drinking water	< 0.25	85	0.23
WPC Zele ruwwater	Surface water	< 0.25	4.4	< 0.20
WPC Zele reinwater	Drinking water	< 0.25	4.5	< 0.20
Overige				
Lobith	Surface water	< 0.25	49	< 0.20

Attachment VI Results suspect screening halogenated methanesulfonic acids

TABLE VI-1 RESULTS SUSPECT SCREENING OF Cl-MSA, Cl₂-MSA, Cl₃-MSA, Br-MSA, Br₂-MSA AND BrCl-MSA

Sample description	Matrix	n	Cl-MSA (ng/L)*	Cl ₂ -MSA (ng/L)*	Cl ₃ -MSA (ng/L)*	Br-MSA (ng/L)*	Br ₂ -MSA (ng/L)*	BrCl-MSA (ng/L)*
Reinwater Kralingen	DW	1	n.d.	5.2	1.4	n.d.	n.d.	n.d.
Reinwater grondwaterzuivering Baanhoek	DW	2	n.d.	11	1.4	n.d.	n.d.	1.0
Reinwater Baanhoek (dw uit ow en gw)	DW	3	n.d.	13	1.5	n.d.	n.d.	1.2
Heel, Reinwaterlevering Helden	DW	4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vessem reinwater	DW	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Waalwijk reinwater (vlijmen)	DW	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reinwater Scheveningen	DW	7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Drinkwater Bergen	DW	8	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reinwater Leiduin	DW	9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reinwater Weesperkarspel	DW	10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vechterweerd rein	DW	11	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb. Engelse Werk rein	DW	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb. Buren rein	DW	13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb. Doorn rein	DW	14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb. Soestduinen rein	DW	15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb. Edese Bos rein	DW	16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb. Dinxperlo rein	DW	17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lekkerkerk rein	DW	18	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Reinwater (WBG)	DW	19	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Noordbargeres rein	DW	20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
WPC Blankaart reinwater	DW	21	n.d.	75	4.0	n.d.	65	108
WPC Zele reinwater	DW	22	n.d.	148	n.d.	n.d.	3.7	52
ruw grondwater Jeugddorp (Dordrecht)	GW	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vessem ruwwater	GW	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Waalwijk ruwwater	GW	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Grondwater secundair Zuid in Laren	GW	4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Noordbargeres ruw	GW	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Keizersveer	SW	1	n.d.	12	3.1	n.d.	1.2	n.d.
Afgeleverd water Biesboschbekkens	SW	2	n.d.	33	1.8	1.1	11	17
Heel, Innamewerk Lateraalkanaal	SW	3	n.d.	10	1.6	n.d.	n.d.	n.d.
Heel, Spaarbekken De lange Vlieter	SW	4	n.d.	3.4	n.d.	n.d.	n.d.	n.d.
Inname Lagedruk pompstation Brakel	SW	5	n.d.	7.5	2.2	n.d.	2.1	n.d.
IJsselmeerwater	SW	6	n.d.	1.4	n.d.	n.d.	1.3	n.d.
Ruw water inlaat WCB (Lekkanaal)	SW	7	n.d.	3.3	1.2	n.d.	n.d.	n.d.
Bethunepolder	SW	8	n.d.	0.9	n.d.	n.d.	n.d.	n.d.
ruwwater (duinfiltraat)	SW	9	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Drentse Aa	SW	10	n.d.	1.7	n.d.	n.d.	n.d.	n.d.
WPC Blankaart ruwwater	SW	11	n.d.	32	3.7	n.d.	10	19
WPC Zele ruwwater	SW	12	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lobith	SW	13	n.d.	3.8	1.0	n.d.	n.d.	n.d.
Heel, gezamenlijk ruwwater Galgenberg	RBF/DF	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Heel, gezamenlijk ruwwater De Reut en Langven	RBF/DF	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Vechterweerd ruw	RBF/DF	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lekkerkerk ruw	RBF/DF	4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Sample description	Matrix	n	Cl-MSA (ng/L)*	Cl ₂ -MSA (ng/L)*	Cl ₃ -MSA (ng/L)*	Br-MSA (ng/L)*	Br ₂ -MSA (ng/L)*	BrCl-MSA (ng/L)*
Duinfiltiraat Meijendell	RBF/DF	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Duinfiltiraat Berkheide	RBF/DF	6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Na duinpassage	RBF/DF	7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RO Feed	PW	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RO Permeaat	PW	2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
RO Concentraat	PW	3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Effluent WPJ	PW	4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Toevoer UV/H2O2-AKF	PW	5	n.d.	1.9	n.d.	n.d.	n.d.	n.d.
Toevoer duin (na passage UV/H2O2)	PW	6	n.d.	1.2	n.d.	n.d.	1.1	n.d.

