Datum	Diarienummer	Ärendetyp
2021-08-18	21FMV5219-1:1	3.4
	Dokumentnummer	Sida
		1(2)

HKV LEDS TF Hållbarhetssektionen
Lidingövägen 24
107 85 Stockholm

Er referens
[redacted]
VerkO Log V Metod

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Rapport *Validation of the TOP-methodology for purpose of quantification of PreFAS in fluorine-free fire-fighting foams*

Försvarets Materielverk (FMV) överlämnar härmed konsultrapporten *Validation of the TOP-methodology for purpose of quantification of PreFAS in fluorine-free fire-fighting foams,* med bilaga (Appendix 1), till Försvarsmakten.

Bakgrund

Försvarets Materielverk har på uppdrag av Försvarsmakten (HKV LEDS TF Hållbarhetssektionen) beställt en konsultstudie i syfte att undersöka om analysmetoden TOP (Total Oxidizable Precursor), optimerad och validerad för matrisen fluorfria (PFAS-fria) släckskumskoncentrat, kan anses vara en tillförlitlig analysmetod att använda i upphandlingsprocessen för att verifiera släckskumskoncentrat som uppges vara PFAS-fria.

Konsultstudien genomfördes av [redacted] (Tekn. Dr., M.Sc.), Intersolia Sweden AB. Validering av analysmetoden TOP har utförts av SGS Analytics Sweden AB (tidigare SYNLAB Analytics & Services) i Linköping, Eurofins Environment Testing Sweden AB i Lidköping samt ALS Global laboratories i Prag (Tjeckien). ALS genomförde även optimeringen av TOP-metoden. Analysarbete avseende ultra-korta PFAS samt EOF/CIC har utförts av [redacted] vid Örebro Universitet. Från FMV har uppdraget letts av miljösamordnarna [redacted] och [redacted]

Note: This English translation is for English readers' convenience only. If there is a difference between this translation and the Swedish original (above), Swedish original supersedes this translation.

The Swedish Defence Materiel Administration (FMV) hereby submit the consultancy report *Validation of the TOP-methodology for purpose of quantification of PreFAS in fluorine-free fire-fighting foams,* with appendix (Appendix 1), to the Swedish Armed Forces.

Background

The Swedish Armed Forces (HKV LEDS TF Hållbarhetssektionen) assigned the Swedish Defence Materiel Administration (FMV) to commission a consultancy study with the purpose of investigating if the TOP assay (Total Oxidizable Precursor), optimized and validated for the matrix fluorine free (PFAS-free) firefighting foams, is a reliable analytical method to use in procurement to verify claims of firefighting foams being PFAS-free.

[redacted] (Eng.D, M. Sc.), Intersolia Sweden AB, conducted the consultancy study. ALS Global laboratories in Prague (Czech Republic), SGS Analytics Sweden AB (former SYNLAB Analytics & Services) in Linköping (Sweden) and Eurofins Environment Testing Sweden AB in Lidköping (Sweden) performed the validation step of the TOP assay. ALS also performed the optimization of the TOP assay. The analyses in regards to ultra-short PFAS and EOF/CIC were conducted by [redacted] at Örebro University. Environmental officers [redacted] and [redacted] from the Swedish Defence Materiel Administration, lead the study.

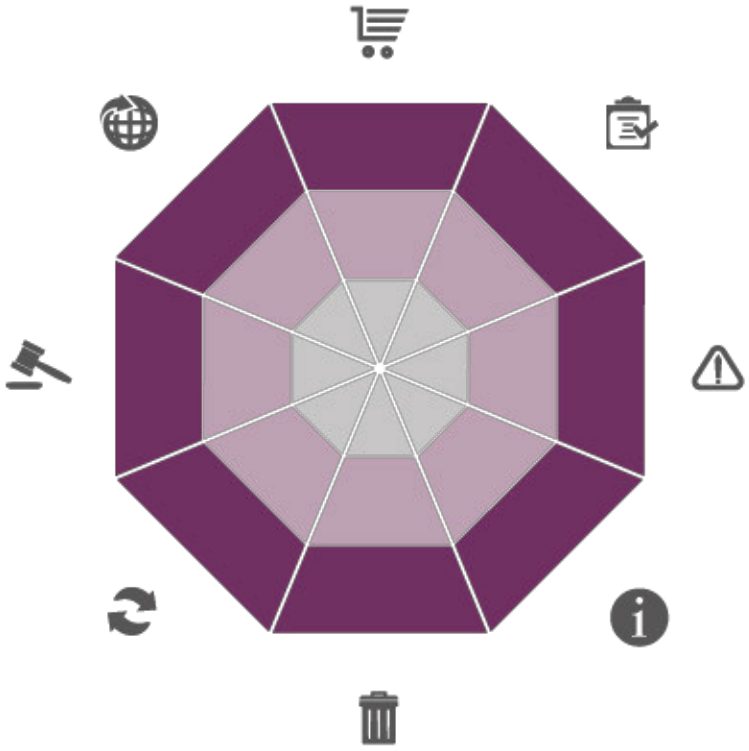
Försvarets Materielverk

[redacted]

Projektledare Miljo- och hållbarhetsarbete

Sändlista:
Arkiv

intersolia
Chemical Responsibility



Validation of the TOP-methodology for purpose of quantification of PreFAS in fluorine-free fire-fighting foams

Contents

1. Sammanfattning (svenska)

I denna fördjupade studie har TOP-metoden undersökts avseende lämplighet och prestanda vid kvantifiering av PFAS i fluorfria (PFAS-fria) släckskumskoncentrat, härefter benämnt F3-koncentrat (F3: fluorine-free foam).

Metodiken har fördelen att även dolda PFAS (främst perfluorerade alkylsyror och fluorerade telomeralkoholer), för vilka det ej nödvändigtvis finns konventionella analysmetoder, via oxidation i TOP-steget omvandlas till kända PFAS för vilka det finns vedertagen analysmetodik HPLC-MS/MS. En ytterligare fördel med TOP-metodiken är att den, genom att instrumentellt bygga på HPLC-MS/MS-instrumentering, erfar en hög känslighet (låga rapporteringsgränser) och hög selektivitet.

Nackdelen med metodiken är främst att ökningen av enskilda, kända PFAS-homologer, efter TOP-oxidationen, ej går att härleda till vilka specifika PFAS-prekursorer som förelegat i skumprovet innan TOP-steget.

TOP-metodiken applicerad på matrisen släckskumskoncentrat ställer krav på rätt dosering av oxidationsmedel då koncentraten har ett högt innehåll av oxiderbart organiskt kol (enligt de säkerhetsdatablad som tillhandahålls av leverantörerna). Huruvida F3-koncentrat innehåller mer oxiderbart organiskt kol jämfört med AFFF-koncentrat var inte utrett innan projektet initierades.

TOP-metodiken som verifikationsmetod för förekomst av PFAS i F3-koncentrat har ej tidigare validerats med avseende på metodprestanda. I en förstudie genomförd av FMV 2017-2018 påvisades PFAS i fem F3-koncentrat vilka av leverantörerna uppgavs vara ”fluor-fria”. Halterna som påvisades var i regel mycket låga men skulle potentiellt kunna leda till spridning av PFAS till miljön vid frekvent övningsverksamhet med släckskum.

I samband med upphandling av fluorfria släckskum är det kritiskt att ha en vetenskapligt etablerad verifikationsmetod för begreppet ”PFAS-fritt”. Syftet med studien har därför varit att statistiskt undersöka om och hur TOP-metoden skulle kunna nyttjas för detta ändamål.

Studien bygger på att sex F3-koncentrat (utvalda av FMV) och ett AFFF-koncentrat provtagits och analyserats med konventionell HPLC-MS/MS-metodik av tre kommersiella laboratorier.

Skumkoncentraten har sedan varit föremål för TOP-oxidation enligt ett specifikt, inom ramen för projektet, optimerat protokoll[1].

Resultaten visar att för de F3-koncentrat där PFAS detekteras både före och efter TOP-oxidation, ökar halterna PFAS efter TOP-steget med 28 – 112 ggr. För AFFF-koncentratet ökade halten PFAS, beräknat som $\sum PFAS_{11}$, på motsvarande sätt ca 660 ggr efter TOP-steget.

Tre av de sex ursprungligt utvalda F3-koncentraten innehöll bedömdes inte innehålla några PFAS, varken före eller efter TOP-steget, medan för de andra tre F3-koncentrat kunde PFAS påvisas i låga halter ($\sum PFAS_{11}$; 10-190 µg/l) innan TOP och i förhöjda halter efter TOP ($\sum PFAS_{11}$; 325-8 100 µg/l).

I de F3-koncentrat där PFAS påvisades var PFAS-homologen 6:2 FTS den enda varianten som detekterades innan TOP-steget. Efter TOP-steget detekterades främst PFBA, PFPeA och PFHxA men även lägre halter av PFHpA.

Avseende AFFF-koncentratet förekom 6:2 FTS och i en replikatanalys även PFHxA innan TOP-oxidationen medan PFBA, PFPeA och PFHpA detekterades enbart efter TOP.

Baserat på ANOVA-variansanalys[2] kunde det konstateras att ett av de deltagande F3-koncentraten skulle bli dimensionerande för hela frågeställningen; *”hur höga PFAS-halter efter TOP-oxidation kan ett F3-koncentrat uppvisa och fortfarande betraktas som ”PFAS-fritt?”*

[1] Se appendix 1 till denna rapport för information avseende optimering av TOP-metoden med avseende på skumkoncentrat.

[2] ANOVA-variansanalys är en statistisk metod för hypotesprövning. Variansanalys kan användas för att undersöka skillnader i medelvärde och varians mellan två eller fler populationer.

När analysresultaten för detta F3-koncentrat analyserades medelst ANOVA kunde det konstateras att den systematiska variansen uppgick till *ca* 74 % av den totala variansen. Beräkningar av ensidiga 95 %-iga konfidensintervall, dels enligt Student's t-fördelning, och dels medelst den i sammanhanget något robustare Studentized Range Distribution enligt Tukey's honestly significant difference-metoden, gav ett ensidigt konfidensintervall om 415 - 1 552 g/l avseende uppmätt halt $\sum PFAS_{11}$ efter TOP. Detta betyder, som rekommenderats i rapporten, att om det bredare intervallet enligt Tukey's honestly significant difference om 1 552 µg/l nyttjas, bör det således ge en statistisk rabatt om *ca* 1 552 µg/l när F3-koncentrat skall verifieras som PFAS-fria, enligt TOP-metoden. Med ett 95 %-igt konfidensintervall är sannolikheten 5% att begå ett s k statistiskt typ I-fel, d.v.s. att felaktig betrakta ett PFAS-fritt F3-koncentrat som PFAS-innehållande.

Givet det 95 %-iga ensidiga konfidensintervallet blir det enbart Foam2 som klassas som ej PFAS-fritt, medan Foam 3 och Foam 5 blir klassade som PFAS-fria. För Foam 1 och Foam 4 redovisar inget av de tre deltagande laboratorierna någon PFAS-förekomst efter TOP-oxidationen varför dessa skall betraktas som PFAS-fria. Foam 6 bedömdes som PFAS-fritt redan efter metodutvecklingssteget då 12 genomförda TOP-oxidationer vid 9 olika TOP-betingelser ej kunde påvisa PFAS i det F3-koncentratet.

Inom ramen för projektet har även den optimerade TOP-metoden validerats m a p repeterbarhet och reproducerbarhet, i enlighet med kraven befästa i standarden ASTM E691-99 ("*Standard practice for Conducting a Interlaboratory study to determine Precision of a Test Method*").

Både TOP-metodens repeterbarhet och dess reproducerbarhet, beräknade som "difference 2-sigma limit", har befunnits variera mellan olika F3-koncentrat och förefaller ej vara koncentrationsberoende (beräknade som relativ standardavvikelse).

En intressant iakttagelse är att repeterbarheten för F3-koncentrat Foam 2 (dimensionerade för det ensidiga konfidensintervallet) uppvisade en repeterbarhet om 1 514 µg/l, således snarlik i storlek med det ensidiga föreslagna konfidensintervallet.

Slutligen har projektet även arbetat med att ta fram en mer generisk fluorbudget för både F3-koncentrat och AFFF-koncentrat.

De olika fluor-innehållande bidragen har uppdelats i;

1) de PFAS som rapporteras i en SCFC-MS/MS[3] analys av ultra-korta PFAS
2) de PFAS som rapporteras i en konventionell $PFAS_{25}$-analys
3) de PFAS som rapporteras efter TOP-oxidation
4) halten organiskt fluor som rapporteras efter en CIC/EOF-analys.

Fraktion 2 och 3 i listan ovan är sannolikt överlappande.

För de skumkoncentrat som ingått i studien var resultaten m a p en fluorbudget delvis svårtolkade. Analys av ultra-korta PFAS medelst SCFC-MS/MS i både outspädda skumkoncentrat och i TOP-oxiderade skumkoncentrat visar att dessa fem ultra-korta PFAS ej förekom i koncentraten (varken i F3-koncentrat eller AFFF-koncentrat). Detta beror troligen på metodikens höga detektionsgränser. Detekterade halter av organiskt fluor däremot, bestämda som summan av organiskt fluor i neutral och i anjonisk fraktion, är svåra att relatera till rapporterade halter $\sum PFAS_{11}$ efter TOP-oxidation;

- för AFFF-koncentratet förklaras ca. 46 % av det fluororganiska innehållet från CIC/EOF-analysen av halten $\sum PFAS_{11}$ efter TOP,
- för F3-koncentratet Foam 2 (det F3-koncentrat som enligt föreslagen metodik ej kan betraktas som PFAS-fritt) förklaras 23,4 % av det rapporterade fluororganiska innehållet från CIC/EOF-analysen av halten $\sum PFAS_{11}$ efter TOP,
- för Foam 5 (F3-koncentrat som den föreslagna metodiken i rapporten klassar som "PFAS-fritt") förklaras 0,1 – 25 % av det rapporterade fluororganiska innehållet efter CIC/EOF-analysen av halten $\sum PFAS_{11}$ efter TOP (beroende på om prover föremål för CIC/EOF-analys

[3] SCFC-MS/MS; Superkritisk fluid kromatografi kopplat till MS/MS.

först varit föremål för TOP-oxidation eller om CIC/EOF-analysen företagits på spädda, ej-oxiderade skumkoncentrat).

I beräkningarna kring fluorbudgeten har rapporterade halter organiskt fluor från CIC/EOF (i ng F/ml) konverterats till 6:2 FTS-ekvivalenter för att CIC/EOF och TOP skall kunna jämföras.

Sammantaget ger detta en bild av två analysmetoder, TOP och CIC/EOF, för att bestämma organiskt fluor som sådant eller som PFAS, som bägge absolut har sina förtjänster men som ej verkar mäta samma storhet – likt två linjaler som har helt olika skalindelning. Detta kan liknas vid att ”TOP-linjalen” möjligen mäter sträckor upp till någon decimeter med hög riktighet och där graderingen är millimeter, medan CIC/EOF-linjalen sannolikt klarar att mäta sträckor på flera meter men där skalindelningen är i centimeter eller decimeter.

Det skall tilläggas att studien ej syftade till att validera eller systematiskt undersöka CIC/EOF-metoden m a p F3-koncentrat och det laboratorium som utförde CIC/EOF-analyserna har ej kunnat påverka försöksupplägg, prover eller spädningar.

Den viktigaste slutsatsen från studien är att när tillverkning av, tillgängliggörandet av, och användningen av skumkoncentrat blir föremål för reglering eller gränsvärden, bör reglerande myndigheter och andra beslutsfattare även specificera med vilken analysmetodik en skumtillverkare eller användare skall verifiera sina skumkoncentrat med. I upphandlingssammanhang bör man på samma sätt definiera vad man menar med exempelvis begreppet ”PFAS-fritt”, samt ge tydliga anvisningar om hur deltagande anbudsgivare/leverantörer skall kunna verifiera sina produkter som just PFAS-fria.

Genom antagande att de sex F3-skum som undersökts i denna studie representerar alla F3-skum på marknaden, bör det ensidiga konfidensintervallet, ’den statistiska rabatten’, om 1 552 µg/l medge en trovärdig verifiering av begreppet ”PFAS-fritt” för F3-koncentrat som analyseras av valfritt kommersiellt laboratorium som genomför en TOP-assay enligt det protokoll som framtagits i projektet.

2. Summary

In this in-depth study, the TOP-oxidation methodology has been assessed for its adequacy in evaluating the presence of PFAS in allegedly PFAS-free foam concentrates (F3-concentrates), as well as having been validated with regard to its performance in terms of the quantification of PFAS in such matrices.

The TOP-methodology itself has the advantage of being able to transform hidden PFAS, *i.e.*, PFAS-homologues that are currently not part of a conventional HPLC-MS/MS method, to known PFAS for which there are HPLC-MS/MS methods. Another advantage of the TOP-methodology is that it benefits from the high selectivity, good sensitivity and low LOD associated with HPL-MS/MS methods. Furthermore, the TOP-methodology does not require any additional expensive equipment nor any unconventional chemicals, in contrast to for instance the EOF/CIC-methodology.

The main advantage of the TOP-methodology resides in the fact that it is a bit of a 'black box' where it is virtually impossible to track or originate the increase of a known PFAS after TOP-oxidation to a certain PFCA[4] precursor. In the context of fire-fighting foams, PFAS and their PFCA-precursors aggregate to several thousand different molecules, potentially added to the foam concentrate to increase the film-forming potential. Executing the TOP-oxidation assay will reveal a large extent of those hidden PFAS but no information will be retrieved as to the chemical identity of the PFCA precursors.

The TOP-oxidation methodology applied to foam concentrates (both F3- and AFFF-concentrates) requires a precise dosing of oxidants since the matrices themselves contain a fair amount of organic carbon and most probably all other carbon-hydrogen species will be oxidized before the oxidants start to transform PFCA precursors to know PFAS. Over-dosing of the oxidants may potentially also be detrimental to the results if PFCA precursors are mineralized or being transformed to ultra-short chain PFAS in the TOP-assay and subsequently lost prior to HPLC-MS/MS quantification.

As a first step, DOC (Dissolved Organic Carbon) was determined in the group of foam concentrates chosen for this study. The six (6) F3-concentrates of the study varied considerably in their DOC contents (between 8 - 210 g/l), while the AFFF-concentrate contained 265 g/l of DOC.

The TOP-methodology has, to the best of the project´s knowledge, not been validated with regard to its prestanda and its precision with respect to F3-concentrates. In a pilot study on the TOP-assay applied to F3-foams executed by FMV in 2017-2018, PFAS was reported in five (5) allegedly "PFAS-free" F3-concentrates. The PFAS concentrations were very low but extensive fire-fighting exercises with these foams would potentially create contaminated areas.

In terms of procurement of fluorine-free foam concentrates, it is deemed necessary to have a scientifically approved method of verification with regard to the concept of PFAS-free. Thus, the aim of this study has been to statistically validate the precision and accuracy of the TOP-assay in terms of F3-concentrates.

In this current study, six (6) F3-concentrates and one AFFF-concentrate (all chosen by technical experts at FMV) were subjected to sampling and chemical analysis by conventional HPLC-MS/MS-methods by three commercial laboratories. The foam concentrates were thereafter subjected to a TOP-oxidation according to an optimized TOP assay protocol developed within this study, and subsequently analysed using HPLC-MS/MS.

Results from the study shows that in the F3-concentrates were PFAS was in fact reported (3 out of 6), the concentrations of PFAS increased post TOP by factor of 28 – 112 times, measured as $\sum PFAS_{11}$. The corresponding increase in PFAS-concentration with regard to the AFFF-concentrate was 660 times post TOP.

In the three F3-concetrates containing PFAS, 6:2 FTS was the only occurring homologue before TOP, while PFBA, PFPeA and PFHxA were reported post TOP. As for the AFFF concentrate, 6:2FTS and,

[4] PFCA; perfluoralkyl acids

in some replicate analysis, also PFHxA were reported before TOP while PFBA, PFPeA, PFHxA and PFHpA were reported post TOP.

Based on an ANOVA[5] analysis (both two- and one-way ANOVA) it became evident that one of the F3-concentrates stood out and became dimensioning and central for the question at issue; "*how high concentrations of PFAS can a F3-concentrate display after TOP-oxidation and still be considered as PFAS-free*"?

Upon scrutinizing the results from the ANOVA analysis, it became evident that the systematic variance amounted to 74 % of the total variance in data. Calculations of a one-sided 95 % confidence interval, both in accordance with the Student's t-distribution and Studentized Range Distribution resulted in in an interval between 415 – 1 552 µg/l with respect to $\sum PFAS_{11}$ after the TOP-assay. This means that if the one-sided Studentized Range Distribution interval by Tukey's honestly significant difference of 1 552 µg/l, which is said to be more robust than Student's t-distribution-based confidence intervals, is employed, a statistical discount of 1 552 µg/l must be given in the TOP-oxidation result. Thus, if the concentration of PFAS post TOP (as $\sum PFAS_{11}$) > 1 552 µg/l, then the F3-concentrate cannot be regarded as PFAS-free. The risk of committing a type I statistical error, erroneously dismissing a F3-concentrate as not being PFAS-free, will have a 5 % probability.

Applying the one-sided confidence limit of 1 552 µg/l renders only Foam 2 of the participating F3-concentrates to be dismissed as not being PFAS-free. Regarding the other F3-concentrates of the study, three of them (Foam 1, Foam 4 and Foam 6) were considered PFAS-free without having to apply the 'statistical discount' while two F3-concentrates (Foam 3 and Foam 5) displayed $\sum PFAS_{11}$-concentrations > 0 but < 1 552 µg/l post TOP.

Within the study, the precision (repeatability and reproducibility) of the TOP-assay with regard to F3-concentrates has also been evaluated in accordance with ASTM E691-99 ("*Standard practice for Conducting a Interlaboratory study to determine Precision of a Test Method*").

Both the precision repeatability and reproducibility, calculated as the "difference 2-sigma limit", vary between the F3-concetrates in a non-concentration dependant manner (calculated as relative standard deviation).

An interesting observation is that both the repeatability of the TOP-assay $\sum PFAS_{11}$-results for Foam2 (1 514 µg/l) is in the same size as the one-sided confidence interval, 1 552 µg/l

Finally, the study also aimed at providing a more generic fluorine budget of the foam concentrates (both F3- and AFFF-concentrates).

The potentially fluorine-containing fractions were divided in to;

1) PFAS reported in an SCFC-MS/MS-analysis of ultra-short chain C_2-C_3 PFAS,
2) PFAS reported in a conventional $PFAS_{25}$-analysis by HPLC-MS/MS,
3) PFAS reported as $\sum PFAS_{11}$ in a TOP-assay executed according to the TOP-assay protocol developed within this study.
4) The concentration of extractable organic fluorine from a CIC/EOF-analysis, converted to 6:2 FTS-equivalents.

The results of the aggregated fluorine budgets were difficult to interpret and incomprehensive. Taking the quite elevated LOD of the analysis of ultra-short chain PFAS into account, ultra-short chain PFAS was not reported in neither F3-concentrates nor the AFFF-concentrate, neither in the non-oxidized nor the TOP-oxidized samples. Thus, ultra-short chain PFAS does not contribute to the fluorine budget.

[5] ANOVA is a statistical technique that used to check if the means of two or more groups are significantly different from each other and furthermore ANOVA checks the impact of one or more factors by comparing the means of different samples.

The reported concentrations of extractable organic fluorine, in the neutral as well as the anionic SPE-fractions respectively from the CIC/EOF-analysis were, however difficult to accommodate when comparing these results with the $\sum PFAS_{11}$-results from the TOP-oxidations.

- In the AFFF-concentrate ca 46 % of the extractable organic fluorine (as 6:2-equivalents) was explained by the $\sum PFAS_{11}$-concentration after TOP (at the best).
- In Foam 2 (the non-"PFAS-free" F3-concentrate), ca 23,4 % of the extractable organic fluorine (as 6:2-equivalents) was explained by the $\sum PFAS_{11}$-concentration after TOP.
- In Foam 5 (a "PFAS-free" F3-concentrate when applying the 'statistical discount') 0,1-25 % of the extractable organic fluorine (as 6:2-equivalents) was explained by the $\sum PFAS_{11}$-concentration after TOP.

In all comparisons between CIC/EOF and TOP-results, reported concentrations have been converted from ng Fluorine/ml in the CIC/EOF to ng/ml 6:2 FTS-equivalents.

Overall, the results when comparing CIC/EOF and TOP give the impression that these two analytical methods may be complimentary and are for sure measuring organic fluorine using different rulers where the TOP-method seem more suitable to provide high precision for the lower to medium PFAS concentration range and the CIC/EOF may provide good precision for very high PFAS concentration ranges.

In fairness, the aim of the study was not to validate the CIC/EOF -method and the fact that using also the CIC/EOF-method in this investigation presented itself late during the study means that no adjustments, dilutions or experimental design to specifically accommodate the samples for CIC/EOF was made.

The main overall conclusion from the study is that when the manufacturing, placing on the market, and use of fire-fighting foams becomes regulated, and regulatory bodies impose limit values for PFAS, then it is important, that extensive regulatory guidelines on analytical methods for verification of the limit values are provided.

With regard to public procurement processes involving "PFAS-free" fire-fighting foams, it will be crucial for the equality of the procurement process to explicitly state the verification method to foam formulators participating.

By assuming that the six (6) F3-concentrates in this study represents all F3-concentrates on the market and that the performance of the three (3) commercial laboratories participating reflects all commercial laboratories, the one-sided confidence interval (the 'statistical discount') of 1 552 µg/l (as $\sum PFAS_{11}$), will provide a credible verification of the concept of "PFAS-free" in these type of matrices when one submits a F3-concentrate for a TOP-assay (executed in compliance with the TOP-protocol provided herein) to any commercial laboratory conducting TOP-assays.

3. Aim of the study

The aim of this study was to test the hypothesis that the TOP assay, optimized and validated for the matrix fluorine free fire-fighting foams (F3), is a reliable analytical method for verifying claims of a fire-fighting foam being "PFAS-free".
The means to the aim in the study was to evaluate the different variance contributions when applying the TOP assay to declared fluorine free fire-fighting foams (sources of variance could be experimental, variance from replicates, variance from the different laboratories conducting the TOP assay etc.) Furthermore, as different variance contributions were estimated, the confidence limit of the systematic variance was calculated.

4. Introduction

In the introductory chapter, the reader of this report is offered a brief orientation as to the overall issue of PFAS in fire-fighting foams including current legislation, proposed thresholds for "PFAS-free" from different stakeholders as well as foreseeable future legislation. Methods of analysis and verification are also described with their pros and cons, respectively.

4.1 What is a "PFAS-free" fire-fighting foam?

The market for PFAS-free fire-fighting foams (often referred to as Fluorine Free Foams (F3)) has grown over the past years, as users of PFAS-based fire-fighting foams are transitioning to PFAS-free alternatives. In light of scientific findings in toxicology and eco toxicology, the continuous use of PFAS-based fire-fighting foams (often referred to as aqueous film forming foams (AFFF)[6]) are considered very problematic from a human health as well as an ecotoxicological perspective. PFAS[7] are used in fire-fighting foams to lower the surface tension and allow the formation of an aqueous film between fuel and foam.

Today there is no globally common definition of a fluorine free/PFAS-free fire-fighting foam, in relation to accepted limit value of traces/impurities of PFAS substances in the foam as well as analytical method(s) accepted to define these levels. It might pose future challenges for foam producers, authorities that procure/purchase foams, as well as end-users of foam concentrates, when different countries/regions/sectors now are developing their own definitions of PFAS-free fire-fighting foams. The lack of validated analytical methods to measure "all PFAS" content in a foam sample also adds to the complexity of the definition. For example, the upcoming EU restriction proposal on PFAS in fire-fighting foams will likely propose a limit value for PFAS that, if exceeded, would trigger restrictions on use and/or placing on the market. Hence, it is important to get an understanding of which analytical methods that can verify if a fire-fighting foam is in or out of scope for restrictions, as a complement to the supplier´s information that the foam is "fluorine free" or "PFAS free".

Examples presented in this chapter of the report demonstrate that the definition of "PFAS-free" can vary between different policies and that a lack of guidance on what analytical method(s) to use for verification leaves this decision to *e.g.* the foam producer, procurement body or end-user – who may lack the specific competence to choose the most appropriate analytical method. It is therefore evident that future regulations/product criteria on "PFAS-free" fire-fighting foams needs to specify a validated analytical method (or a combination of such) for verification of the requirement "PFAS-free".

[6] Class B fire-fighting foams are formulated to be most efficient at extinguishing liquid hydrocarbon fuel fires. Class B foams currently available on the market either contain fluorinated surfactants/fluoropolymers (PFAS) (*e.g.* in aqueous film-forming foams (AFFF)) or are fluorine free.
[7] The definition of 'PFAS' seems to have undergone a change from previously being read as *perfluorinated alkyl substances* to nowadays more adhering to the definition of *perfluoroalkyl and polyfluoroalkyl substances that are man-made chemicals with at least one fully fluorinated carbon atom*.

4.2 The EU regulatory landscape

The propensity of PFAS substances to persist in the environment once released (*i.e.* 'forever chemicals') in combination with their high bioaccumulation potential, has rendered in the inclusion of PFAS in the EU chemicals strategy8 (published in 2020), with commitments on actions to phase out the use of PFAS in the EU, unless their use is essential.
However, the use of AFFF was identified as problematic long before the EU chemicals strategy was published, and certain individual PFAS substances have been subjected to regulation and phase-out from AFFF use (as well as other uses) due to their SVHC[9]-properties, such as PFOS and PFOA (already restricted) but also PFHxS and PFHxA (upcoming restrictions).

The current regulatory approach in the EU, evaluating the human health and environmental risks and socio-economic pros and cons of individual PFAS substances (or small groups of closely related substances), is of course unsatisfactory for a substance group with over 4 700 individual substances[10].

A shift in the one-by-one PFAS substance regulatory approach is now emerging in the EU, as seen in the ongoing work by ECHA on drafting a restriction proposal for (all) PFAS in fire-fighting foams (Annex XV restriction dossier expected in October 2021), as well as the ongoing work by a group of Member States and Norway on a broad restriction dossier covering all uses of all PFAS, except those uses that are considered "essential" (dossier expected in 2022-2023).

To ensure a proper implementation and enforcement of PFAS restrictions, validated analytical methods to analyze "all PFAS" in samples from different matrices (with sufficiently high sensitivity) needs to be readably accessible for suppliers, downstream users as well as Member States authorities responsible for the enforcement of the REACH provisions.

Out of the allegedly 4 700 different PFAS substances, commercial laboratories have only analytical methods to quantify approx. 50-55 different PFAS and dedicated research laboratories approx. 75-80 different PFAS substances in foam concentrate matrices.

Thus, for end-users of AFFF that are committed to comply with the currently regulated PFAS as well as with the soon-to-be regulated PFAS substances, current analytical methods are enough to support legal compliance and also stipulated PFAS-levels in procurement.

However, for end-users that are committed to the procurement and use of only PFAS-free foams (Fluorine Free Foams (F3), the problems associated with such an undertaking may prove insurmountable.

For formulators of foam concentrates that aim to reduce or phase-out PFAS substances from their foam concentrates, buying the tenside contents from specific suppliers of tenside mixtures, rather than buying the actual surface active/film-forming chemical substances as such, may find it very difficult to guarantee and verify that the tenside mix from their supplier in fact does not contain any (of the 4 700 or so) PFAS substances, other than to fully rely on statements from their suppliers.

In October 2021, the EU restriction proposal on PFAS in fire-fighting foams is expected. As previous EU restriction proposals on chemicals, it is expected to define the scope of "PFAS" as well as a limit value that will define when restrictions on use and/or placing on the market of fire-fighting foams will come into effect. As the EU regulatory landscape for the use of PFAS in different applications are clearly moving towards a total ban of all PFAS (except for essential uses), the need for sensitive, validated analytical methods to measure "all PFAS" content, rather than individual PFAS substances, has rapidly emerged.

[8] https://ec.europa.eu/environment/strategy/chemicals-strategy_en
[9] SVHC = Substances of very high concern, as defined in EC nr 1906/2006 article 33.
[10] OECD made in 2018 a survey as to the number of PFAS substances currently being put on the market as such, in mixtures, and in articles and concluded that the number of PFAS substances surmounts of 4 700 individual substances

4.2.1. Proposed Swedish national legislation on fire-fighting foams containing "highly fluorinated substances"

In a short pilot study conducted by the Swedish Defence Materiel Administration (FMV) in 2017-2018, a small number of fluorine free foams (F3) (according to supplier´s information) were investigated and analysed. The pilot study was initiated by the FMV pursuant to proposed national legislation in Sweden by the Swedish Chemicals Agency (KemI) in 2016[11] to reduce the amounts of PFAS reaching the environment by the use of fire-fighting foam by implementing an application of national authorization for users of fire-fighting foams containing "highly fluorinated substances > 0,0001 % (w/w) in the foam concentrate", obligating the users to collect all water and foam residues from fire-fighting exercises and irreversibly destroy it.
"Highly fluorinated substances", the wording in the proposal from KemI meaning all organic moieties containing fluoro-carbon bonds, thus not only restricting it to PFAS but also including fluoro-telomer compounds (substances where not all carbon-hydrogen bonds have been replaced with fluorine-carbon bonds).
The pilot study was aiming at addressing whether the TOP-methodology (explained in chapter 4.4.2) could distinguish a F3 concentrate from a foam concentrate with a concentration of "highly fluorinated substances" > 0,0001 % (w/w). The fluorine free foams in the pilot study were determined to contain ca 0,00005 % (w/w) as a sum of "highly fluorinated substances" after the TOP-oxidation step and thus, none of them exceeded the trigger value in the proposed national foam legislation (*i.e.* their use would not be affected by the obligations in the proposed legislation).

When using conventional analysis/quantification methods (LC-MS/MS) with regard to PFAS (24 different PFAS substances were included in the method), only minute concentrations (1-25 µg/l) of a couple of PFAS substances (normally, 6:2 FTS and PFOA) could be detected in the F3-concentrates. The detected/quantified concentrations of PFAS were 6-10 times the method limit of detection and would most likely have passed as "contaminants" or "method bias".

However, when the very same F3 concentrates were subjected to an oxidation step prior to the final chemical analysis/quantification, the reported concentrations of PFAS in the F3-concentrates were magnified by a factor of 10-100 and the number of detected PFAS increased to 4-5 substances (normally PFBA, PFPeA, PFHxA, and 6:2 FTS).

A corresponding oxidation treatment and subsequent analysis of a known PFAS-containing AFFF concentrate also revealed that the concentrations of PFAS increased by a factor of 2 200 and the number of detected PFAS substance before and after the oxidation step increased from five to eight.

The number of samples in the study were limited and no replicate analysis were conducted but the study still gave one valuable insight.

- The methodology of oxidizing the foam concentrates prior to analyzing them by LC-MS/MS, called the TOP (Total Oxidizable Precursor) analysis methodology, could reveal and quantify "hidden" PFAS substances (precursor PFCAs) which were not included among the 24 PFAS substances of the analytical method, by converting them to one or several of the known ones (one or several of the 24 PFSA substance included in the analytical method).

The study of course raised a need for further investigations in the following aspects, which formed the foundation for the study described in this report.

- To what extent were hidden PFAS converted in to known ones in the TOP-oxidation step (could there still be "hidden" PFAS in the samples after the TOP-oxidation step)?
- Common analytical method performance data was not investigated in the pilot study such as the TOP method precision, accuracy, repeatability and reproducibility.

[11] "*Förslag till nationella regler för högfluorerade ämnen i brandsläckningsskum*", Kemikalieinspektionen, 2016, ISSN 0284-1185. Artikelnummer: 361 178. The proposed national legislation has to date (June 2021) not entered into force presumably because of the prioritization towards a common EU restriction on PFAS in fire-fighting foams.

- The pilot study used the very same TOP-oxidation parameter settings regardless of type of foam concentrate and the TOP-settings was not by any means optimized.

4.2.2 Transition to fluorine-free fire-fighting agent - US Department of Defense

Forthcoming restrictions on the US Department of Defense´s use of PFAS-based firefighting foams was included in the National Defense Authorization Act for Fiscal Year 2020 (NDAA 2020)[12] section. 322 "Replacement of Fluorinated Aqueous Film-Forming Foam with Fluorine-Free Fire-Fighting Agent"). Section 322 states that before January 31, 2023, the Secretary of the Navy must publish a military specification for a fluorine-free fire-fighting agent for use at all military installations and ensure that such agent is available for use by not later than October 1, 2023. Furthermore, it is stated that *"no amount authorized to be appropriated or otherwise made available for the Department of Defense may be obligated or expended after October 1, 2023, to procure fire-fighting foam that contains in excess of one part per billion of perfluoroalkyl substances and polyfluoroalkyl substances"*.
A "one part per billion (1 ppb) of PFAS" thus represents a summed concentration (summed over all variants of PFAS) of 1 µg/l in the foam concentrate. Section 322 in the Act does not state the method of verification, which makes this a decision that needs to be made at the course of the transition period, probably during the drafting of the military specification. A conventional LC-MS/MS method would easily meet the sensitivity requirements needed to quantify a finite number of PFAS substances below 1 ppb (= 1 µg/l) per corresponding PFAS substance. However, one should only expect to gain concentration information on 50-55 different PFAS substances, which is far from covering the 4 700 known PFAS substances.

Could the TOP methodology, used in the FMV pilot study described in the previous chapter, provide more comprehensive analytical data to verify the requirement of a maximum of one ppb of PFAS in a future procurement of fluorine-free fire-fighting foams? Alternatively, could the combination of the TOP methodology and any of the TOF (Total Organic Fluorine)-methods (CIC, AOF and EOF) (described in chapter 4.4.3) be the most suitable alternative to characterize such complex matrices as fire-fighting foams with respect to the PFAS-contents? The study presented in this report is an attempt to answer these questions.

4.2.3 The GreenScreen criteria for PFAS-free fire-fighting foams

The GreenScreen™ for Safer Chemicals (GreenScreen) was developed by the non-profit organization Clean Production Action (CPA) and the methodology provides a structured approach to evaluate a comprehensive set of human and environmental health and safety data, related to chemical substances.

The GreenScreen methodology claims to;

- distill complex hazard evaluations down to an easy-to-understand hazard table,
- place chemicals along a continuum of concern and assigns a chemical one of four possible benchmarks,
- can be used to select environmentally preferable, safer chemicals for use in products and processes, supporting the health of users and the environment.

With regard to fire-fighting foam concentrate, GreenScreen has laid out a set of criteria, "*The GreenScreen Certified™ Firefighting Foam standard (version 2.0) for Class A Foam Concentrates, Class B Foam Concentrates, Class A Wetting Agents and Class A & B Wetting Agents*"[13].

In the criteria set it is stated that "*PFAS-free is defined as zero intentionally added PFAS to the product and PFAS contamination in the product must be less than 0.0001 percent by weight of the product (1 part per million) total organic fluorine as measured by combustion ion chromatography*".

[12] https://www.congress.gov/116/bills/s1790/BILLS-116s1790enr.pdf
[13] https://www.greenscreenchemicals.org/certified/fff-standard

Thus, the GreenScreen criteria for PFAS-free is verified by yet another analytical methodology (TOF-metodology), combustion ion chromatography (CIC), which in many aspects differ from the TOP methodology, used in the FMV pilot study.

In comparison with other policies on "PFAS-free" fire-fighting foams presented in the previous chapters in this report, the GreenScreen criteria for fire-fighting foams is the only one specifying an analytical method for verification of the requirement "PFAS-free".

The next chapter in this report will discuss today´s different available methods for quantifying and identifying PFAS, as well as their pros and cons.

4.3 Analytical methods to quantify and characterize PFAS

Not all fluorine present in fire-fighting foams is PFAS, or conventional PFAS (the typical C_4-C_o perfluoro alkyl acids or sulphonic acids) but can also be present as ultra-short chain PFAS (C < 4), as fluorotelomer substances, as highly fluorinated precursors with a great variation in chain length, linearity/degree of branching, type of functional groups, or as inorganic fluoride salts. In the figure below (see figure 1) a conceptual interpretation of the forms of fluorine in fire-fighting foams has been compiled.

This subchapter tries to summarize the available analytical techniques and their pros and cons.

4.4 Methods of quantification and identification of PFAS

4.4.1 Conventional methods of quantification and identification of PFAS

The analytical methods used to identify and quantify trace levels of PFAS in various environmental and regulatory relevant matrices such as ground water, surface water, drinking water, foodstuff, biosolids, sediment, soil, etc., are normally based on a solid phase extraction step where the PFAS contents of the sample is up-concentrated in a solid adsorbent, and then being eluted in a small aliquot of a suitable solvent. This up-concentrated sample is then injected into a Liquid Chromatography (LC)-column (providing a separation in the space domain), and then being detected and quantified using a mass spectrometric detector such as a time-of-flight detector (providing a separation in the time domain) or an ion trap.

Furthermore, ms/ms-fragmentation data (m/z for the mother ions and the corresponding m/z for daughter ions) may also reveal structurally important information in the identification and structural alignment of unknown PFAS substances.

Over the last 10-15 years the PFAS analysis methodology (both techniques for sample work-up as well as the instrumental analysis part) has improved tremendously and it is possible to quantify sub nanogram/l[14] concentrations of PFAS in drinking water and ground water samples albeit most PFAS substances would normally have limit of quantification (LOQ) of 1-40 ng/l in such matrices using commercially available methods and laboratories.

However, the conventional PFAS analysis using a LC-system has a limitation as to the number of different PFAS substances that can be quantified in a single analysis (currently some 50-55 PFAS substances). Since this limitation essentially stems from fundamental chromatographic limitations with respect to band broadening in the packed bed column format of the LC-column, only minor improvements as to the number of PFAS substances that can be resolved and quantified in a single analysis can be anticipated in the future. Thus, it is only possible to identify and quantify some 50-55 different PFAS substances out of 4 700 possible ones, in a single chemical analysis.

[14] 1 ng/l = $1*10^{-9}$ gram/l, 1 ng/l = 1 000 ppt (parts per trillion)

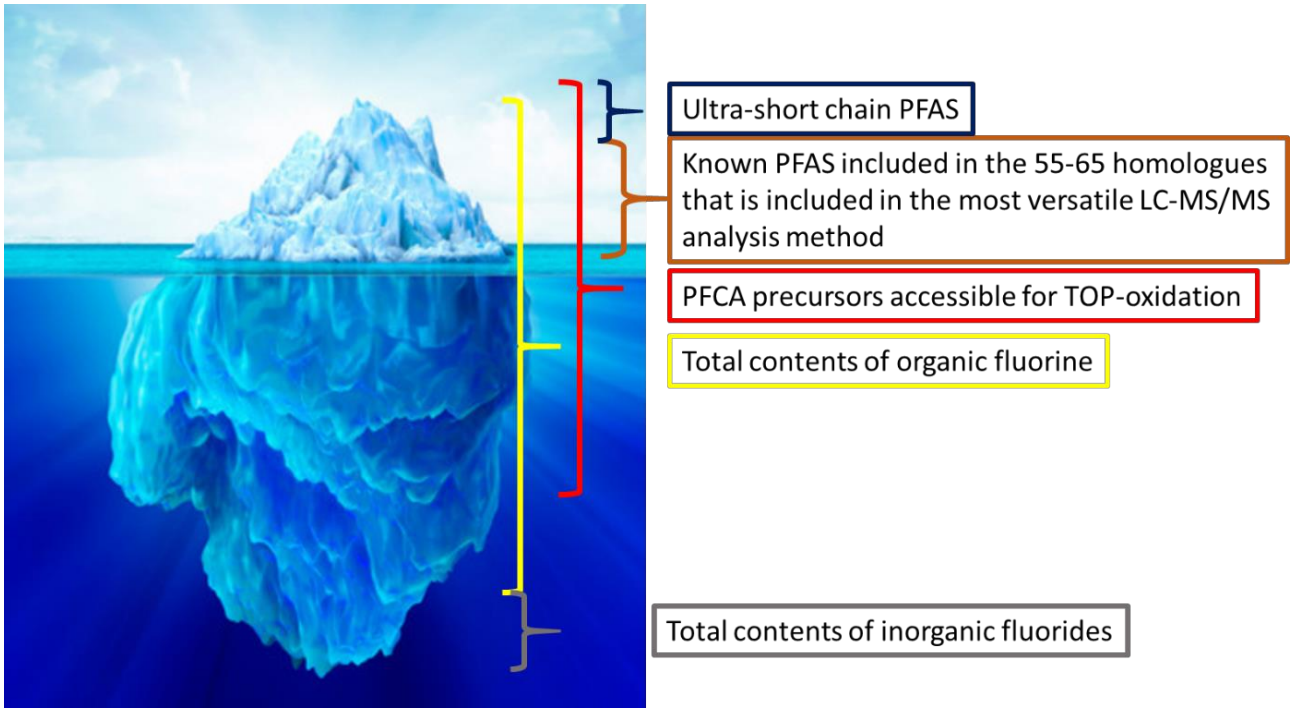


Figure 1. Graphical interpretation of the occurrence of the element of fluorine in foam concentrates. The size/length of the staples have been assigned arbitrarily and may not reflect the situation in all foams.