* = F₃-MSA equivalents

Attachment VII Toxicological evaluation: structural alerts and Cramer classification

STRUCTURAL ALERTS AND CRAMER CLASSIFICATION INDICATED BY OECD QSAR TOOLBOX (V3.4.0.1.7) PROFILING

Technical name	CAS	Structural alerts	Cramer class
HPFO-DA	13252-13-6	<p><u>DNA Binding by OASIS v.1.4:</u></p> <p>AN2 >> Schiff base formation by aldehyde formed after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>Radical >> Radical mechanism via ROS formation (indirect) >> Geminal Polyhaloalkane Derivatives</p> <p>SN1 >> Carbenium ion formation >> Alpha-Haloethers</p> <p>SN2 >> Acylation involving a leaving group after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> Nucleophilic substitution at sp3 carbon atom after thiol (glutathione) conjugation >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> SN2 at sp3-carbon atom >> Alpha-Haloethers</p> <p><u>In vivo mutagenicity (Micronucleus) alerts by ISS:</u></p> <p>H-acceptor-path3-H-acceptor</p>	High (Class III)
FRD-902	62037-80-3	<p><u>DNA Binding by OASIS v.1.4:</u></p> <p>AN2 >> Schiff base formation by aldehyde formed after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>Radical >> Radical mechanism via ROS formation (indirect) >> Geminal Polyhaloalkane Derivatives</p> <p>SN1 >> Carbenium ion formation >> Alpha-Haloethers</p> <p>SN2 >> Acylation involving a leaving group after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> Nucleophilic substitution at sp3 carbon atom after thiol (glutathione) conjugation >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> SN2 at sp3-carbon atom >> Alpha-Haloethers</p> <p><u>In vivo mutagenicity (Micronucleus) alerts by ISS:</u></p> <p>H-acceptor-path3-H-acceptor</p>	High (Class III)
F ₃ -MSA	1493-13-6	No structural alerts	High (Class III)
FOSA	754-91-6	<u>DNA Binding by OASIS v.1.4:</u>	High (Class III)

	<p>AN2 >> Schiff base formation by aldehyde formed after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>Radical >> Radical mechanism via ROS formation (indirect) >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> Acylation involving a leaving group after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> Nucleophilic substitution at sp3 carbon atom after thiol (glutathione) conjugation >> Geminal Polyhaloalkane Derivatives</p> <p><u>DNA alerts for AMES by OASIS v.1.4:</u></p> <p>AN2 >> Schiff base formation by aldehyde formed after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>Radical >> Radical mechanism via ROS formation (indirect) >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> Acylation involving a leaving group after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> Nucleophilic substitution at sp3 carbon atom after thiol (glutathione) conjugation >> Geminal Polyhaloalkane Derivatives</p> <p><u>DNA alerts for CA and MNT by OASIS v.1.4:</u></p> <p>AN2 >> Schiff base formation by aldehyde formed after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>Radical >> Radical mechanism via ROS formation (indirect) >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> Acylation involving a leaving group after metabolic activation >> Geminal Polyhaloalkane Derivatives</p> <p>SN2 >> Nucleophilic substitution at sp3 carbon atom after thiol (glutathione) conjugation >> Geminal Polyhaloalkane Derivatives</p> <p><u>Carcinogenicity (genotox and nongenotox) alerts by ISS:</u></p> <p>Perfluorooctanoic acid (PFOA) (Nongenotox)</p> <p>Structural alert for nongenotoxic carcinogenicity</p>	
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