4.4.2 The TOP Assay – state of the art knowledge of the methodology

Total oxidizable precursor (TOP) assay as briefly described in 4.4.2, was developed by Houtz and Sedlak in 2012 with the purpose of estimating the total concentration of unidentified PFAS and Perfluoralkyl acids (PFAA) precursors present in urban runoff and storm water. The basic workflow of the TOP assay is based on the thermolysis of persulfate at alkaline conditions (pH > 12) and elevated temperatures (85 °C) to produce hydroxyl radicals (OH•).

$$S_2O_8^{2-} + heat \rightarrow 2SO_4^-$$

$$2SO_4^- + OH^- \rightarrow 2SO_4^{2-} + \cdot OH$$

In the presence of hydroxyl radicals (OH•), PFAA precursors are oxidized to form perfluorocarboxylic acids (PFCAs), while the original PFCAs and PFSAs present in the samples remain unchanged. The total concentration of precursors can then be estimated by calculating the change in PFCA concentration before and after TOP assay.

So far, the TOP assay has been applied to urban runoff (Houtz and Sedlak, 2012), groundwater (Casson and Chiang, 2018), and wastewater (Houtz et al. 2016) but to a lesser extent on fire-fighting foam concentrates.

While the TOP assay is one most selective method for measuring unidentified PFAS, it is of course not without shortcomings. One of the limitations is that it is limited to only precursors that can transform to PFCAs, but any precursor that is oxidized to other forms will be missed unless these other forms are represented among the PFAS substances of the LC-MS/MS method. Another limitation is that matrix complexity, e.g., co-contaminants and natural organic matter, could affect the oxidative conversion of the precursors (Casson and Chiang, 2018). The high salt content of TOP samples could contribute to unwanted instrumental matrix effects in the post-TOP analysis since the LC-system is sensitive to high salt concentrations in the samples; therefore, post-oxidation samples may require cleanup to remove interferences ([redacted] et al., 2019).

The TOP assay is very useful in estimating the total concentration of precursors present in the sample, but the structural information of the precursors is partially or entirely lost. Essentially the conversions taking place during the TOP oxidation step is a bit of a black box.

However, Houtz and Sedlak (2012) demonstrated that when samples containing C_8 sulfonamide-containing precursors (*i.e.*, perfluorooctane sulfonamide (FOSA), N-ethyl-perfluoroctane sulfonamido acetic acid (N-EtFOSAA) and N-methyl-perfluoroctane sulfonamido acetic acid (N-MeFOSAA)) were subject to top assay conditions, only PFOA was produced at a conversion yield of 97 ± 3%, 92 ± 4%, and 110 ± 8%, respectively.

When fluorotelomer-based precursors, *i.e.* 6:2 fluorotelomer sulfonic acid (6:2 FTSA) and 8:2 fluorotelomer phosphate diester (8:2 diPAP) were subjected to TOP assay, each produced a suite of PFCAs of varying chain lengths. While the increase in the concentration of a C_n PFCAs indicates the presence of a C_n precursor, the total increase in the concentration of PFCAs does not necessarily equate to the total concentration of the C_n precursor. The conversion of precursor oxidation during TOP assay can be summarized by the following reactions:

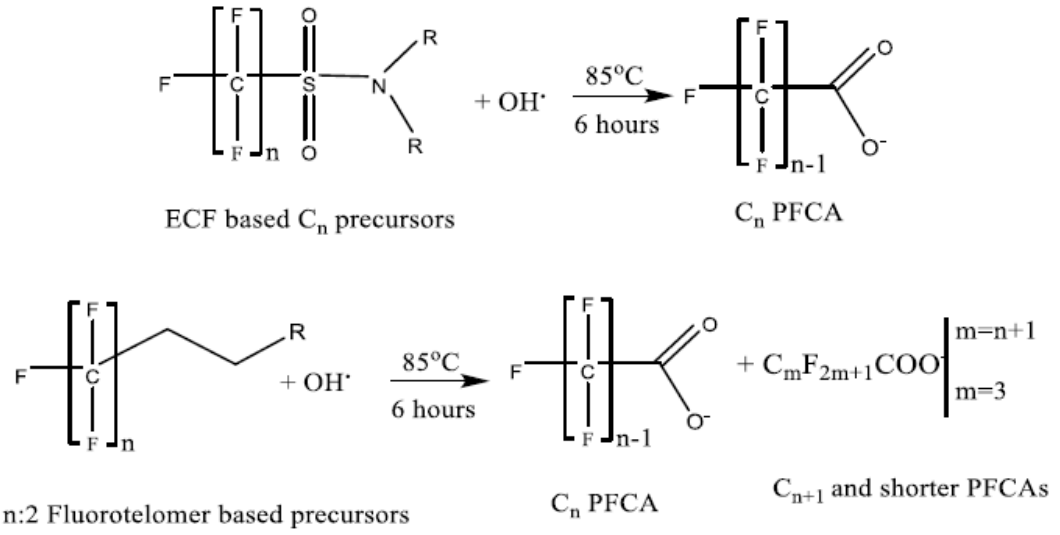


Figure 2. Transformation pathway of PFAA precursors oxidation during the TOP assay. Adapted from (Houtz and Sedlak, 2012)

As a final remark on the TOP assay methodology compared to the standard LC-MS/MS method, the standard method reveals the tip of the iceberg with good accuracy, precision and repeatability, while the TOP assay reveals a substantial part of the sub-surface part of the iceberg (in terms of its total PFAS contents). However, the analytical performance of the TOP assay with respect to foam concentrates have, to the best of the project´s knowledge, not yet been evaluated nor validated.

4.4.3 Total organic fluorine (TOF) analysis methods – CIC, AOF, EOF

Combustion ion chromatography (CIC) is used for bulk organo-fluorine measurement. In CIC, samples are combusted at about 900-1 000 °C, resulting in the release/thermal degradation of all bonded fluorine ato ms present in the molecule as fluoride ions, which are then converted to hydrofluoric acid (HF). The HF is absorbed into an absorption solution of sodium hydroxide (NaOH). Ion chromatography is subsequently used to measure the fluoride ion concentration.

CIC can be applied to both liquid or solid samples, but one major pitfall to this method is its inability to differentiate between organic and inorganic fluorine (McDonough et al., 2018).

Combustion ion chromatography modified for fluorine analysis (CIC-F) has been applied to measure the bulk organo-fluorine contents in an attempt to close the mass balance of PFAS in human blood samples, seawater samples (Miyake et al. 2007), blood (Miyake et al. 2007), surface waters (D'Agostino and Mabury 2017), sediments (Yeung et al. 2013), soils (Codling et al. 2014; Wang et al. 2013), fish tissue (Yeung and Mabury 2013) and liver tissue (Yeung et al. 2009).

The success of using CIC to estimate the total concentration of unknown PFAS is largely determined by how effectively inorganic and organic fluorine are separated. Therefore, pretreatment steps to separate organic and inorganic fluorine must be applied before measuring bulk organo-fluorine in samples.

Two types of sample pre-treatment methods are typically used: extractable organic fluorine (EOF) and adsorbable organic fluorine (AOF) assays. The extractable organic fluorine assay is the most used method (an also used in this particular study). The exact extraction method depends on the sample matrix. For example, a weak anion solid-phase extraction (SPE) method, which included a sequential

elution procedure with an organic solution and a very elaborate washing step with 0.01% NH_4OH has been used to isolate organo fluorine in water samples (Yeung et al. 2008; Miyake et al. 2007).

As a concluding remark with regard to 1) the TOP assay and 2) the TOF-methods (CIC, AOF and EOF), these methods would be very suitable to characterize complex matrices with respect to the PFAS-contents since they are orthogonal (does not measure the same parameters) and hence they could combined prove valuable in order establish a mass balance or budget of organic fluorine in the particular sample matrix. However, so far, the TOF-methods have suffered from poor sensitivity and LOQs have typically been a couple of orders of magnitude higher than comparable LOQs with respect to the TOP assay methods.

4.4.4 Analysis of ultra-short chain PFAS

Ultra-short chain PFAS, normally defined as perfluorinated compounds having a carbon chain length of 2-3 carbons have long been over-looked with regard to their occurrence, mobility, toxicological properties and subsequently the methods to remediate ground water and soil contaminated by these substances. However, the recent regulations and restrictions on the use of long-chain PFAS has resulted in a significant shift in the industry towards short-chain alternatives. The understanding of the environmental fate and remediation of these ultra-short-chain PFAS is still fragmentary, however.

Analysis of ultra-short chain PFAS typically involves non-conventional analytical methods using ultra performance convergence chromatography (UPC2) coupled to a tandem mass spectrometer have also yielded measurements of the PFAS including the ultrashort-chain (C_2-C_3) forms (Yeung et al. 2017).

Despite the advancements in these instrumentation techniques, analytical separation and detection of short-chain PFAS compounds remains a challenge. These polar short-chain compounds are less compatible for the simultaneous extraction and quantitation along with the long-chain PFAS analytes in routine reverse-phased analytical methods. Indeed, short-chain compounds most often elute early and exhibit poor peak resolutions (Ruan and Jiang, 2017). As of now, the use of supercritical fluid chromatography coupled to MS/MS seem to be the best option to quantify ultra-short chain PFAS in various matrices such as water, sediment, soil and products.

5. Methodology

This chapter describes the overall experimental methodology of the study such as how to decide TOP-conditions providing a maximal yield of PFCA precursor transformation (design and evaluation), the type of F3 concentrates included in the study, additional analysis and characterization, the participating laboratories, and the internal standards used to verify TOP-oxidation.

As described in the introduction chapter, the aim of this study was to test the hypothesis that the TOP assay, optimized and validated for the matrices fluorine free fire-fighting foams, is a reliable analytical method for verifying claims of a fire-fighting foam being "PFAS-free".

Both from a regulatory perspective as well as from a procurement perspective it would be very beneficial to have an analytical technique to verify and quantify as much of the "PFAS iceberg" (see Figure 1) as possible with regard to fire-fighting foam concentrates.

However, fire-fighting foam concentrates constitute rather complex samples with a high content of organic carbon, and the organic carbon contents of these type of chemicals may vary from manufacturer to manufacturer, possibly influencing the results of a TOP assay since the basis of the TOP assay is to oxidize all organic carbon as well as the precursor PFCAs.

In order to use the TOP assay to select/discriminate a certain foam concentrate from a group of fire-fighting foam concentrates, it is important to determine the performance of the TOP assay for these types of matrices including the different sources of variance of the results. The pilot study of FMV (2017-2018), presented in chapter 4.2.1, was indeed illustrative as to the potential of the TOP assay for detecting and quantifying "hidden PFAS" (precursor PFCAs), but the study was limited and of no statistical value and the TOP-settings employed in that study may not have been optimal for foam concentrates.

The study presented in this report was therefore designed to evaluate the different variance contributions when applying the TOP assay to F3 concentrates under optimized TOP-conditions and in a way where the different sources of variance (could be experimental, variance from replication, variance from the different laboratories conducting the TOP assay etc.) could be evaluated. Furthermore, as variances is estimated, a further aim was to be able to quantify the confidence limit of the systematic variance.

5.1 Experimental set up

The fluorine free fire-fighting foams (F3)

In order to expand the experimental space of F3 concentrates (organic carbon contents, specific tenside chemistry employed, differences in viscosity, density etc.) technical experts at FMV was consulted and six different F3 concentrates was purchased (see table 1 below). All foam concentrates were purchased in 25 l jeericans and these were sampled at one particular data. The sample bottles were then kept in a fridge at 5-6 ºC prior to sample work-up and analysis.

Table 1. Summary of the F3 concentrates chosen for the study. The fluorine free foams were chosen by technical expertise at FMV to cover as many applications of fire-fighting as possible. The PFAS containing AFFF concentrate was used as a positive in the experiments.

Foam nr	Name	Remarks from the sampling	General description of the F3 concentrates (extracted from producer´s information)
Fluorine Free foams (F3)			
1	JetFoam ICAO-C 3%	Not noted	Synthetic low-expansion fluorine free foam (F3) concentrate, alternative to aqueous film forming foams (AFFF).
2	Meteor T-10/Training	Transparent liquid	Synthetic high-expansion foam concentrate. Flexible fire-fighting agent, for use both indoors and outdoors. Does not contain any fluoro surfactants and is considered a fluorine free foam.
3	HOTFOAM 2%	Transparent liquid	Synthetic high-expansion foam concentrate, mostly used for inside air foam systems in enclosed spaces. Does not contain any fluoro surfactants and is considered a fluorine free foam.
4	Trainer E-lite	Foaming liquid	Fire training foam (should not be used as fire-fighting foam for life saving). Totally free from fluorinated surfactants and is considered a fluorine free foam.
5	ECOPOL A 3%	Green liquid	Synthetic low-expansion fluorine free foam (F3) concentrate, alternative to aqueous film forming foams (AFFF).
6	Respondol[15] ATF 3-3%	White, milky liquid	Synthetic low-expansion fluorine free foam (F3) concentrate, alternative to aqueous film forming foams (AFFF).
PFAS-containing AFFF concentrate			
7	Sthamex AFFF 3% F-15	Not noted	Synthetic aqueous film forming fire extinguishing foam concentrate. Contains fluorinated components. Used for both low and medium expansion foam applications

[15] Foam 6 was not analysed in the validation part of the study since being omitted in the optimization step as "PFAS-free". No PFAS was reported after extensive TOP-oxidation experiments using 9 different TOP-settings.

5.2 The three participating laboratories

ALS Global laboratories in Prague (Czech Reublic), SGS Analytics Sweden AB (former SYNLAB Analytics & Services) in Linköping (Sweden) and Eurofins Environment Testing Sweden AB in Lidköping (Sweden) were the three participating commercial laboratories. In the TOP assay optimization step, ALS Global laboratories in Prague executed all TOP assays. In the validation step, repeatability and reproducibility of the TOP assay was evaluated by the results from all three participating laboratories.

5.3 The TOP assay parameters -Optimization of the TOP assay

The TOP assay parameters most often used are the ones proposed by Houtz & Sedlak; a 120 mM solution with respect to $K_2S_2O_8$ and 250 mM with respect to NaOH, which is then added to a small aliquot of sample and then let to "boil" at 85 ºC for 8 hours.

A possible source of variance and possibly also a source for poor precision of the study could be that the TOP assay parameters would not be suitable for F3 concentrates. Either the concentration of the persulfate could be too low, or the concentration of sodium hydroxide could be too low, the temperature could be too low, as well as the boiling/oxidation time could also be too short, in order to oxidize all organic carbon of the F3 concentrates prior to oxidizing any possible precursor PFCAs.

The influence of the TOP assay parameters on two response variables (concentration of $\sum PFAS_{11}$ and concentration of PFOA) was evaluated in a factorial design experiment (see Table 2 below).

Table 2. In the factorial design the four parameters (concentration of persulfate, concentration of hydroxide, oxidation temperature and oxidation time) was evaluated at three different levels using a reduced factorial experiment with replicate runs in a centrum point (see table below for the design of the 2^{4-1}-experiment).

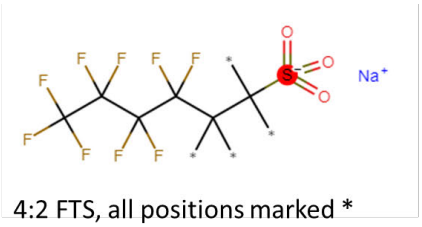
Experiment	[$K_2S_2O_8$] (mM)	[NaOH] (mM)	Temperature, ºC	Time, hr
1	Low (-)	Low (-)	Low (-)	Low (-)
2	High (+)	Low (-)	Low (-)	Low (-)
3	Low (-)	High (+)	Low (-)	Low (-)
4	High (+)	High (+)	High (+)	Low (-)
5	Low (-)	Low (-)	High (+)	High (+)
6	High (+)	Low (-)	High (+)	High (+)
7	Low (-)	High (+)	Low (-)	High (+)
8	High (+)	High (+)	High (+)	High (+)
9	Centrum (0)	Centrum (0)	Centrum (0)	Centrum (0)
10	Centrum (0)	Centrum (0)	Centrum (0)	Centrum (0)
11	Centrum (0)	Centrum (0)	Centrum (0)	Centrum (0)
12	Centrum (0)	Centrum (0)	Centrum (0)	Centrum (0)

The 2^{4-1} central composite design used to optimize the TOP assay conditions. The design was set up to determine if the parameters proposed by Houtz and Sedlak were the optimal parameters also for fire foam matrices or if another set would result in a higher yield of $\sum PFAS_{11}$ and concentration of PFOA. Low (-) represented 60 mM $K_2S_2O_8$, 125 mM NaOH, 80 ºC, and 6 hours of oxidation time. High (+) represented 180 mM $K_2S_2O_8$, 375 mM NaOH, 90 ºC, and 10 hours of oxidation time. Centrum (0) represented the 120 mM $K_2S_2O_8$, 250 mM NaOH, 85 ºC, and 8 hours of oxidation time (the Houtz & Sedlak conditions).

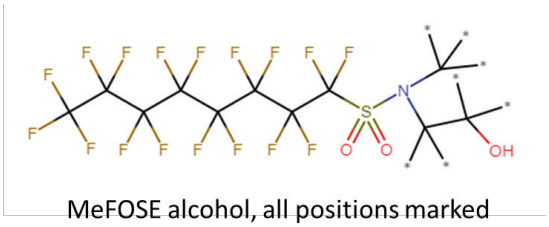
5.4 Internal standards

In order to verify TOP conditions (high redox potential of the TOP-cocktail), two internal standards were added to the TOP assay mixtures; D2-labelled 4:2 FTS as well as D2-labelled MeFOSE alcohol (see figure 3 below).

Rational; if the two internal standards were consumed to 100 % during the TOP assay, the redox potential of the TOP assay cock tail was considered high enough.



4:2 FTS, all positions marked * represents deuterium-labelled carbons.



MeFOSE alcohol, all positions marked * represents deuterium-labelled carbons.

*Figure 3. The internal standards used in the TOP assay optimization step. Positions marked * represents hydrogens now exchanged for deuterium. The 4:2 D2-labelled standard was retrieved from Cambridge Isotope Laboratories while the D2-labelled MeFOSE alcohol was retrieved from Wellington Labs Inc.*

5.5 F3-concentrates subjected to TOP assay optimization

It was decided to submit Foam nr 1, 5 and 6 for the TOP assay optimization, and the optimization experiments were conducted by ALS Global Laboratories in Prague (Czech Republic) during the summer 2020.

5.6 Establishment of final TOP assay method.

The results of the TOP optimization 2^{4-1} experiment were statistically evaluated using the Software Design Expert 12 (StatEase Corporation, Minneapolis, US) as well as the software SIMCA (Umemetrics, Sweden).

5.7 Determination of DOC

Dissolved Organic Carbon (DOC) was determined in all fire-fighting foam concentrates, in accordance with SS-EN 1484, by ALS Global Laboratories (Prague, Czech Republic).

It could be of importance to determine the DOC of the foam concentrates since the TOP-oxidation method involves oxidizing all organic material in the foam concentrate, as well as all PFCA precursors. It is likely that PFCA precursors are only quantitatively transformed to known PFAS when the excess of oxidant present is high enough to also oxidize all organic carbon in the foam concentrate.

5.8 Evaluation of the TOP assay method by a three lab Round-[redacted] exercise

Six[16] different foam concentrates (five different F3-concentrates and one AFFF-concentrate) were submitted to three different commercial laboratories. Each laboratory executed 4 top assays per foam concentrate according to the TOP assay protocol established from the TOP assay optimization step and reported their corresponding results (concentrations of different PFAS substances before and after the TOP assay) as in an interlaboratory study (ILS) in accordance with ASTM E691-99 "Standard practice for Conducting a Interlaboratory study to determine Precision of a Test Method".

Statistical evaluation of the results (one way ANOVA variance analysis) was conducted on the XLSTAT software (AddinSoft AS, Paris France).

6. Deviations from and additions to the experimental design

This chapter describes the major deviations needed to be implemented in the experimental set-up due to different unforeseen circumstances.

6.1 Choice of fire-fighting foam concentrates for the TOP optimization

As stated in 5.5, the three F3 concentrates subjected to the TOP assay optimization 2^{4-1}-experiment were submitted to ALS Prague. However, after a full experiment of Foam 6 (12 TOP assays conducted at 9 different TOP-settings) and yet some 4-5 TOP assays with respect to Foam 1, the conducting laboratory could not report any PFAS substances > LOD in any of the TOP assay runs.

It was then decided that the TOP assay optimization (all 12 TOP assays of the experimental design) was to be repeated with a new foam concentrate, Foam 7, which is a known AFFF concentrate containing PFAS substances. By using a AFFF concentrate (containing elevated concentrations of PFAS) the optimization experiments were regarded as fool-proof in providing data for method optimization.

Foam 6, thus already established as a PFAS-free foam after TOP, was replaced with Foam 7 in the validation study.

6.2 CIC/EOF analysis of fire-fighting foams

The CIC/EOF-analysis of a sub-selection of foam concentrates were conducted using both TOP-oxidized foams as well as original foam concentrates (both F3- as well as AFFF-concentrates). The samples were initially subjected to a solid phase extraction step and the SPE-columns were sequentially eluted to form a neutral fraction as well as anionic fraction.

The eluted fractions were the subjected to a thermal combustion in a ceramic sample holders introduced into a furnace at 900 -1 000 ºC. At the elevated temperatures, complete pyrolysis of the sample occurs in a humid oxygen-enriched atmosphere. The samples oxidized under these conditions, experience a quantitative breaking all strong carbon-fluorine bonds, and the vapours are sparged through an absorption solution using Argon gas. The HF evolved from combustion of organic fluorine dissociates to form H^+ and F^--ions in the absorption solution, which also contains an internal standard to calibrate the analytical results. The samples were then transferred to the ion chromatograph for analysis where fluoride was measured. A more detailed account of the EOF and CIC-procedures are provided in chapter 6.4.

[16] Foam 6 was not analysed in the validation part of the study since being omitted in the optimization step as "PFAS-free". No PFAS was reported after extensive TOP-oxidation experiments using 9 different TOP-settings. Foam 6 was thus replaced with Foam 7 in the validation part of the study.

6.3 Analysis of ultra-short chain PFAS substance

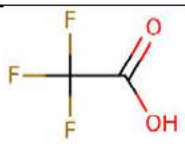
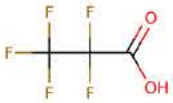
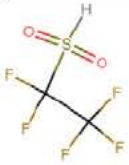
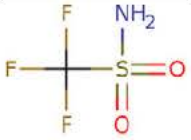
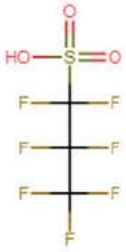
The analysis of ultra-short chain PFAS in both foam concentrates as well as in samples from TOP-oxidized concentrates were executed by super critical fluid chromatography-MS/MS, as described in Björnsdotter et al., 2019, but with the exception that a Quattro Premier XE MS/MS system (Waters Corporation, Milford, USA) were used for the mass spectrometry.

The F3 foam concentrates were diluted 1:200 (1:100-dilution with MQ-water and subsequently a 1:1 with methanol) and the AFFF concentrate was diluted 1:2 000 (1:1 000-dilution with MQ-water and subsequently a 1:1 with methanol).

The TOP-oxidized samples were diluted 1:10 000 after the TOP-assay and was then diluted 1:1 with methanol, thus a 1:20 000-dilution.

The method was optimized for the quantification of four (4) different short-chain PFAS (see Table 3 below). All analysis of ultra-short chain PFAS was executed by [redacted] Ingrid Erikson Jogsten at Örebro University.

Table 3. Ultra-short chain PFAS included in the analysis.

Name	Structure	CAS	Mw
TFA (trifluoro acetic acid)		76-05-1	114,02
PFPrA (perfluoro propanoic acid)		422-64-0	164,03
PFEtS (perfluoro ethane sulfonate)		354-87-0	202,07
TFMS (trifluoro methyl sulfonamide)		421-85-2	149,09
PFPrS (perfluoro propane sulfonate)		-	250,09

6.4 Details on the CIC/EOF-analysis of TOP-oxidized and non-oxidized foam samples

The sample work-up procedure has previously been described in KemI, 2021. In short, all samples subjected to EOF were filtered through glass fiber filters (Whatman Grade GF/B) prior to extraction using this following procedure: after baking the filters at 450 °C for at least 12 h prior the filtration apparatus was setup and cleaned with MeOH followed by ultrapure water prior to use.

The samples were loaded then onto the filter and the filtrate was collected in a flask and poured back into the original container. MeOH (3 x 3 mL) was used to rinse the walls of the filtration flask and then added to the filtered sample.

All samples, including blanks, were subsequently extracted by SPE using a method adopted from Miyake et al. (2007) with some modifications. Briefly, Oasis WAX cartridges (Waters; 150 mg, 6 cc) were conditioned with 4 mL of 0.1% NH_4OH in MeOH, followed by 4 mL of MeOH and 4 mL of Milli-Q water. All samples were adjusted to pH 4 using glacial acetic acid and then loaded onto the cartridge at a rate of ~2 drops/sec and then rinsed with 20 mL of 0.01% NH_4OH in water followed by 10 mL of water, 4 mL of ammonium acetate (pH 4), 4 mL of 20% MeOH, all of which were discarded. The cartridges were then dried by centrifugation (2 min, 1000 x g) and finally eluted with 4 mL of 0.1% NH_4OH in MeOH. The extract was reduced to 600 µL under a gentle nitrogen stream which was subsequently used in the CIC-analysis.

This CIC system had a combustion module and an autosampler (both from Analytik Jena, Germany), an absorber module (920 Absorber Module) and an ion chromatograph (IC; 930 Compact IC Flex), both from Metrohm, Switzerland. The combustion tube and absorber unit are made of quartz glass. The anions formed during the combustion were separated with an ion exchange column (Metrosep A Supp 5–150/4), carbonate buffer (64 mmol/L sodium carbonate and 20 mmol/L sodium bicarbonate) as eluent and isocratic elution.

The autosampler injected 100 µL of the extract on a quartz boat. The boat was inserted into the oven (1000–1050 °C) under a flow of oxygen (300 mL/min), argon (100 mL/min), and argon mixed with water vapor (100 mL/min) under hydropyrolytic conditions monitored by a flame sensor followed by 2 minutes of post-combustion time with the flow of oxygen (400 mL) only. The hydrogen fluoride (HF) formed during combustion was absorbed in ultrapure water (in the absorber module). Once the combustion was complete, a 2 mL subsample of the absorber solution was injected into a trap column before introducing to the ion chromatograph. The F^- concentration was measured via conductivity. A five-point calibration curve at using PFOS standards was constructed by using the same combustion method as for the samples and exhibited good linearity. The size of the sampling tubing at the absorption unit was changed from 100 µL to 2 mL in order to allow large volume injection onto the IC unit.

All EOF/CIC-analysis was executed by [redacted] Ingrid Erikson Jogsten at Örebro University.

7. Results

This chapter discloses all results of the study - results from the method development step where optimal TOP-oxidation settings were established, as well as results from the validation part of the study where the three different participating laboratories executed four (4) replicate analysis of the F3 concentrates before and after the TOP-oxidation step. It also describes how results from the method development step have been evaluated statistically. The statistical evaluation of the validation step has been covered in chapters of its own (see Chapter 8-9).

7.1 ∑$PFAS_{25}$ and ∑$PFAS_{11}$

In the post-TOP-analysis, the laboratory reported concentrations of 25 different PFAS[17] (hence ∑$PFAS_{25}$ is possible to calculate), however only 5-6 different PFAS have concentrations > LOQ in the 12 TOP-assays and thus it seems more appropriate to consistently report ∑$PFAS_{11}$.

7.2 TOP optimization assays with regard to foam concentrate 7

Before the TOP assay a $PFAS_{25}$-analysis showed that foam concentrate 7 (diluted 10 000 times with MQ water) contained ∑$PFAS_{25}$ of 4 950,6 µg/l (PFBA 23,4 µg/l, PFHxA 135,7 µg/l and 6:2 FTS 4 791,5 µg/l).

After the TOP assay the ∑$PFAS_{25}$ varied between 1584,7 to 4934,9mg/l and in the TOP assay runs also PFPeA, PFHxA, PFHpA and PFOA was reported in various concentrations.

In the table below, the results after TOP-oxidation have been summarized (see Table 4 below).

[17] ∑$PFAS_{25}$: Perfluorobutanoic acid (PFBA), Perfluoropentanoic acid (PFPeA), Perfluorohexanoic acid (PFHxA), Perfluoroheptanoic acid (PFHpA), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA), Perfluorodecanoic acid (PFDA), Perfluoroundecanoic acid (PFUnDA), Perfluorododecanoic acid (PFDoDA), Perfluorotridecanoic acid (PFTrDA), Perfluorotetradecanoic acid (PFTeDA), Perfluorobutane sulfonic acid (PFBS), Perfluoropentane sulfonic acid (PFPeS), Perfluorohexane sulfonic acid (PFHxS), Perfluoroheptane sulfonic acid (PFHpS), Perfluorooctane sulfonic acid (PFOS), Perfluorodecane sulfonic acid (PFDS), Perfluorododecane sulfonic acid (PFDoDS), 6:2 Fluorotelomer sulfonic acid (6:2 FTS), 8:2 Fluorotelomer sulfonic acid (8:2 FTS), Perfluorooctane sulfonamide (FOSA), N-Methyl perfluorooctane sulfonamide (MeFOSA), N-Ethyl perfluorooctane sulfonamide (EtFOSA), N-Methyl perfluorooctane sulfonamidoethanol (MeFOSE) and N-Ethyl perfluorooctane sulfonamidoethanol (EtFOSE).

Table 4. Results from the TOP-optimization experiments. The parameters $\sum PFAS_{11}$ and the formation of PFOA where subsequently used as response variables to optimize the yield in the TOP-oxidation step.

Name of PFAS homologue/Concentration in experiment nr (mg/l)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Perfluorobutanoic acid (PFBA)	1 038, 9	776, 4	831 , 3	1 113,5	1 117,6	959,8	1 027,8	1 035,8	750,9	707,1	751,3	742,9
Perfluoropentanoic acid (PFPeA)	1 916,7	591,9	1 135,4	1 895,3	2 046,3	633,66	1 473,6	2 631,7	1 215,6	1 291,8	1 315,4	1 274,1
Perfluorohexanoic acid (PFHxA)	693,6	184,6	493,2	735,7	800,3	229,3	612,2	942,4	350,1	340,2	362,3	353,5
Perfluoroheptanoic acid (PFHpA)	118,7	31,68	130,7	199,8	118,1	31,47	161,1	325,0	40,91	42,59	41,64	40,70
Perfluorooctanoic acid (PFOA)	1, 282	6, 269	9, 112	8, 193	12,60	1, 120	20,22	7, 090	<0,50	<0,50	<0,50	<0,05
6:2 FTS	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0	< 1,0
$\sum PFAS_{11}$: PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS, 6:2-FT	3 767,97	1 584,65	2 590,58	3 944,40	4 082,28	1 854,15	3 274,72	4 934,87	2 357,533	2 381,69	2 470,67	2 411,20

In three of the TOP assay experiments conducted in the same run (corresponding to experiment 5, 6 and 8 according to table 2, the conversion of the internal standards did not reach a 100 % conversion. These TOP-assays were repeated and in the replicate runs, all internal standard conversions reached 100 %. The results of the 12 TOP assays in the optimization step are tabulated in Table 4 above. All results (also from the omitted runs) are shown in figure 4 below.

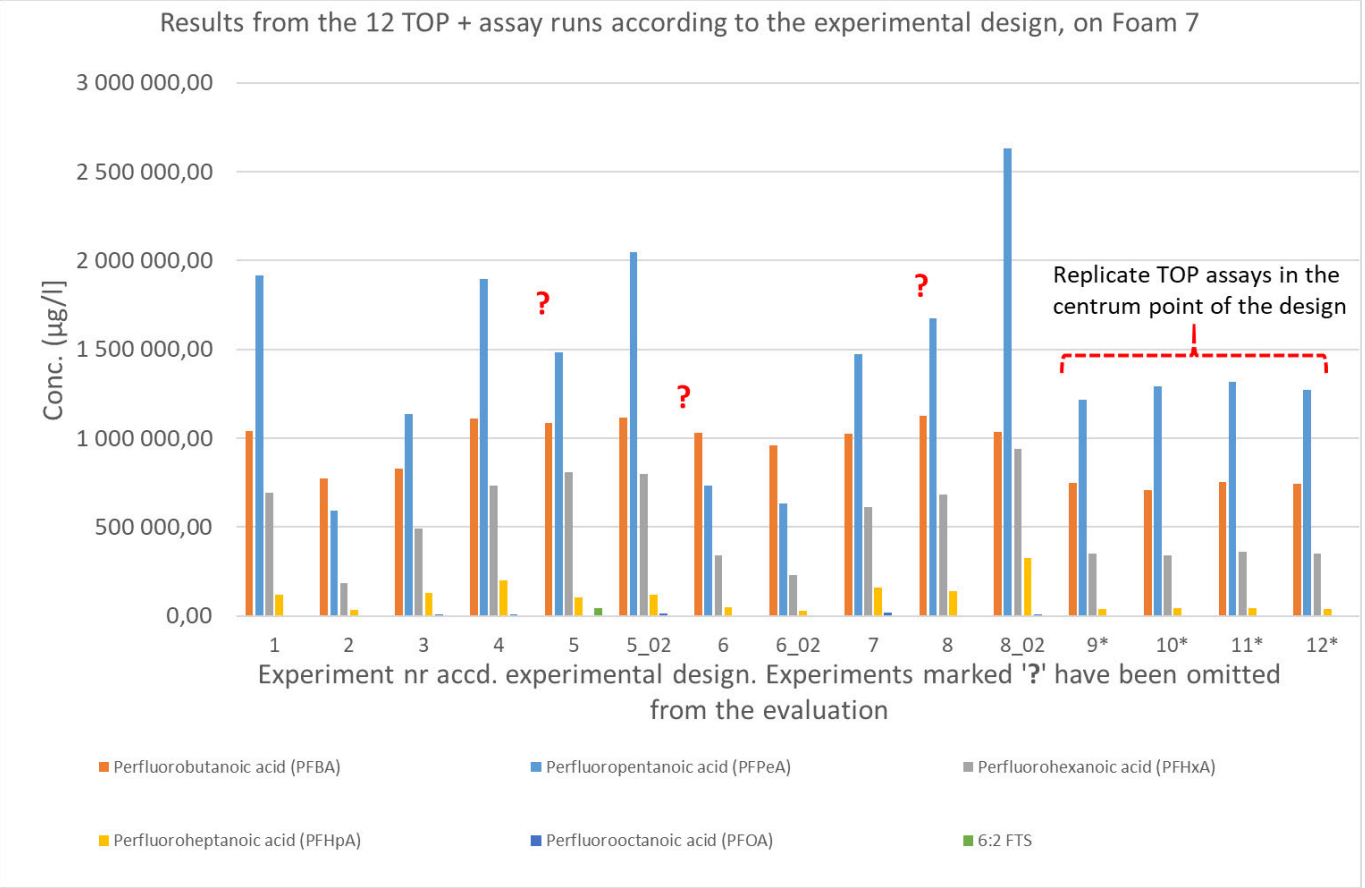


Figure 4. Overall results of the 15 TOP assay experiments (including the omitted runs) in the optimization step. The size of the staples illustrates the concentrations of different PFAS-homologues formed during the TOP-oxidation step. Please note that PFPeA is the dominant PFAS-homologue formed during the TOP-step. Comparing the omitted experiment nr 8 (where the added internal TOP-oxidation standards were not fully transformed) from experiment nr 8_02 (when the added internal standards in fact was 100 % transformed) provides some information as to the importance of correct temperature settings when executing the TOP-assay. Experiment nr 8_02 gave the highest yield with respect to $\sum PFAS_{11}$. The runs marked with a '?' refers to runs where the internal oxidation standard was not converted 100 % (omitted runs). These runs have not been used in the method development/optimization statistical evaluation. The last four runs (experiment 9-12) represent the four replicate TOP-oxidations on Foam 7 in the central point of the experimental design. Note the very benign repeatability variance in TOP-experiment 9-12. The standard deviation (RSD of 2,03 %) from the central point were used to represent the 'variance' of the whole evaluated domain.

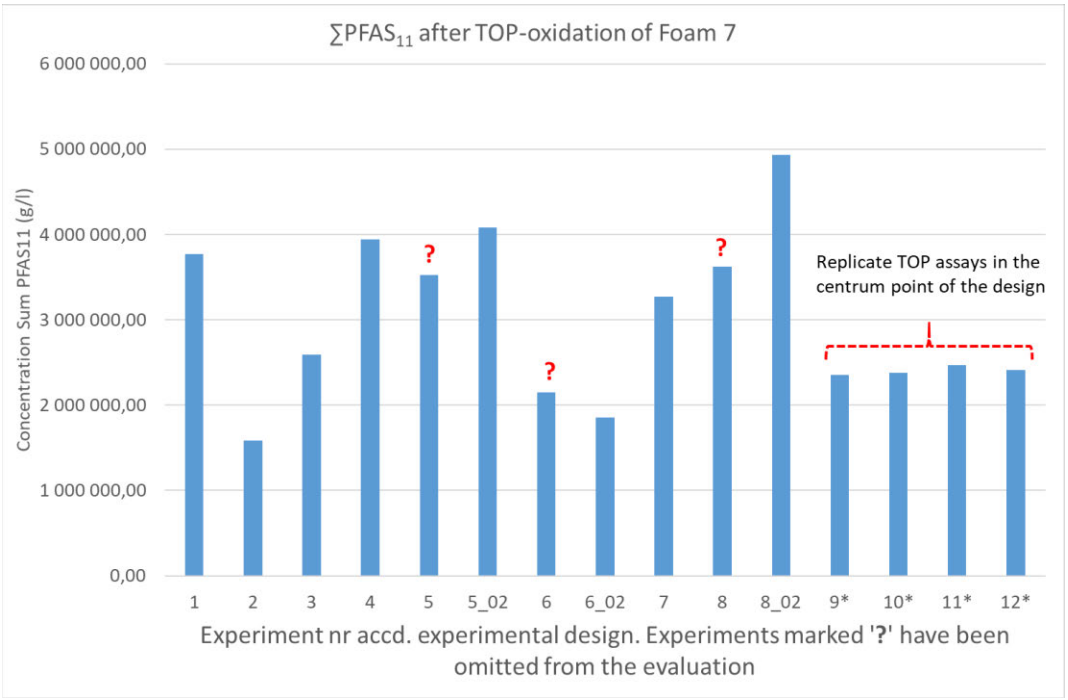


Figure 5. Similarly, as in the previous Figure 4, the results of the TOP-optimization runs, depicted as ∑PFAS$_{11}$-concentrations. Experiment no 8 gave the highest yield in terms of ∑PFAS11.

The results were then statistically evaluated with regard to two variables; concentration of ∑PFAS$_{25}$ and the concentration of PFOA *(i.e.* the peak area of PFOA eluted after 2.42-2.43 minutes at the m/z of 413, rather than the concentration).

7.2.1 Response variable; Area of the PFOA peak

The model hypothesis was that there was a quadratic dependence of the response variable with regard to all four TOP parameters evaluated (concentration of persulfate, concentration of hydroxide, temperature and time).

The statistical treatment showed that all four main parameters where significant at the 95 %-significance level.

The model being evaluated for significancy was thus;

1) A model where the response variable was the formation of PFOA, measured as the peak area of the PFOA-peak

Peak area PFOA (R1) = intercept + k_1*[Persulfate] + k_2*[hydroxide]+k_3*[temperature]+k_4[time]+ k_5[Persulfate]*[hydroxide]+ k_6*[Persulfate]*[temperature]+ k_7*[persulfate]*[time]+k_8[hydroxide]*[temperature]+k_9*[hydroxide]*[time]+k_{10}*[temperature]*[time]+k_{11}*[Persulfate]^2+k_{12}*[hydroxide]^2+ k_{13}*[temperature]^2+k_{14}*[time]^2 + ….+ error.

Since the experimental design was a reduced factorial design, only the intercept and cofficients k_1-k_7 as well as k_{10} can be determined. The remaining coefficients will be coupled to to k_1-k_7 and k_{10}. Thus, the model was simplified into;

Peak area PFOA (R1) = intercept + k_1*[Persulfate] + k_2*[hydroxide]+k_3*[temperature]+k_4[time]+ k_5[Persulfate]*[hydroxide]+ k_6*[Persulfate]*[temperature]+ k_7*[persulfate]*[time]+ k_{10}*[time]*[temperature].

With regard to the concentration of persulfate a lower concentration affects the formation of PFOA from "hidden" PFAS (precursor PFCAs) better, while a higher concentration of hydroxide is favourable, as is higher temperature and a lower oxidation time. The interaction effect of the parameter persulfate*temperature affects the response variable "Peak area PFOA" positively while the interaction between persulfate and oxidation time affects the response variable negatively.

7.3 Response variable; concentration of $\sum PFAS_{11}$

In a similar fashion as was the case with regard to the response variable "Peak area PFOA", the response variable $\sum PFAS_{11}$, the best fit of the statistically significant coefficients was used to determine the "optimal" experimental settings for the TOP-oxidation of Foam 7.

Concentration $\sum PFAS_{11}$ = intercept + k_1*[Persulfate] + k_2*[hydroxide]+k_3*[temperature]+k_4[time]+ k_5[Persulfate]*[hydroxide]+ k_6*[Persulfate]*[temperature]+ k_7*[persulfate]*[time]+ k_{10}*[time]*[temperature].

"Optimum" with regard to achieving the highest concentration of $PFAS_{11}$ and presumably the highest transformation of precursor PFCAs to PFAS, was similar to, but not identical to the results with regard to the response variable "Peak are of PFOA".

It was determined that the highest overall yield of PFAS-formation in the TOP assay of the Foam 7 AFFF formulation, was achieved using the TOP-settings;
Low (-), Low (-), Low (-), High (+) with respect to the parameters of Persulfate (60 mM), Sodium hydroxide (125 mM), temperature (80 ºC) and oxidation time (12 hr).

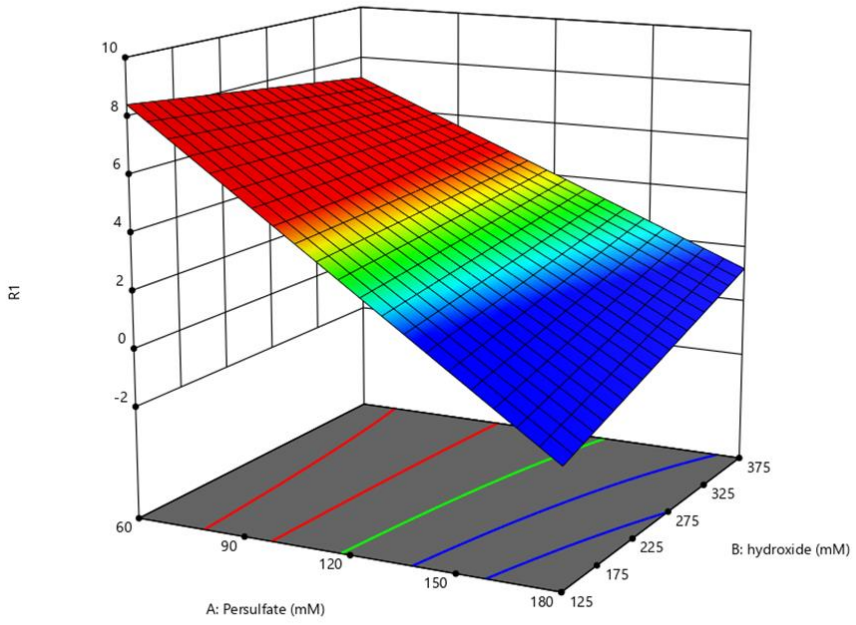


Figure 6. Evaluation of the TOP-oxidation method development experiments. In the response surface plot, the response variable (R1) of $\sum PFAS_{11}$, was plotted as a function of concentration of persulfate and concentration of sodium hydroxide at the fixed temperature of 85 ºC and the fixed oxidation time of 10 hr.

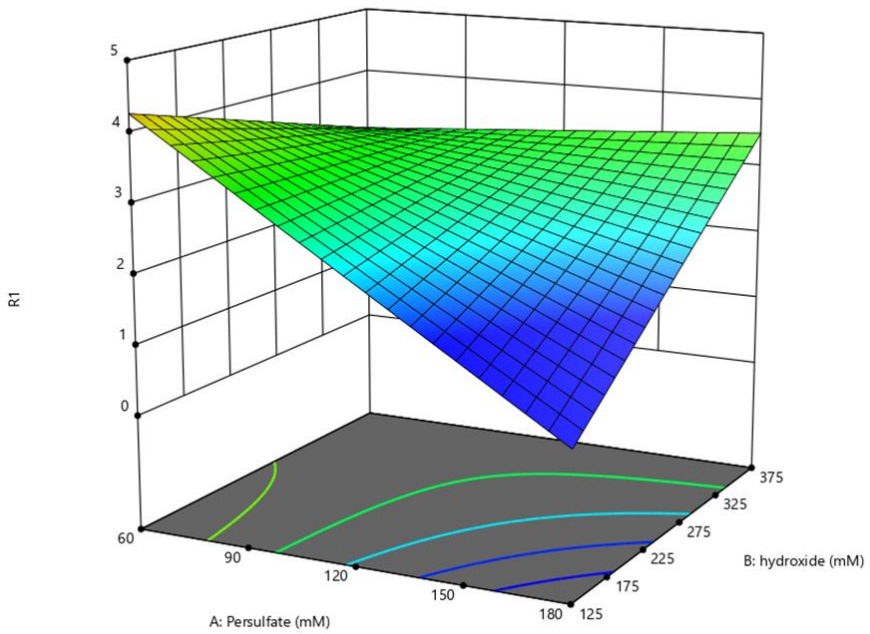


Figure 7. Evaluation of the TOP-oxidation method development experiments. In the response surface plot, the response variable (R1) of $\sum PFAS_{11}$, was plotted as a function of concentration persulfate and concentration of sodium hydroxide at the fixed temperature of 85 ^{o}C and the fixed oxidation time of 6 hr. Please note that with these settings, it seems as if optimal yield in the TOP-oxidation step would require a maximum concentration of persulfate (375 mM), however the full evaluation of the optimization runs revealed that slightly higher PFAS-yield would be accomplished at the settings (-), (-), (-), (+) and that the studies of response surfaces revealed a saddle point in the centrum point of the experimental design (at the settings (0), (0), (0), (0)).

A full evaluation of the factorial design optimization step is provided in appendix 1 to this report.

7.4 DOC determination of the seven participating foam concentrates

The DOC of all the foam concentrates were determined using the SS-EN 1484 method and the results are summarized in Table 5 below.

Table 5. DOC-determination of six F3 concentrates and one AFFF concentrate.

Foam #	Dissolved organic Carbon (DOC, g/l)
1 (JetFoam ICAO-C 3%)	104
2 (Meteor T-10/Training)	34,9
3 (HOTFOAM 2%)	210
4 (Trainer E-lite)	8,02
5 (ECOPOL A 3%)	117
6 (Respondol ATF 3-3%)	85,8
7 (Sthamex AFFF 3% F-15)	265

7.5 Analysis of ultra-short PFAS

In none of the foam concentrates (F3-concentrates 1-5[18], nor AFFF concentrate 7) ultra-short PFAS was quantified.
LOQs are summarized in Table 6 below.

Table 6. Reported LOQs for the five (5 ultra-short PFAS included in the study.

Sample name	Concentration (µg/l)	Comment
Foam 1 concentrate	< 400 with regard to TFA and PFPrA < 80 with regard to TFMS, PFEtS and PFPrS	Results reported for 1:200 dilution. Foam diluted 1:100 in water. Further dilution to 1:1 MeOH/Water.
Foam 2 concentrate	< 400 with regard to TFA and PFPrA < 80 with regard to TFMS, PFEtS and PFPrS	Results reported for 1:200 dilution. Foam diluted 1:100 in water. Further dilution to 1:1 MeOH/Water.
Foam 3 concentrate	< 400 with regard to TFA and PFPrA < 80 with regard to TFMS, PFEtS and PFPrS	Results reported for 1:200 dilution. Foam diluted 1:100 in water. Further dilution to 1:1 MeOH/Water.
Foam 4 concentrate	< 400 with regard to TFA and PFPrA < 80 with regard to TFMS, PFEtS and PFPrS	Results reported for 1:200 dilution. Foam diluted 1:100 in water. Further dilution to 1:1 MeOH/Water.
Foam 5 concentrate	< 400 with regard to TFA and PFPrA < 80 with regard to TFMS, PFEtS and PFPrS	Results reported for 1:200 dilution. Foam diluted 1:100 in water. Further dilution to 1:1 MeOH/Water.
Foam 7 concentrate	< 4 000 with regard to TFA and PFPrA < 800 with regard to TFMS, PFEtS and PFPrS	Results reported for 1:2000 dilution. Foam diluted 1:1000 in water. Further dilution to 1:1 MeOH/Water.

[18] Foam 6 was not analysed in the validation part of the study since being omitted in the optimization step as "PFAS-free". No PFAS was reported after extensive TOP-oxidation experiments using 9 different TOP-settings.

Sample name	Concentration (µg/l)	Comment
Foam 1 TOP-sample	< 40 000 with regard to TFA and PFPrA < 8 000 with regard to TFMS, PFEtS and PFPrS	Dilution of TOP extract 1:20 000 (according to information from Eurofins) Further dilution 1:1 MeOH/Water for injection on SFC-MS/MS Dilution 1:40 000
Foam 1 TOP-sample	< 40 000 with regard to TFA and PFPrA < 8 000 with regard to TFMS, PFEtS and PFPrS	Dilution of TOP extract 1:20 000 (according to information from Eurofins) Further dilution 1:1 MeOH/Water for injection on SFC-MS/MS Dilution 1:40 000
Foam 2 TOP-sample	< 40 000 with regard to TFA and PFPrA < 8 000 with regard to TFMS, PFEtS and PFPrS	Dilution of TOP extract 1:20 000 (according to information from Eurofins) Further dilution 1:1 MeOH/Water for injection on SFC-MS/MS Dilution 1:40 000
Foam 3 TOP-sample	< 40 000 with regard to TFA and PFPrA < 8 000 with regard to TFMS, PFEtS and PFPrS	Dilution of TOP extract 1:20 000 (according to information from Eurofins) Further dilution 1:1 MeOH/Water for injection on SFC-MS/MS Dilution 1:40 000
Foam 4 TOP-sample	< 40 000 with regard to TFA and PFPrA < 8 000 with regard to TFMS, PFEtS and PFPrS	Dilution of TOP extract 1:20 000 (according to information from Eurofins) Further dilution 1:1 MeOH/Water for injection on SFC-MS/MS Dilution 1:40 000
Foam 5 TOP-sample	< 40 000 with regard to TFA and PFPrA < 8 000 with regard to TFMS, PFEtS and PFPrS	Dilution of TOP extract 1:20 000 (according to information from Eurofins) Further dilution 1:1 MeOH/Water for injection on SFC-MS/MS Dilution 1:40 000
Foam 7 TOP-sample	< 40 000 with regard to TFA and PFPrA < 8 000 with regard to TFMS, PFEtS and PFPrS	Dilution of TOP extract 1:20 000 (according to information from Eurofins) Further dilution 1:1 MeOH/Water for injection on SFC-MS/MS Dilution 1:40 000

7.6 Results of the CIC/EOF-analysis

The results of the CIC/EOF analysis are summarized in the Table 7 below.

Table 7. Results of the CIC/EOF-analysis of some of the participating foam concentrates. Please note that some of the results reflect CIC/EOF-analysis of diluted foam concentrates, some reflect the analysis of CIC/EOF-analysis of TOP-oxidized samples (thus extensively diluted samples), and some results reflect the CIC-analysis only where no SPE-pretreatment have been used. Some of the results have been aggregated (summation of SPE fraction of neutral eluate and SPE fraction of anionic eluate).

Sample	TOP-pretreatment	SPE/EOF	µg F/ml	mg F/l	Converted to 6:2 FTS-equivalents, mg/l	Comment
Foam 2	N/A	N/A	20	20	34,7	Foam concentrate diluted 1:100. Result compared with correspond. value for ∑PFAS11 after TOP, see Table 9.
Foam 2	Yes	N/A	< LOD			Sample diluted 1:100 000
Foam 5	Yes	Yes, neutral fraction	70	70	121,3	
Foam 5	Yes	Yes, anionic faction	149	149	258,2	
Foam 5	Summation of neutral and anionic fraction from SPE		219	219	379,4	Result compared with correspond. value for ∑PFAS11 after TOP, see Table 9.
Foam 5	N/A	Yes, neutral fraction	0,46	0,46	0,80	
Foam 5	N/A	Yes, anionic faction	0,32	0,32	0,55	
Foam 5	Summation of neutral and anionic fraction from SPE 0,78		0,78	0,78	1,4	Result compared with correspond. value for $\sum PFAS_{11}$ after TOP, see Table 9.
Foam 5	N/A	N/A	5,1	5,1	8,84	
Foam 7	Yes	Yes, neutral fraction	160	160	277,2	
Foam 7	Yes	Yes, anionic fraction	29	29	50,2	
Foam 7	Summation of neutral and anionic fraction from SPE 189		189	189	327,5	
Foam 7	N/A	Yes, neutral fraction	2 250	2 250	3 898,4	Result compared with correspond. value for $\sum PFAS_{11}$ after TOP, see Table 9.
Foam 7	N/A	Yes, anionic fraction	1 490	1 490	2571, 6	
Foam 7	Summation of neutral and anionic fraction from SPE		3 740	3 740	6 479,9	Result compared with correspond. value for $\sum PFAS_{11}$ after TOP, see Table 9.
Foam 7	N/A	N/A	12 000	12 000	20 791	Result would correspond to a concentration of non-extractable organic fluorine and inorganic fluorine of (20 791-6479,9) 14 311 mg/l

7.7 The final proposed TOP assay protocol

From the optimization step (the TOP-conditions providing the most increase in $\sum PFAS_{11}$ in Foam 7), the critical steps from dilution of foam concentrates, addition of different internal standards, transfer of foam samples between utensils, the TOP-oxidation step itself, as well as final quantification of PFAS using LC-MS/MS, was iterated with the three participating laboratories.

A prerequisite of the whole project, enabling three commercial laboratories, normally being competitors, to participate, was the fact that each participating laboratory could use their own quantitative method for the determination of PFAS in water samples, using LC-MS/MS as long as the method already was validated.

All participating laboratories had in-house developed, and validated methods based on the EPA method 537.1 or the ISO/DIS 21675-method. Each participating laboratory could deviate from the standard method in terms of solvents used, number of PFAS quantified in the analysis, the LC-column used, the gradient used, internal- and surrogate standards used, MS/MS-conditions used etc., but the method had to be under all circumstances extensively validated for precision (RSD < 20%), repeatability, accuracy (recovery of replicate samples within 30 % of true value), Peak asymmetry factors, Minimum Reporting Level (MRL), Recovery range (50-150 % at the Minimum Reporting Level etc.)

One could argue that if the participating laboratories used slightly different PFAS quantification methods, an additional variance factor is introduced. This is true. However, the TOP-oxidation step in itself was considered to be a by far larger variance factor, thus permitting the participating laboratories to utilize their own in-house method.

7.8 Brief description of the sample preparation method

First the laboratories did a quantitative determination of PFAS in the non-oxidated foam sample at a dilution of 1: 10 000 with regard to Foam 1-5 and a dilution of 1 : 100 00 to 1:1 000 000 with respect to Foam 7 (executed as two consecutive 1:1 000-dilutions). The foams had to be diluted with MQ-water. Internal- and surrogate standard selection could be chosen freely by the laboratory.

The laboratories could choose to either clean-up the sample using solid phase extraction or proceed with a direct injection. If direct injection were chosen, the laboratory added internal standards to a 9 ml of diluted foam solution, then 3 ml acetonitrile, 120 µl of a 0,5 M acetate buffer were added. The sample was filtered through a 0,22 µm cellulose filter prior to the direct injection.

7.9 Brief description of the TOP-oxidation sample preparation

25 ml of the diluted foam samples (Foam 1-5 diluted 1:10 000 in MQ water, Foam 7 diluted 1 : 100 000 to 1: 1 000 000 in MQ water), was transferred to 50 ml polypropylene tubes. Please note that the participating laboratories were instructed to only use tubes that had septum-free lids.

50 µl of a 100 ng/ml TOP-oxidation internal standard was added to the sample.

25 ml of TOP-mixture was then added to the sample. The TOP-mixture contained 120 mM potassium persulfate ($K_2S_2O_8$) and 250 mM sodium hydroxide (NaOH).

The polypropylene tube was then transferred to a water bath, set at 80 ºC and the oxidation reactions were allowed to proceed fort 12 h.

After 12 hours the oxidation reaction was terminated by addition of 200 µl of formic acid (98 % w/w).

The polypropylene tube was then subjected to at least 3 min of ultra-sound treatment (to desorp PFAS from the test tube inner surfaces).

Additional internal standards were added to the sample to accommodate for losses in the filtering. The test tube was then centrifuged (3 000 rpm, 3 min), and then filtered through the 0,22 µm cellulose filter.

The filtered solution was then quantified using direct injection LC-MS/MS.

7.10 The evaluation of the proposed TOP assay protocol

Results of the quantification of non-oxidized foams can be briefly summarized as;

- SGS Analytics reported minute concentrations of 6.2 FTS in all the F3 concentrate (Foam 1-5). 6:2FTS-concentration in Foam 2; 190 µg/l. 19 µg/l in Foam 3, 11 µg/l in Foam 4, and 25 µg/l in Foam 5. Furthermore, the laboratory reported branched PFOS in Foam 1 (concentration 4,5 µg/l).
- Eurofins reported 6:2 FTS in Foam 2 and Foam 3. 6:2FTS-concentration in Foam 2; 143 µg/l and 15 µg/l in Foam 3.
- ALS reported 6:2 FTS in Foam 2. 6:2 2 3FTS-concentration in Foam 2; 198 µg/l.
- All three laboratories reported similar concentrations of 6:2 FTS in Foam 7; SGS Analytics reported 5 552 µg/l, Eurofins reported 6 500 µg/l and ALS reported 5 070 µg/l.

The results of the quantitative analysis of non-oxidized samples are summarized in Table 8 below.

Table 8. Results of the PFAS-analysis of the participating laboratories and foams before the TOP-oxidation step.

Laboratory	**Foam #**	**PFAS-homologue**	**Concentration (µg/l]**	**Comment**
SGS Analytics	1	6:2 FTS and branched PFOS	7,5 and 4,5 respectively	Method LOD for branched PFOS 6 µg/l
	2	6:2 FTS	190	
	3	6:2 FTS	19	
	4	6:2 FTS	11	
	5	6:2 FTS	24	
	7	6:2 FTS	5 552	
Eurofins	1			
	2	6:2 FTS	143	
	3	6:2 FTS	15	
	4			
	5			
	7	6:2 FTS	6 500	
ALS Global	1			
	2	6:2 FTS	198	
	3			
	4			
	5			
	7	6:2 FTS and PFHxA	5 070 and 159 respectively	

As evident from Table 8 above;

- the results of the quantitative analysis are quite consistent between laboratories,

- all foams but Foam 7 would have passed the proposed Swedish national legislation from Kemikalieinspektionen (Swedish Chemicals Agency) (assuming densities around 1 kg/l for the foam concentrates)[19] and furthermore,
- all foams pass the GreenScreen-criteria as fluorine-free[20] (albeit the fact that data does not stem from TOF-analysis),
- notably, none of the foams will pass the "one ppb of PFAS" (1 µg/l) threshold as defined in section 322 in the National Defense Authorization Act for Fiscal Year 2020[21].

7.11 The TOP-oxidation results

The participating laboratories were requested to execute four (4) TOP-oxidations per foam concentrate and report the quantified PFAS after the oxidation procedure.

The results after the TOP-oxidation can briefly be described as;

- none of the laboratories reported any formation of PFAS in Foam 4,
- neither Eurofins nor ALS Global reported any formation of PFAS in Foam 1, while SGS Analytics reported minute formation of PFHxA and PFHpA in one of the four conducted TOP-oxidation samples (19,4 and 28,6 µg/l respectively).
- all the three laboratories reported formation of the PFAS-homologues of PFBA, PFPeA, PFHxA and PFHpA.
- all three laboratories reported similar $\sum PFAS_{11}$-concentrations for Foams 2, 3 and 5.
- ALS Global and SGS Analytics did not report any formation of 6:2 FTS in any of the F3 concentrates (Foams 1-5), while Eurofins reported some formation of 6:2 FTS in Foam 2 (average 6:2 FTS-concentration after TOP was 1 125 µg/l while the corresponding 6:2 FTS concentration before TOP was reported to be 143 µg/l).
- differences in the reported $\sum PFAS_{11}$-concentrations with regard to Foam 7 was to be expected. However, neither ALS Global nor SGS Analytics reported any residual concentration of 6.2 FTS after the TOP-oxidation in Foam 7, while in the case of Eurofins, the concentration of 6:2 FTS increased slightly after the TOP-treatment (from 6 500 µg/l in the non-oxidized foam to 7 750 µg/l in the TOP-treated foam).

One important apparent conclusion can be drawn from the analytical work conducted;

- Foam 4 and most probably also Foam 1 could be regarded as PFAS-free since no formation of PFAS is detected in the TOP-experiments. Also, Foam 6, sorted out from further investigation already in the TOP-optimization step (when it by necessity of the project, was replaced with Foam 7) must be regarded as PFAS-free in accordance with all criteria previously discussed. Thus, there seems to be F3 concentrates presently on the market that upon scrutiny are in fact PFAS-free (given previously discussed limitations of the TOP-method; are all precursor PFCAs transformed to PFAS?).

[19] See 4.2.1.
[20] See 4.2.3
[21] See 4.2.2

The results of the TOP-oxidation step have been summarized Table 9 below.

Table 9. Results and the occurrence/distribution of different PFAS-homologues appearing after the TOP-oxidation step. The foam that demonstrated lowest concentration of ∑$PFAS_{11}$was Foam 1 (only minute concentrations of PFAS reported by one laboratory only). The PFAS-homologue with the highest concentrations was Foam 2 (∑$PFAS_{11}$ between 7 950 – 10 119 µg/l).

Laboratory	Foam #	Concentration of ∑$PFAS_{11}$, µg/l	PFAS-homologue reported	Concentration (µg/l]	Comment
SGS Analytics	1	Up to 47,9	PFHxA and PFHpA	19,4 and 28,6	PFHxA and PFHpA were only reported above LOQ (6 µg/l) in one of the four conducted TOP-oxidations.
	2	7 508- 9 492	PFBA, PFPeA, PFHxA and PFHpA	1 969 – 2 533, 3 895 – 4 710, 1 471 – 1 991, 173 – 258 respectively	
	3	356 – 448	PFBA, PFPeA, PFHxA and PFHpA	85 – 144, 205 – 244, 57 – 86, 6 – 31 respectively	
	5	397 – 423	PFBA, PFPeA, PFHxA and PFHpA	74 – 107, 221 – 242, 73 – 86, 7 – 11 respectively	
	7	2 538 – 2 738 mg/l	PFBA, PFPeA, PFHxA and PFHpA	594 – 652 mg/l, 1 100 – 1 201 mg/l, 668 – 770 mg/l, 97 – 115 mg/l respectively	
Eurofins[22]	2	7 950 – 8 720	PFBA, PFPeA, PFHxA and PFHpA and 6:2 FTS	1 600 – 2 000, 3 100 - 3 900, 1 200 - 1 600, 200 – 250, 990 – 1 200 respectively	Average degree of oxidation (quantified as the conversion of the internal TOP-standard) was 85,5 %
	3	290 – 410	PFPeA & PFxA	150 – 270 and 120 – 150 respectively	Average degree of oxidation (quantified as the conversion of the internal TOP-standard) was 63,3 %
	5	310 – 410	PFPeA & PFxA	170 – 240 and	Average degree of oxidation

[22] Upon request from the laboratory, the degree of oxidation was quantified with another TOP internal standard, ^{13}C8-PFOSA, as compared with the other participating laboratories.

Laboratory	Foam #	Concentration of ∑$PFAS_{11}$, µg/l	PFAS-homologue reported	Concentration (µg/l]	Comment
				140 – 170 respectively	(quantified as the conversion of the internal TOP-standard) was 75,0 %
	7	1 130 – 1 830 mg/l	PFBA, PFPeA, PFHxA and PFHpA and 6:2 FTS	580 – 710 mg/l, 1 400 - 1 800 mg/l 1 100 – 1 200 mg/l 150 – 200 mg/l respectively.	Average degree of oxidation (quantified as the conversion of the internal TOP-standard) was 81,0 %
ALS Global	2	9 785 - 10 119	PFBA, PFPeA, PFHxA and PFHpA respectively	2 633 – 2 782, 5 433 – 5 755, 1 358 – 1 411 223 – 255 respectively	
	3	222 - 239	PFPeA	222 - 239	
	5	279 -300	PFPeA	279 -300	
	7	3 422 – 3 851 mg/l	PFBA, PFPeA, PFHxA and PFHpA respectively	850 – 911 mg/l, 1 783 – 2 044 mg/l, 663 – 768 mg/l, 117 – 127 mg/l respectively	

Some basic statistical treatment of the results of the TOP-oxidations with respect to the response variable ∑$PFAS_{11}$ is provided in Table 10 below.

Table 10. Average concentrations (of four replicate determinations) of results reported by each participating laboratory, for each corresponding F3 concentrate after TOP-oxidation. The results provided in table 9 (an average- a median concentration, along with the standard deviation) would normally be satisfactorily to judge whether the confidence interval of the mean would encompass 0. However, rejecting a F3 concentrate on that merit alone would not take between-laboratory variance in to account.

Laboratory	Foam #	Average concentration of $\sum PFAS_{11}$, µg/l	Median concentration of $\sum PFAS_{11}$, µg/l	Standard deviation
Foam 2				
SGS Analytics/Synlab	2	8 254	8 009	862,0
Eurofins	2	8 270	8 205	364,0
ALS Global	2	9 932	9 911	139
Foam 3				
SGS Analytics/Synlab	3	400	398	39,67
Eurofins	3	347,5	344,9	61,27
ALS Global	3	230	229	8,36
Foam 5				
SGS Analytics/Synlab	5	413,0	416,0	11,4
Eurofins	5	355,1	350,0	44,3
ALS Global	5	285,7	282,0	2,3

8. ANOVA – analysis of variance in the reported results from the validation study.

This chapter describes the statistical evaluation method used to evaluate variances in the data from the validation study. As such, the chapter, inevitably discusses and applies some mathematical statistical theory that can be omitted by the reader when results are further discussed and scrutinized in chapter 9.

Analysis of variance (ANOVA) is a collection of statistical models and their associated estimation procedures (such as the "variation" among and between groups) used to analyse the differences among means. ANOVA is a statistical technique that is used to check if the means of two or more groups are significantly different from each other and furthermore ANOVA checks the impact of one or more factors by comparing the means of different samples.

ANOVA was developed by the statistician Ronald Fisher (Fisher 1925). ANOVA is based on the law of total variance, where the observed variance in a particular variable is partitioned into components attributable to different sources of variation. In its simplest form, ANOVA provides a statistical test of whether two or more population means are equal, and therefore generalizes the t-test beyond two means.

In the typical application of ANOVA, the null hypothesis is that all groups are random samples from the same population. Rejecting the null hypothesis is taken to mean that the differences in observed effects between treatment groups (participating laboratories in this case) are unlikely to be due to random chance. It is not the scope of this report to provide a lengthy description on ANOVA and the interested reader is referred to books on the matter (see for instance Scheffé's "The Analysis of Variance or Vik's "Regression, ANOVA, and the General Linear Model")

ANOVA can provide;

- As exploratory data analysis, an ANOVA employs an additive data decomposition, and its sums of squares indicate the variance of each component of the decomposition (or, equivalently, each set of terms of a linear model).
- Comparisons of mean squares, along with an F-test allow testing models/hypotheses.

Closely related to the ANOVA is a linear model fit with coefficient estimates and standard errors.

In a first attempt to evaluate the different forms of variance from the TOP-oxidations, all F3-concentrates where PFAS-homologues were reported by all three participating laboratories (Foam 1, Foam 4 and Foam 6 thus not included in the statistical evaluation) were subjected to an initial 2-way ANOVA analysis of variance. The depending variables thus being the foam brand and the laboratory conducting the TOP-assay.

Results of the 2-way ANOVA clearly show that the biggest source of variance is the foam type (since the average $\sum PFAS_{11}$-concentrations of the three foams (Foam 2, Foam 3 and Foam 5) differ to such a great extent). From the 2-way ANOVA analysis it is also clear that there is considerable variance between conducting laboratories executing the TOP-assay on the very same foam. Furthermore, the 2-way ANOVA also shows the interaction variance (foam type * laboratory) significant albeit difficult to interpret. All these three factors are significant on the 95 % confidence level (see the F-values of Table 11 below). This implies that the means differ significantly, and the so-called null hypothesis (all three of them) must all be rejected.

Table 11. Results of a 2-way ANOVA analysis of variance on the three F3-foams that all three participating laboratories reported to contain PFAS (Foam 1, 4 and 6 thus omitted from further statistical evaluation).

Source of variation	Degrees of freedom (DF)	Sum of Squares	Mean Squares	F-value	P-value	F-crit.
Foams (replicate)	2	575293321,2	287646660,6	2868,412	3,58E-32	3,354131
Laboratories	2	1816700,291	908350,1454	9,058066	0,000977	3,354131
Interaction	4	5708097,58	1427024,395	14,23028	2,28E-06	2,727765
Residual	27	2707581,71	100280,8041			
Totalt	35	585525700,8				

Since the foam concentrates 2, 3 and 5 differ so much in their reported $\sum PFAS_{11}$-concentrations, it was decided that Foam 2 would have to represent a worst-case scenario of the population of F3-foams and hence a 1-way ANOVA analysis was conducted on Foam 2.

The results of Table 10 were subjected to an analysis of variance in accordance with the ANOVA-methodology and the one-way ANOVA-calculations are summarized in Table 12 below.

The objective of the exercise is to compare different contributions of variance in order to establish a level of certainty by which the TOP-results may result in either classifying a F3 concentrate as "PFAS free" or not. From the results in Table 10 one must assume that the results of Foam 2 presents a worst

case with regard to the criteria of a F3 concentrate being "PFAS-free", since the average concentration of $\sum PFAS_{11}$ is by far the highest of the F3 concentrates in this study.

If one would choose for instance Foam 3 or Foam 5 and calculate the confidence interval for the average $\sum PFAS_{11}$-concentration, Foam 2 would automatically be considered a non-PFAS-free foam.

Table 12. ANOVA on the average $\sum PFAS_{11}$-concentration in Foam 2, analyzed by three different laboratories, where each laboratory has made 4 TOP-oxidations/determinations on $\sum PFAS11$-concentration. The ANOVA calculations show that the systematic variance between the three participating laboratories executing 4 TOP-determinations on the $\sum PFAS_{11}$-concentrations is larger than the variance experimental variance within each laboratory.

Source	**Degrees of Freedom (DF)**	**Sum of Squares**	**Mean squares**	**F-value**	**Pr>F**
Variance between laboratories	2	7 431 603,04	3 715 801,52	12,46	**0,003**
Variance within laboratories	9	2 684 824,32	298 313,81		
Total variance	11	1 011 6427,4			
Calculated against model Y=Mean(Y)					

By dividing the variance between laboratories with the total variance, the r^2-value (between 0 and 1) is obtained, which is a measure as of how much of the overall variance that is attributed to the variance between laboratories. In this case (see Table 12) the r^2-value is 0,735, thus 73,5 % of the overall variance is attributed to the variance between the three laboratories (and thus 26,5 % of the total variance is attributed to the variance between replicate TOP-oxidations at the very laboratory).

By calculating the mean squares (thus dividing the sum of squares by the degrees of freedom), and then subsequently dividing mean squares of the variance between laboratories with the mean squares of the variance within laboratories, one calculates the F-value for the Fisher-test. In this case the F-value is 12,46 (see Table 12). If the null hypothesis is formulated that "all three participating laboratories executing 4 consecutive TOP-oxidations on Foam 2, their respective average $\sum PFAS_{11}$-concentration are the same", it can be phrased as;

H_0; Average $\sum PFAS_{11}$(ALS) =Average $\sum PFAS_{11}$(Eurofins) = Average $\sum PFAS_{11}$(SGS Analytics)

The P-value of table Table 12 is the probability of obtaining a result more extreme (bigger) than the observed F- ratio, assuming the null hypothesis is true, or as;

$P [F(2, 9) > 12,46]$.

The F-distribution renders the value 0,997, interpreted as the probability of null hypothesis to be true is 1-0,997 = 0,003 or 3 % probability (see Table 8).

At a 95 % significance level the null hypothesis is thus rejected. There is a systematic difference between the laboratories conducting the TOP-oxidations of Foam 2.

The mathematical model of the ANOVA is that the independent variable, $\sum PFAS_{11}$ is a function of both the particular laboratory executing the TOP-oxidation as well as a term that reflects "the effect of all other extraneous variables" (the random error), which can be phrased as;

$$\sum PFAS11_{ij} = \mu + \beta_j + \varepsilon_{i(j)},$$

where μ is the true value of the total average of $\sum PFAS_{11}$, notation ***j*** goes from 1-3 (the number of participating laboratories and the notation ***i*** goes from 1-4 (the number of replicate determinations each laboratory conduct).

By calculating the standard error pair-wise for the different two-pair combinations of participating laboratories the equation describing the independent variable $\sum PFAS11_{ij}$ can be written as;

$$\sum PFAS11_{ij} =$$

$$\sum PFAS11_{SYNLAB,AVERAGE} + Q1_{ALS}*(\sum PFAS11_{ALS,\ AVERAGE} - \sum PFAS11_{SYNLAB,AVERAGE}) +$$

$$Q1_{EUROFINS}*(\sum PFAS11_{EUROFINS,AVERAGE} - \sum PFAS11_{SYNLAB,AVERAGE})$$

The calculated coefficients $Q1_{ALS}$ and $Q1_{EUROFINS}$ are tabulated in Table 13 below with its corresponding standard error. From the 95% confidence interval of the Q1-coefficients it is evident that only the coefficient $Q1_{ALS}$ is significant.

Table 13. Calculated model coefficients along with standardized standard errors and corresponding confidence intervals.

Source	Value	Standard error	t	Pr > |t|	Lower bound (95%)	Upper bound (95%)
Q1-ALS	0,861	0,198	4,343	**0,002**	0,413	1,310
Q1-Eurofins	0,008	0,198	0,040	0,969	-0,441	0,457
Q1-Synlab	0,000	0,000				

Using the coefficient Q1ALS and the equation above, one can thus calculate the $\sum PFAS11_{ij}$ for j = 1-3, and compare the prediction with the reported value of $\sum PFAS11_i$ of the laboratory for i = 1 - 4, which has been tabulated in Table 14 below.

Table 14. The predictions, residuals and the 95 % confidence limit for the reported value of $\sum PFAS11_{ij}$.

Exp.	$\sum PFAS_{11}$	Pred. of $\sum PFAS_{11\ ij}$	Residual	Std. residual	Std. dev. on pred. (Mean)	Lower bound 95% (Mean)	Upper bound 95% (Mean)	Std. dev. on pred. (Observation)	Lower bound 95% (Observation)	Upper bound 95% (Observation)
1	7950,0	8270,0	-320,0	-0,59	273,1	7652,2	8887,8	610,6	6888,6	9651,4
2	8720,0	8270,0	450,0	0,82	273,1	7652,2	8887,8	610,6	6888,6	9651,4
3	8000,0	8270,0	-270,0	-0,49	273,1	7652,2	8887,8	610,6	6888,6	9651,4
4	8410,0	8270,0	140,0	0,26	273,1	7652,2	8887,8	610,6	6888,6	9651,4
5	9784,9	9931,5	-146,7	-0,27	273,1	9313,8	10549,3	610,6	8550,1	11312,9
6	9932,4	9931,5	0,85	0,002	273,1	9313,8	10549,3	610,6	8550,1	11312,9
7	9890,2	9931,5	-41,4	-0,08	273,1	9313,8	10549,3	610,6	8550,1	11312,9
8	10118,7	9931,5	187,2	0,343	273,1	9313,8	10549,3	610,6	8550,1	11312,9
9	9491,9	8254,4	1237,6	2,266	273,1	7636,6	8872,2	610,6	6873,0	9635,8
10	7508,4	8254,4	-746,0	-1,37	273,1	7636,6	8872,2	610,6	6873,0	9635,8
11	7907,8	8254,4	-346,6	-0,64	273,1	7636,6	8872,2	610,6	6873,0	9635,8
12	8109,4	8254,4	-145,0	-0,26	273,1	7636,6	8872,2	610,6	6873,0	9635,8

It is quite obvious that the models suffer from the poor lack of fit with regard to the TOP-oxidations executed and reported by ALS Global (see Figure 8 below were predicted versus reported values of $\sum PFAS11_{ij}$ have been plotted). It should be emphasized, however, that it is not possible to state that the results reported by ALS Global are erroneous, only that the coefficient-based ANOVA model cannot incorporate them nor their variance. In fact, the lowest within-laboratory variance in this study is shown from the ALS laboratory.

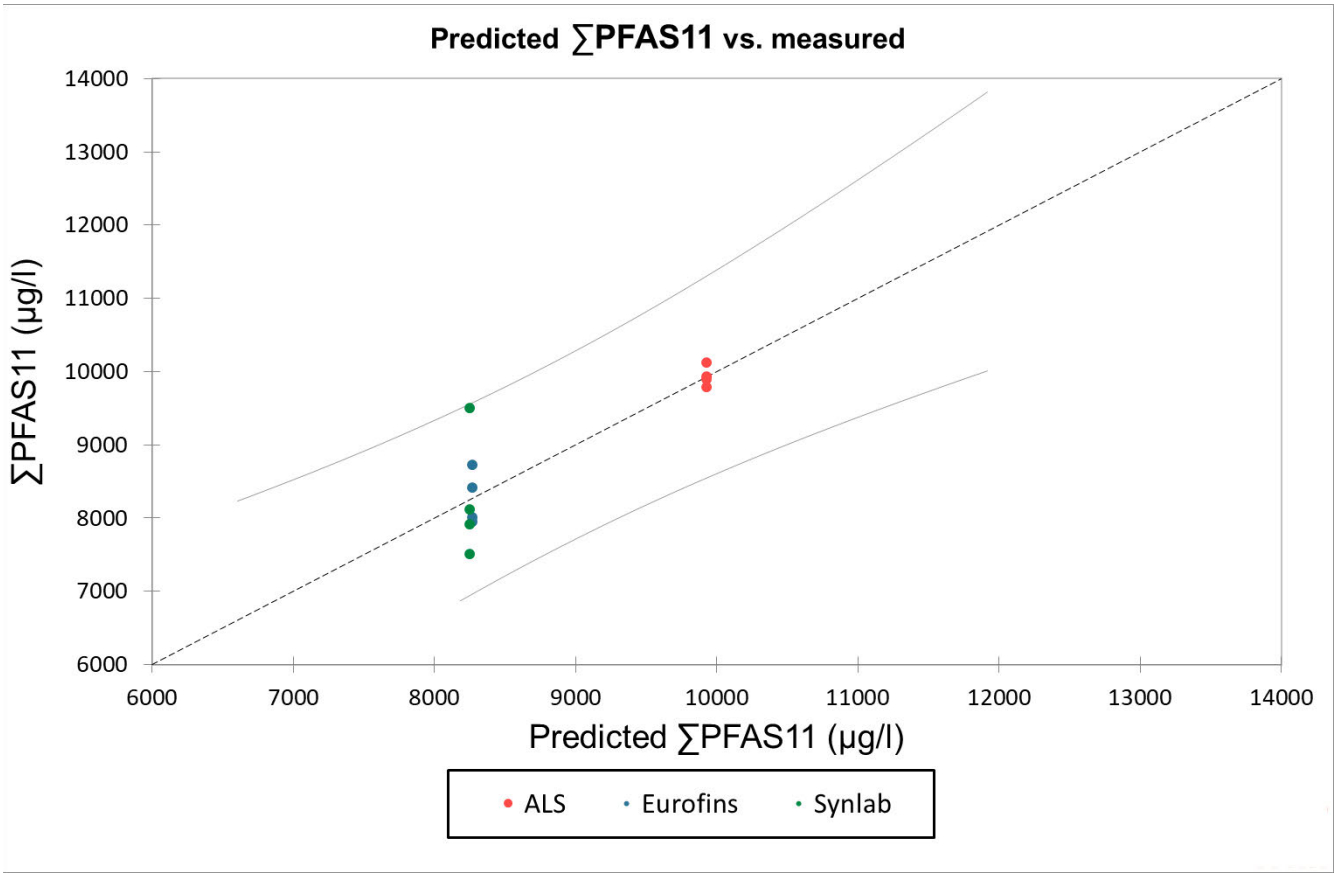


Figure 8. Predicted-vs-reported values of $\sum PFAS11_{ij}$, with a double-sided 95 % confidence interval.

From the double-sided confidence interval calculated in Table 14, it is evident that one would need to accommodate individual TOP-oxidation results, with regard to the concentration of $\sum PFAS_{11}$ (in µg/l) of a F3 concentrate (with the characteristics of Foam 2), analysed at an arbitrary laboratory, with a substantial statistical "discount", of **2 762,7 µg/l** before claiming with a 5 % risk of being wrong, that the F3 concentrate is not PFAS-free.

However, the calculation in Table 14 is based on the Tukey's honestly significant difference (TSD), which provides a double-sided 95% confidence interval that all population mean differences of the parameter $\sum PFAS_{11}$, are contained within. This confidence interval is a bit wider than a confidence interval strictly based on the t-distribution since it is based on the Studentized range distribution.

Furthermore, a one-sided confidence interval would be more appropriate. Hence from the tabulated values of the Studentized range distribution a correction factor taking into account the difference between the α = 0,05/2 for (N, k) with the α = 0,05 (N, k) of 1,78. Thus, the one-sided Tukey's honestly significant difference -value would decrease to **1 552,1 µg/l**.

A corresponding one-sided 95% confidence interval (based on N = 12,) and Student's t-distribution would narrow down the interval to;

$$t_{(1-\alpha)} * \left(\frac{Std.\,on\,pred.}{\sqrt{N}}\right)_{from\ the\ ANOVA} = 1{,}796 * \frac{610.649}{\sqrt{12}} = 414{,}8\ \mu g/l$$

Thus, for a reported mean $\sum PFAS_{11}$-concentration stemming from a TOP-oxidation according to the TOP-settings employed in this study, a discount of 414,8 µg/l would be appropriate to use in order to judge whether the F3 concentrate in fact is PFAS-free.

Basically the 'statistical discount' provides the null hypothesis;

H_0; $\sum PFAS_{11\ (arbitrary\ lab)}$ + 'one-sided confidence interval > 0

H_0; $\sum PFAS_{11}$+1552 µg/l > 0 then the F3 concentrate is not PFAS-free (by implementation of a one-sided Studentized range distribution confidence interval, or

H_0; $\sum PFAS_{11}$+418 µg/l > 0 (by implementing a one-sided Student's t distribution confidence interval)

Using the "statistical discount" calculated herein on the F3 concentrate investigated in the study the fallout would be as tabulated in Table 15 below.

Table 15. Application of the TOP-assay 'statistical discount' (both based on the Student's t-distribution as well as based on Tukey's honestly significant difference) on the reported grand averages of $\sum PFAS_{11}$ in Foam 2, 3 and 5.

Foam no	**Reported grand average, µg/l**	**Standard deviation from the measurements**	**Reported grand average adjusted with "statistical discount" of 414,8, µg/l**	**Reported grand average adjusted with "statistical discount" of 1 552,1 µg/l from the one-sided confidence interval of Tukey's honestly significant difference (from the Studentized range distribution).**
2	8818,637	958,997	8403,837	7 266,5
3	325,914	83,619	-88,886	-1 226,2
5	351,267	59,573	-63,533	-1 200,8

Since Foam 3 and Foam 5 will have adjusted grand average concentrations of $\sum PFAS_{11} < 0$, the confidence interval of the actual measurements (the standard deviation and number of replicates) would not matter (it would still encompass 0), and thus with a 5 % risk of being wrong, one would need to pass these two foams as "PFAS-free", while the "statistical discount" would single out Foam 2 as non-compliant as to being "PFAS-free", having a "adjusted" grand average $\sum PFAS_{11} > 0$.

As evident from Table 15 above, two of the foams (Foam 3 and Foam 5) would, after applying the statistical discount, also pass the "one ppb of PFAS" (1 µg/l) threshold as defined in section 322 in the National Defense Authorization Act for Fiscal Year 2020.

9. Evaluation of TOP-method precision

This chapter discusses the value of the study from a precision/accuracy point of view along with minimum quality criteria established in scientific guidelines when conducting interlaboratory studies.

In order to verify that an analytical technique/method provides accurate enough results, the method needs to be tested with respect to its precision. Determination of precision, of course, would be easily accomplished if one knows the true value of the parameter the method aims to measure, such as the $\sum PFAS_{11}$-concentration of a certain foam matrix. The precision would then be measured as the standard deviation of x number of replicate measurements on a prepared sample where the $\sum PFAS_{11}$-concentration was known.

One would distinguish between repeatability precision (*the variability between results obtained within a single laboratory in the shortest practical period of time by a single operator at the lab on a certain set of apparatus*) and reproducibility precision (*the variability between test results obtained in different laboratories, each of which has applied the test method to test the test material, taken at random*). A sample with a known $\sum PFAS_{11}$-concentration would optimally be a certified reference material.

Certified Reference Materials (CRMs) are 'controls' or standards used to check the quality and metrological traceability of products, to validate analytical measurement methods, or for the calibration of instruments. A certified reference material is a particular form of measurement standard. Reference materials are particularly important for analytical chemistry and clinical analysis. Since most analytical instrumentation is comparative, it requires a sample of known composition (reference material) for accurate calibration. These reference materials are thus, produced under stringent manufacturing procedures and differ from laboratory reagents in their certification and the traceability of the data provided.

Unfortunately, certified reference materials of F3 concentrate (or any other fire-fighting foam matrix) are not available (and not likely to be produced in the future, either), thus, the only way to try to quantify the precision of the TOP-method would be to execute a Round [redacted] testing approach where several laboratories conduct the TOP-analysis of a set of well-defined samples (an interlaboratory study, ILS).

This has been tried in this study with regard to F3 concentrate and the variable $\sum PFAS_{11}$ but how does it compare with the scientific guideline of an interlaboratory comparison, as described in the ASTM E691-99 "*Standard practice for Conducting a Interlaboratory study to determine Precision of a Test Method*"? This question will be answered in the Chapter 9.1- 9.3 below.

9.1 The number or participants

Besides the fact that an interlaboratory study (ILS) of precision should include defined key roles such as a "coordinator", a "statistician" and a "laboratory supervisor", the ASTM guideline explicitly states that the number of participating laboratories should be between "8 – 30" ("*Under no circumstances should the final statement of precision of the test method be based on acceptable test results for each test material from fewer than 6 laboratories. This would require that the ILS begin with 8 or more laboratories in order for allow for attrition*").

The validation study executed herein used three (3) laboratories, partly from a budgetary consideration and partly because not many more commercial laboratories qualified to participate could be identified in Sweden when the study was planned for (autumn 2019).

The ASTM guideline defines "qualified" as a "*proper laboratory facilities and testing equipment, competent operators, familiarity with the test method, a reputation for reliable testing work and sufficient time and interest to do a good job*".

The three participating laboratories in this study was considered "qualified" in all aspects of the word.

9.2 The test material

The ASTM guideline emphasizes that "*An ILS of a test method should include at least three (3) materials representing different test levels, and for the development of broadly applicable precision statements, six (6) or more materials should be included in the ILS*".

Originally the interlaboratory study involved six (6) different F3 concentrate and while one F3 (Foam 6) was excluded from the ILS since it already in the TOP-optimization step proved to be PFAS-free regardless of TOP-oxidation settings, yet an additional AFFF concentrate (Foam 7) was added.

Furthermore, the ASTM guideline offers the possibility to divide a pre-set ILS of a too broad variety of test material (when different classes of the test material exhibit different precision statements), into several smaller ILS's.

Finally, the ASTM guideline underlines the importance of a test material being "*as homogenous as possible prior to its division in to test material samples*".

The test materials (the F3 concentrates) of the ILS were all liquids and thus homogenous as such. Each participating laboratory were distributed foam concentrate samples that prior to their distribution had been stored cold and dark.

9.3 Number of replicate determinations

The ASTM guideline concludes that "*it is generally sound to limit the number of test results on each material in each laboratory to a small number such as three (3) or four (4)*" and that "*the time and effort invested in an ILS is better spent examining more materials across an increased number of participating laboratories, than recording a large number of test results per material within a few laboratories*".

The number of replicate TOP-oxidations and $\sum PFAS_{11}$-determinations in the ILS were four (4).

9.4 Calculations of repeatability and reproducibility precision

Based on the individually reported results for each laboratory, for each F3 concentrate (from 4 replicate determinations), a set of statistical parameters where calculated.

$$\bar{x} = \sum_{1}^{n} x/n$$

where the average x is basically the average $\sum PFAS_{11}$-concentrations for laboratory j for foam i (from 4 replicates, n = 4).

$$s = \sqrt{\sum_{1}^{n} (x - \bar{x})^2/(n-1)}$$

where s is the standard deviation of the average x (above),

$$\bar{\bar{x}} = \sum_{1}^{p} \bar{x}/p$$

is the grand average of all determinations of the ∑PFAS$_{11}$-concentration on foam I from all three (3) participating laboratories (p = 3),

$$d = \bar{x} - \bar{\bar{x}}$$

where d is the deviation from the average x (one laboratory, 4 replicates) from the grand average (3 laboratories, 4 replicates) on foam i,

$$s_{\bar{x}} = \sqrt{\sum_{1}^{p} d^2/(p-1)}$$

where s_x is standard deviation of average x,

$$s_r = \sqrt{\sum_{1}^{p} s^2/p}$$

where s_r is the repeatability standard deviation,

$$(s_R)^* = \sqrt{(s_{\bar{x}})^2 + (s_r)^2 (n-1)/n}$$

where $(s_R)^*$ is the reproducibility standard deviation,

$$h = d/s_{\bar{x}}$$

where h is the Mandel's between-laboratory consistency h-statistic and

$$k = s/s_r$$

where k is the Mandel's within-laboratory consistency k-statistic.

From the calculations both the repeatability precision s_r and the reproducibility precision (s_R)*, along with the calculated repeatability and reproducibility, was tabulated in Table 16 below;

Table 16. Compilation of repeatability and reproducibility precision as well as calculation of TOP-method repeatability and reproducibility for Foam 2, 3 and 5.

Foam #	s_r (µg/l)	(s_R)* (µg/l)	r (µg/l)	R (µg/l)
2	546,18	959,40	1 513,9	2 659,3
3	42,42	83,92	117,6	232,6
5	27,01	59,93	74,9	166,1

Expressing precision in terms of confidence limits at a certain probability level allows for a straightforward interpretation; the difference between results obtained in the same laboratory is significant if it is greater than **r**. Reproducibility (**R**) applies to differences between laboratories. The calculation methods (for **r** and **R** respectively) use the critical difference between two results at the 95% confidence level. They are sometimes referred to as the ‘‘difference two-sigma limit’’ (d2s). The limits are calculated by multiplying the standard deviation with the factor 1:96*(2)^0,5 (thus, ~ 2,8).

This means that **if the difference** between two samples of, for instance Foam 2, subjected to TOP-oxidation and $\sum PFAS_{11}$-determination in the same laboratory, **exceeds 1 513,9 µg/l**, it might be suspected either that the TOP-oxidation and subsequent $\sum PFAS_{11}$-determination was erroneous in some sense or that the two samples of Foam 2 were from different populations/batches.

The Mandel’s consistency statistics has been used to evaluate ***h*** and ***k*** respectively and check for outliers in the data (see figure 9 and 10 below).

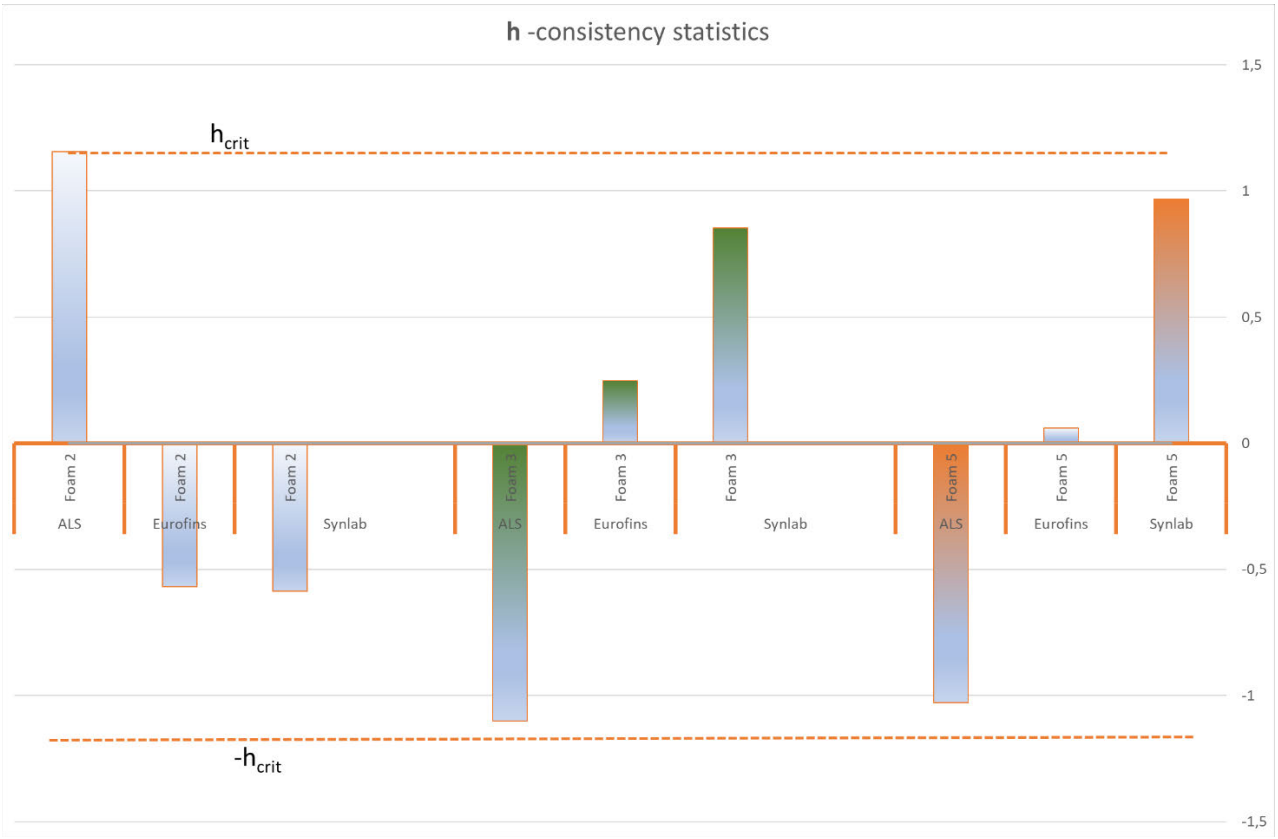


Figure 9. The between-laboratory consistency has been calculated as Mandel's consistency h-statistics. It provides means to assess the overall character of variability of the TOP-method as well as singling out particular combinations of laboratory and foam that might be outliers. The h_{crit}- and -h_{crit}-line represents 0,5 % significance level of an average of four replicates being an outlier (i.e. if the dotted line is crossed, there is a 50 % probability that that average is an outlier). However, since all three participating laboratories have h-values which are both positive as well as negative, none of the laboratories seem yield biased average determinations of $\sum PFAS_{11}$ in F3 concentrates.

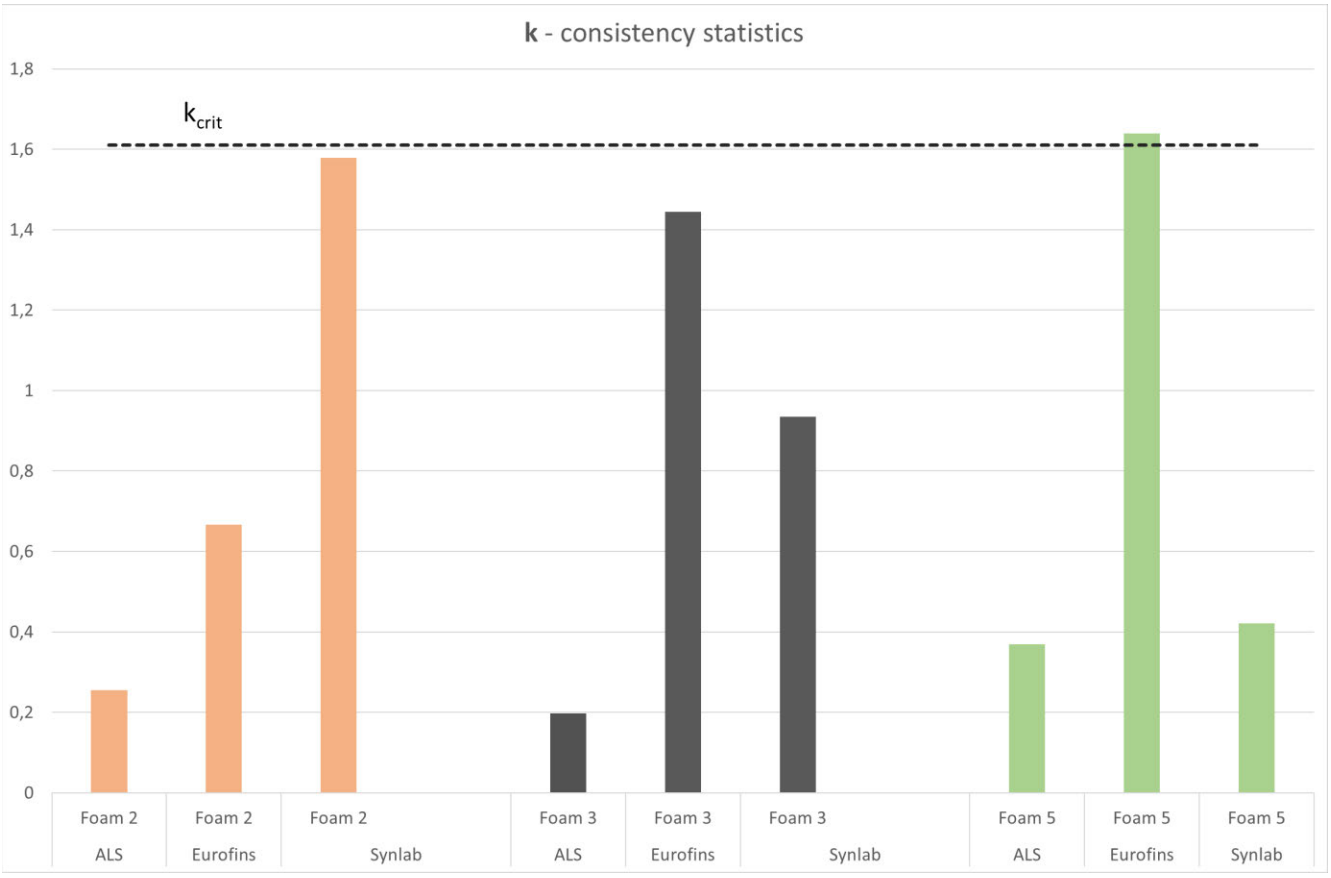


Figure 10. The within-laboratory consistency has been calculated as Mandel's consistency k-statistics. It provides means to assess variability between replicates or as in this case, between averages. At the significance level of 0,5, none of the averages reported seem to be an outlier. Please notice the consistent (for all F3 concentrates) very low variability of ALS in this plot as compared to the other participating laboratories.

10. Discussion

As described already in the introductory chapter of this report, there is a rapidly increasing interest, primarily from authorities that conduct public procurements to be able to verify statements such as "PFAS-free" for articles and chemicals (such as fire-fighting foams) when evaluating tenders.

It is evident from the data presented herein that evaluating the performance and precision of the TOP-assay with respect to F3 concentrates is a complex task. Another validation of the method and some of the performance numbers of the TOP-assay using several participating laboratories and several F3-concentrates, will likely strengthen the acceptance for using the method in the analysis of alleged PFAS-free foam concentrates.

The legislative/regulatory pressure on PFAS-free foam concentrates, described in chapter 4.2 are likely to be accompanied with further EU-regulations in the upcoming years. Consequently, foam formulators will, in the near future, need to adopt to procurement requirements, while submitting their foams for verification analysis. Thus, a common ground shared between all stakeholders of the "PFAS-free foam sector", in interpreting the analysis results and associated uncertainties, either from conventional PFAS-analysis, from the TOP-assay, or from TOF-analysis needs to be implemented, otherwise procurement requirements with regard to "PFAS-free" will result in a mush of legal uncertainties.

As far as the project knows, no attempts of validating the TOP-assay with regard to F3 concentrates have been made in the ILS-type of way before, and as the work in this study has progressed a lot of valuable knowledge/lessons learned in the pursuit of this type of study has been gained;

- With regard to the optimization of the TOP-method it would have been sensible to expand the experimental domain further in order to more clearly establish optimal conditions with regard to the $\sum PFAS_{11}$-yield. In this study a "saddle point" in the $\sum PFAS_{11}$-yield could be observed but this may be an artefact of the rather narrow experimental domain evaluated, or at least a larger domain would have enabled the determination of the response surface with some more clarity.

- Another complicating factor with regard to the methodology is the fact that within the study, optimal TOP-conditions were established with respect to an AFFF concentrate, and these settings were then used in the validation step of F3 concentrates. The rational for this was that the AFFF concentrate (Sthamex AFFF 3% F-15) contained 265 g/l of organic carbon and the F3 concentrates contained significantly lower concentrations of organic carbon. Hence, if the dosing of oxidants, the pH of the oxidant cocktail, the temperature and the oxidation time employed, was enough to oxidize the AFFF concentrate and its higher concentration of organic carbon, it would probably be enough to oxidize the F3 concentrates. However, upon executing the TOP-oxidations in the validation part of the study, the AFFF concentrate was diluted a factor of 100 00 - 1 000 000, while the F3 concentrates were only diluted a factor of 1 000 – 10 000. Thus, in theory, the dosing of oxidants in the diluted AFFF concentrate could be well enough to oxidize the AFFF-mixture containing ca 0,265 – 2,65 mg/l of organic carbon in solution, while the concentration in the F3-mixture in the TOP-step, on the average ca 94 mg/l of organic carbon, could prove not potent/concentrated enough. However, the disappearance of the added internal TOP conversion standard in the TOP-oxidations of the F3 concentrates suggest that the concentration of added oxidant (persulfate and sodium hydroxide) was high enough to account for a complete oxidation.

- Yet another apparent weakness the study is of course that each participating laboratory were free to utilize their own in-house analytical method with regard to PFAS and were not forced to use a certain, pre-defined analytical protocol such as EPA method 537.1 or the ISO/DIS 21675-method. However, all methods of the participating commercial laboratories are based on these methods and are validated in-house. The variance between-laboratory variances were indeed higher than the within-laboratory variance but that will almost always be the case in these type of ILS-validation studies, and the data indicate that the between-laboratory variance reported herein is to a large part accounted for in the TOP-oxidation itself, rather than the LC-MS/MS method used to quantify the outcome of the TOP-step.

As for the proposed 'statistical discount' of 419 µg/l (from the one-sided confidence interval of the Student's t-distribution) and the 1 552 µg/l (from the one-sided confidence interval of the Studentized range distribution) both stem from the assumption that the three commercial laboratories participating in the ILS represent the population of available laboratories that can execute the TOP-oxidation method with respect to fire-fighting foam concentrates, and the five F3 concentrates evaluated represent the population of commercially available F3.

The consequence of applying the 'statistical discount' would result in approving Foam 1 and 3-5 as "PFAS-free", with a 5 % probability of being wrong, while rejecting Foam 2 from being "PFAS-free", also with a 5 % probability of being wrong.

As tempting as it would be to propose the 419 µg/l 'statistical discount' rather than a 1 552 µg/l-discount (all based on the ANOVA -calculation on Foam 2), the calculation of the TOP-method repeatability and reproducibility with regard to Foam 2 of 1 514 and 2 659 µg/l, strongly suggest that the 1 552 µg/l-'statistical discount' should be considered (*i.e.*, it is in the same order of magnitude as the precision of the TOP-method for the critical Foam 2).

To confirm the concept of a 'statistical discount' of 1 552 µg/l in evaluating TOP-oxidation results from F3 concentrates, a repeated study including at least six (6) participating laboratories would be

desirable and the repeated study should aim at including > 10 different F3 concentrates[23]. If the results of the repeated study in terms of the variances (between-laboratory and within-laboratory) are in the same order of magnitude as in this study, the foam industry, procurement organisations/authorities, regulatory authorities and the international commercial laboratories would benefit from a joint agreement on the concept of "PFAS-free" foams as well as a standardization of the execution of the TOP-assay for F3 concentrates.

However, with the assumptions above in mind (the representativeness of the participating laboratories and the representativeness of the five F3 concentrates), the study shows that it would in fact be possible to utilize optimized TOP-assay and the statistical discount of 1 552 µg/l as a direct verification tool for the procurement of "PFAS-free" foams. The methodology would then distinguish a PFAS-free foam from a non-PFAS-free foam with a 5 % probability of being wrong (making a statistical type I error). If the significance level is not deemed good/safe enough, the methodology could possibly be improved as to provide the possibility to distinguish the non-PFAS-free foam with only a 1 % probability of being wrong, by expanding the number of laboratories, replicates and F3 foams included.

The exact level of significance (of the confidence limit) required in a court of administrative law to substantiate a claim such as not being "PFAS-free" is out of the scope of this study and strongly depend on the system in a specific country and that country's precedents. However, the assumption from the project is that a 95 % statistical confidence limit should be acceptable to verify if a foam can be considered "PFAS-free" in public procurement.

In a procurement situation, the procuring body can establish any requirements that tenderers need to adhere to as long as the requirements meet the fundamental principles for public procurement in the EU. Thus, in such a situation requiring the verification of "PFAS-freeness" using the optimized TOP-assay in combination with the statistical discount when evaluating the results, would be perfectly acceptable.

It should be noted that a foam being singled out as not being "PFAS-free" (Foam 2 in this study) by the proposed methodology (the optimized TOP-assay in combination with the 'statistical discount'), is by no means indicative that the foam producer has purposely added PFAS to an allegedly PFAS-free F3-concentrate. A foam concentrate having a $\sum PFAS_{11}$-concentration of 1 553 µg/l (and hence being singled out as not being "PFAS-free"), are not likely to benefit from the film-forming properties of PFAS since the concentration of fluoro tensides would be too low. Rather, it could be indicative that the producer may have produced the foam at a PFAS-contaminated production site, presumably from previous/historic production cycles. Remember that an AFFF-foam after TOP-oxidation seem to have a $\sum PFAS_{11}$-concentration of 1,1 – 3,8 g/l (see Foam 7, Table 9).

What are then the inherent drawbacks of the TOP-methodology with respect to fire-fighting foams?

The method can, as stated previously, be considered a "black box"; classical PFAS-homologues may appear after the oxidation step but the method does not provide any information on the chemical identity of the precursors.

Another draw-back is that highly fluorinated precursors (not quantifiable by a commercially available $PFAS_{25}$ or $PFAS_{35}$-method) may degrade to other fluorine species upon oxidation, for instance they may be mineralized rather than being transformed to classical PFAS. Very volatile precursors may for instance be lost in the TOP-assay due to vaporization. In this particular study though, vaporization of ultra-short chain PFAS in the TOP-step do not seem to account for the miss-matching fluorine budget, since no ultra-short chain PFAS was detected in the analysis of non-oxidized concentrate samples either.

[23] As recommended in precision validation by ASTM E691-99 "*Standard practice for Conducting a Interlaboratory study to determine Precision of a Test Method*"

From a method development point of view, the TOP-data as such, would benefit from being complemented by TOF-data. The TOF-results indicate that for the foam concentrates participating in this study only a fraction of the total organic fluorine (from TOF) is represented within the TOP-results, it would obviously cast a shadow of implementing the optimized TOP-assay for F3 concentrates since that could indicate that a vast fraction of organo-fluorine substances in the F3 concentrates are in fact not transformed to classical PFAS in the TOP-oxidation.

One could easily argue that ultra-short PFAS, presumably not transformed in the TOP-oxidation, might be lost upon handling of the TOP-samples post-oxidation, i.e., ultra-short PFAS with a high vapour pressure would probably concentrate as vapour in the head space between the liquid surface and the lid in the test tubes. If no special attention is given to this fact upon transferring a subsample for LC-MS/MS analysis after TOP, these homologues could easily be vented out when opening the lid. The fact that the study indicated that neither the F3-concentrates nor the AFFF-concentrate contained any ultra-short PFAS, neither in the diluted concentrates as such nor in the TOP-oxidized samples, is somewhat comforting.

Pursuant to the attempt to establish a total fluorine budget for the foam concentrates by also employing CIC/EOF-analysis (a variant of the TOF-analysis) and then compare results from CIC/EOF and reported $\sum PFAS_{11}$ from the TOP-assay reveals some peculiarities and possibly suggest that the CIC/EOF-analysis of foam concentrates would probably benefit from a validation procedure similar to the one conducted for the TOP-assay in this report.

However, for the AFFF-concentrate (denoted ‘Foam 7’) comparing the CIC/EOF-analysis, at the best giving a total fluorine concentration of 3 740 mg F/l, which corresponds to a concentration of 6:2 FTS equivalents of 6479,9 mg/l, with the reported average $\sum PFAS_{11}$-concentration after TOP (3 laboratories, 4 replicates) of 3 007,8 mg/l, the TOP-result only account for 46,4 % of the PFAS reported in the CIC/EOF.

As for Foam 2, the corresponding value of reported $\sum PFAS_{11}$-concentration after TOP /Concentration of 6.2 Equivalents after CIC/EOF is 25,4 %, and with regard to ‘Foam 5’ best match corresponds to 23,2 %.

The CIC/EOF-data presented herein must be regarded with some caution since the aim of the study was not to systematically investigate that method and anomalies (?) observed such as the huge difference in results executing the CIC/EOF-analysis of TOP-oxidized Foam 5 compared to non-oxidized Foam 5-concentrate. This may very well be attributed to artifacts/problems with dilutions and sample handling.

Finally, it can be concluded that it is of outmost importance that proposed (legal) limit values of PFAS are also accompanied with explicitly stated method(s) of verification, when implementing regulations regarding these types of chemicals (foam concentrates). As outlined in the initial chapter of this report, both EU, the US Department of Defense (US DOD), as well as voluntary industrial certification standards (Green Screen) have or are in the process of setting cut-off values for “PFAS-free” in fire-fighting foams. These cut-off values vary tremendously from 1 ppb (US DOD) to 1 ppm (Green Screen), while only Green Screen provides a method of verification.

It is in the best interest of the legislator that all actors on the market verify their corresponding foams using the same method, be that TOP, CIC/EOF or a combination thereof. If no method(s) of verification accompanies new regulations on PFAS in fire-fighting foam, it is conceivable that some actors on the foam market may utilize a conventional HPLC-MS/MS method albeit the fact that the method may not include the PFAS-homologues/fluoro-surfactants present in the foam, and thus provide a false negative result.

11. Conclusions

It can be concluded that the TOP-oxidation assay is suitable to verify claims regarding “PFAS-free” firefighting foams, which was the aim of the study to investigate. The methodology of TOP benefits from the high general accuracy, sensitivity and selectivity provided by the LC-MS/MS instrumentation while the oxidation step itself does not require any expensive laboratory utensils or chemicals.

To accommodate for variances between different laboratories conducting the TOP-assay, a one-sided confidence limit of 1 552 µg/l for the reported results (a ’statistical discount’) could be used.

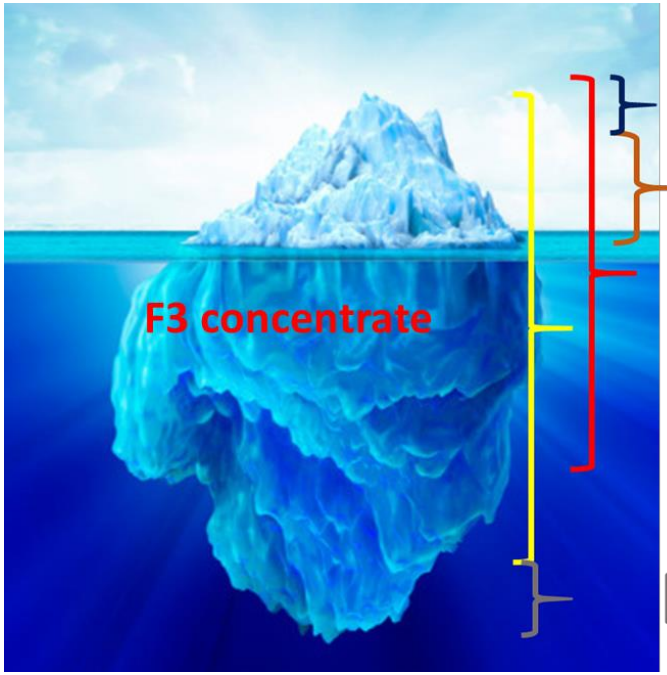
Generally, in F3-concentrates, subjecting the concentrate to a TOP-oxidation step magnifies the $\sum PFAS_{11}$-concentrations by a factor of 28-112 times. Thus, using a conventional HPLC-MS/MS method optimized for 50-55 different PFAS-homologues will probably not detect the vast plethora of possible PFAS-homologues that might be present in foams.

Hence, the TOP-assay is very much needed as a verification tool for ”PFAS-free” fire-fighting foams.

The major drawback of the TOP-method is its inherent ”black box”-behavior, *i.e.* it is impossible to identify the PFCA precursors that have been transformed to known PFAS-homologues. Furthermore, there might be PFCA precursors that is not being transformed quantitatively in the TOP-assay or PFCA precursors that is even being mineralized/transformed into non-perfluorinated molecules in the TOP-assay.

In an attempt to estimate the completeness of the TOP-reaction, all TOP-assays were conducted using specific internal TOP-conversion standards. Besides that, a comparison between TOP-results and CIC/EOF-analysis data was also conducted. The attempt to establish a fluorine budget of the typical F3-concetrate using a combination of all analytical results (ultra-short PFAS, quantitative HPLC-MS/MS analysis before and after TOP as well as CIC/EOF-analysis) proved very difficult.

As can be seen from the ‘iceberg image’ (see Figure 11 below) the fluorine budget contains gaps and discrepancies rendering it difficult to answer the questions regarding the completeness of the TOP-oxidation in terms of converting PFCA precursors to known PFAS. This may actually suggest that the CIC/EOF-method could also benefit from a validation with regard to the F3-matrices, similar to the attempt to validate the TOP-assay presented herein.



Ultra-short chain PFAS

Known PFAS included in the 55-65 homologues that is included in the most versatile LC-MS/MS analysis method

PFCA precursors accessible for TOP-oxidation

Total contents of organic fluorine

Total contents of inorganic fluorides

< 400 µg/l to < 40 mg/l [A]

$\sum PFAS_{25}$ ~ 20 – 200 µg/l [B]

$\sum PFAS_{11}$ up to 7 270 µg/l [C] (6 F3-foams)

0,78 [D] -20 [E] mg F/l**(LOD~ 5-10 µg/l) which correpond. to 1,35 – 34,6 mg/l of 6.2 FTS-equivalents

µg/l-to-mg/l-levels?

Figure 11. The "PFAS-iceberg" of F3 concentrates, after executing the study. Please note that ultra-short chain PFAS does not seem to contribute to the overall fluorine contents in these foams, neither when analyzed prior to TOP, nor when analyzed post TOP. As for the PFCA-precursor contents it seems to range from "1 852 µg/" (the 'statistical discount') to 7 270 µg/l (see Foam 2).

[A] reflects data from the analysis of ultra-short chain PFAS (as concentrate and as TOP-oxidized samples) and the extraordinarily poor limit of detection reflect the high degree of dilution of the TOP-oxidized samples.

[B] reflects the ∑PFAS11-concentration of Foam 2 before TOP-oxidation.

[C] reflects the reported ∑PFAS11-concentration of Foam 2 adjusted with the 'statistical discount'.

[D] reflects the measured concentration of extractable organic fluorine from then CIC/EOF-analysis of Foam 5 while [E] reflects the total organic fluorine from the CIC-analysis of Foam 2. Both these concentrations have then been re-calculated in to 6:2 FTS-equivalents.

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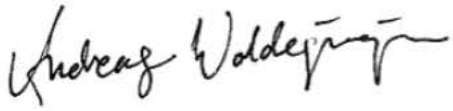
Annexes

See Annex 1 to this report.

Stockholm 2021-07-07



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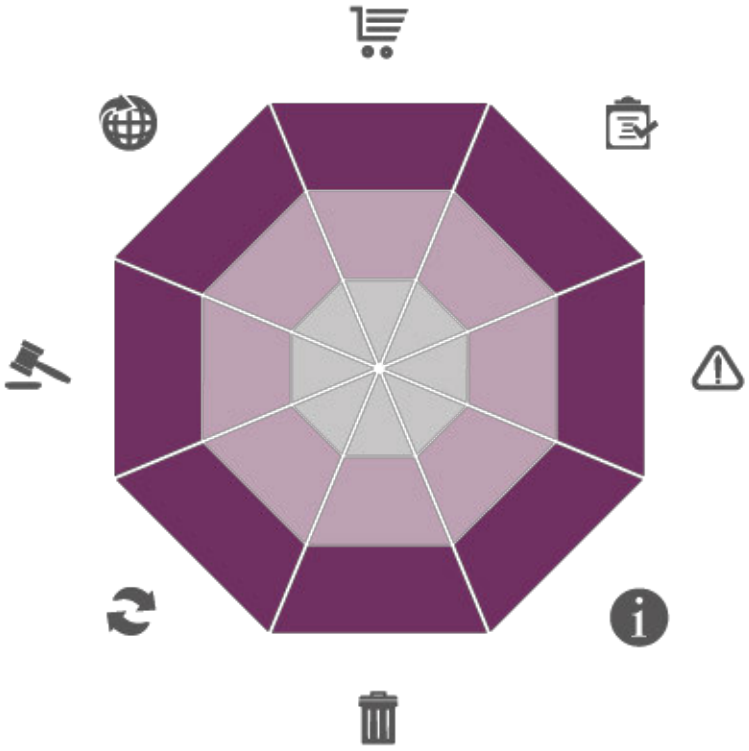

Appendix to report “Validation of the TOP-methodology for purpose of quantification of PreFAS in fluorine-free fire-fighting foams”

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Contents

Introduction

This appendix serves the purpose of illustrating how and why the TOP-method was optimized with respect to the yield of PFAS, when optimized for fire-fighting foam.

The methodology of the TOP-assay has previously been described by Houtz & Sedlak[1], but the method may not necessarily be optimized for matrices such as fluorine fire-fighting foams.

Theory

By evaluating one or several response variables with respect to the experimental settings of the parameters of the TOP-assay, when all the TOP-parameters were varied simultaneously, in a controlled manner, it is possible to statistically evaluate the experimental domain of the TOP-assay and how the different parameters of the TOP-assay influence the response variables. An alternative to this experimental design would off course be to vary one parameter at the time and then evaluate the influence of the response variables (OFAT-experiments, 'one-actor-at-the-time'). However, it can be shown (see Montgomery[2] and Ohlert[3] or other textbooks on experimental planning) that in majority of cases it is faster and more rational (less expensive) to utilize the factorial design study rather than the OFAT-approach in localizing an optimum.

Thus, this type of investigation/optimization is typically executed using a factorial design. In theory, varying four (4) parameters at two levels (low and high) would require 16 different experiments, a 2^4-experiment (simultaneously varying 4 different parameters at 2 levels). Furthermore, if every experiment also should be replicated in order to assess the average response variable as well as the standard deviation, the number of experiments rapidly grows unfortunately.

The advantage of executing a full 2^4-study is that not only the influence of the four main parameters on the response variables can be assessed but also how interaction parameters influence the response variables.

Instead of a full factorial design study, a reduced factorial design can be used. Reducing the factorial design, limits the number of experiments that need to be conducted. However, it will no longer be possible to quantity the main parameters independently of all interaction parameters.

To reduce the number of experiments to be conducted to fit the project budget, a 2^{4-1}-design was suggested. In this design basically 8 experiments are necessary. In order to still be able to quantify the standard deviation of the response variables in the experimental domain investigated, the 2^{4-1} experimental design was complemented by adding four (4) replicate measurements in a central point of the parameter settings (between 'low' and 'high'). The experimental design is depicted in table 1 below.

The four (4) TOP-parameters

The four parameters identified as important to assess in optimizing the yield with respect to PFAS in the TOP-assay were;

1) the concentration of potassium persulfate ($K_2S_2O_{8)}$ in the TOP-cocktail,
2) the concentration of sodium hydroxide in the TOP-cocktail,
3) the temperature of the TOP-assay
4) the oxidation time of the TOP-assay

[1] Houtz E F & Sedlak D L (2012), "Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff", Environ. Sci. Technol. Vol. 46(17), pp 9342–9349

[2] Montgomery, Douglas C. (2013). "Design and Analysis of Experiments" (8th ed.). Hoboken, New Jersey: Wiley. ISBN 978-1119320937.

[3] Oehlert, Gary (2000). A First Course in Design and Analysis of Experiments (Revised ed.). New York City: W. H. Freeman and Company. ISBN 978-0716735106.

One can easily jump to the conclusion that as high concentration of persulfate, as high concentration of sodium hydroxide, as high temperature as practically possible and as long oxidation time as practically possible, would be beneficial for the PFAS-yield. However, to automatically assume that 'the more, the merrier' also applies to the TOP-assay and the response variable of PFAS-yield could prove wrong, thus optimize the TOP-settings for the matrix of fire-fighting foam seemed necessary.

Table 1. The experimental domain of the TOP-assay evaluated in the factorial design experiment.

Experiment	$[K_2S_2O_8]$ (mM)	[NaOH] (mM)	Temperature, °C	Time, hr
1	Low (-)	Low (-)	Low (-)	Low (-)
2	High (+)	Low (-)	Low (-)	Low (-)
3	Low (-)	High (+)	Low (-)	Low (-)
4	High (+)	High (+)	High (+)	Low (-)
5	Low (-)	Low (-)	High (+)	High (+)
6	High (+)	Low (-)	High (+)	High (+)
7	Low (-)	High (+)	Low (-)	High (+)
8	High (+)	High (+)	High (+)	High (+)
9	Centrum (0)	Centrum (0)	Centrum (0)	Centrum (0)
10	Centrum (0)	Centrum (0)	Centrum (0)	Centrum (0)
11	Centrum (0)	Centrum (0)	Centrum (0)	Centrum (0)
12	Centrum (0)	Centrum (0)	Centrum (0)	Centrum (0)

Houtz & Sedlak advised a TOP-assay of consisting of 120 mM $K_2S_2O_8$, 250 mM of NaOH, 85 °C as oxidation temperature and an 8-hour oxidation time. But these settings may not provide optimal yield of PFAS in matrices such as fire-fighting foam, sediment, water and sewage sludge.

In this design, the Houtz & Sedlak original settings was utilized for the 'centrum point', while 'Low' is represented by 60 mM $K_2S_2O_8$, 125 mM NaOH, 80 °C and 6 hour, while 'High' is represented by 180 mM $K_2S_2O_8$, 375 mM NaOH, 90 °C and 10 hour oxidation time.

Optimized for F3-concentrate or any fire-fighting foam?

In a first attempt, three F3- concentrate was submitted to ALS Global in Prague, Czeck Republic (foam 1, foam 4 and foam 6). These three foams were arbitrarily chosen without any preconceived knowledge on their TOP-behaviour respectively.

However, after a full experimental study (12 TOP-oxidations executed in accordance with table 1), on foam 6 and a couple of TOP-oxidations on foam 4, no PFAS was detected neither prior to the TOP-step, nor afterwards.

Thus, the project needed to improvise, and it was chosen to submit a AFFF-foam to ALS Global for completion of the 12 TOP-experiments depicted in table 1. The argument was that the difference in TOP-behaviour between an AFFF-concentrate and a F3-concentrate would be less significant as compared to the difference in PFAS-yield using the non-optimal TOP-settings.

It can of course be argued that this assumption is wrong, and that the chemical differences between AFFF- concentrate and F3- concentrate are such that a common TOP-optimum is not to be expected.

It should thus be noted that the optimization of the TOP-conditions has been executed with respect to an AFFF- concentrate rather than a F3- concentrate although the main aim of the project was to assess

different variance contributions of the TOP-assay on F3- concentrate and subsequently validate the TOP-methodology for the analysis of F3- concentrate answering the question of what is "PFAS-free" in terms of the typical F3-matrix.

Results and evaluation of data

The results of the 12 TOP-experiments on the AFFF- concentrate ('foam 7') are summarized in the figure below (see Table 1). As the primary response variable $\sum PFAS_{11}$ was chosen.

The concentration of $\sum PFAS_{11}$ (in g/l) reflects the aggregated concentrations after the TOP-assay of PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFBS, PFHxS, PFOS and 6:2 FTS.

Table 1. Results of the TOP-assays conducted, using the outlined parameter settings (see table 1).

Std	Run	Factor1 (A: Persulfate, mM)	Factor 2 (B: Hydroxide, mM)	Factor 3 (Temp, °C)	Factor 4 (D: Time, hr)	Response ($\sum PFAS_{11}$, mg/l)
7	1	60	125	90	10	4,08228
8	2	180	125	90	10	1,85415
9	3	180	375	90	10	4,93487
5	4	120	250	85	8	2,35753
10	5	120	250	85	8	2,38169
11	6	120	250	85	8	2,47067
12	7	120	250	85	8	2,4112
6	8	60	375	80	10	3,27472
1	9	60	125	80	6	3,76797
4	10	180	375	90	6	3,9444
2	11	180	125	80	6	1,58465
3	12	60	375	80	6	2,59058

It was assumed that the response variable $\sum PFAS_{11}$ (in g/l) could mathematically be described as the polynomial.

$\sum PFAS_{11}$ (R1)=

$$\text{intercept} + k_1*[\text{Persulfate}] + k_2*[\text{hydroxide}]+k_3*[\text{temperature}]+k_4[\text{time}]+ k_5*[\text{Persulfate}]*[\text{hydroxide}]+ k_6*[\text{Persulfate}]*[\text{temperature}]+ k_7*[\text{persulfate}]*[\text{time}]+k_8[\text{hydroxide}]*[\text{temperature}]+k_9*[\text{hydroxide}]*[\text{time}]+k_{10}*[\text{temperature}]*[\text{time}]+$$

$$k_{11}*[\text{Persulfate}]^2+k_{12}*[\text{hydroxide}]^2+ k_{13}*[\text{temperature}]^2+k_{14}*[\text{time}]^2 + k_{15}*[\text{Persulfate}]*[\text{hydroxide}]*[\text{temperatur}]e + k_{16}*[\text{Persulfate}]*[\text{hydroxide}]*[\text{time}]+k_{17}*[\text{Persulfate}]*[\text{temperature}]*[\text{time}]+k_{18}*[\text{hydroxide}]*[\text{temperature}]*[\text{time}]+k_{19}*[\text{Persulfate}]^2*[\text{hydroxide}]+k_{20}*[\text{Persulfate}]^2*[\text{temperature}]+ k_{21}*[\text{Persulfate}]^2*[\text{time}]+k_{22}*[\text{Persulfate}]*[\text{hydroxide}]^2+k^{23}*[\text{Persulfate}]*[\text{temperature}]^2+k_{24}*[\text{Persulfate}]*[\text{time}]^2+k_{25}*[\text{hydroxide}]^2*[\text{temperature}]+k_{26}*[\text{hydroxide}]^2*[\text{time}]+k_{27}*[\text{hydroxide}]*[\text{temperature}]^2+k_{28}*[\text{hydroxide}]*[\text{time}]^2+k_{29}*[\text{time}]^2*[\text{temperature}]+k_{30}*[\text{time}]*[\text{temperature}]^2+k_{31}*[\text{Persulfate}]^3+k_{32}*[\text{hydroxide}]^3+k_{33}*[\text{time}]^3+k_{34}*[\text{temperature}]^3+k_{35}*[\text{Persulfate}]*[\text{hydroxide}]*[\text{time}]*[\text{temperatur}] + .. + E.$$

"E" represents the model error.

However, due to the number of TOP-experiments conducted using different settings of the 4 TOP-parameters is limited, the polynomial needs to be approximated to;

$\sum PFAS_{11}$ (R1) = intercept + k_1*[Persulfate] + k_2*[hydroxide]+k_3*[temperature]+k_4*[time]+ k_5*[Persulfate]*[hydroxide]+ k_6*[Persulfate]*[temperature]+ k_7*[persulfate]*[time]+ k_{10}*[time]*[temperature].

The best fit between the response variable and the results was achieved with the following selection of coefficients;

Intercept = 2,41

k_1 = -0,0383

k_2 = 0,4758

k_3 = -0,2727

k_4 = 0,4187

k_5 = 1,06

k_6 = 0,805

k_7 = -0,8162

k_{10} = 0,8928

The interpretation of the coefficients is that the concentration of persulfate will thus affect the $PFAS_{11}$-yield slightly negative (the $PFAS_{11}$-yield will actually decrease with increasing concentrations of persulfate), since the coefficient k_1 is a small number in comparison and < 0).

The contrary seems to be true with respect to the concentration of sodium hydroxide – increasing the sodium hydroxide concentration lends an increased $PFAS_{11}$-yield ($k_2 > 0$).

The temperature is negatively correlated with the $PFAS_{11}$-yield ($k_3 < 0$) and the oxidation time is positively correlated with the $PFAS_{11}$-yield ($k_4 > 0$).

As for the interaction parameters (parameters not possible to assess using the OFAT-methodology), the picture becomes complex. The interaction between the concentration of persulfate and the concentration of sodium hydroxide is positively correlated with increasing $PFAS_{11}$-yields ($k_5 > 0$) and so is the interaction parameter/factor between the concentration of persulfate and the temperature ($k_6 >0$), while the interaction factor between the concentration of persulfate and oxidation time ($k_7 <0$).

Finally, the last factor that is possible to assess, given the number of TOP-experiments conducted, the interaction between temperature and oxidation time, is positively correlated with the $PFAS_{11}$-yield ($k_{10} > 0$).

Of the above accounted coefficients (k_1-k_7 and k_{10}), all coefficients but k_1 (the concentration of persulfate) are significant at the 95 % confidence level. In Table 2 below, all confidence intervals for the coefficients have been listed.

Table 2. Coefficients in term of coded factors/parameters and their corresponding confidence intervals (double-sided at 95 % confidence level).

Factor	Coefficient estimate	Degrees of freedom (df)	Standard Error	95 % CI low	95 5 CI high	VIF
Intercept	2,41	1	0,0244	2,33	2,48	
A-Persulfate	-0,0383	1	0,0244	-0,1160	0,0393	2,0
B- hydroxide	0,4758	1	0,0244	0,3982	0,5535	2,0
C-Temperature	-0,2727	1	0,0345	-0,3825	-0,1629	4,0
D-Time	0,4187	1	0,0244	0,3410	0,4963	2,0
AB	1,06	1	0,0244	0,9869	1,14	2,0
AC	0,8050	1	0,0345	0,6952	0,9149	3,33
AD	-0,8162	1	0,0345	-0,9261	-0,7064	4,0
BC	Aliased					
BD	Aliased					
CD	0,8928	1	0,0345	0,7830	1,00	3,33

A coefficient estimate represents the expected change in response per unit change in factor/parameter, when all remaining factors are held constant. The intercept in the orthogonal design is the overall average response ($PFAS_{11}$-yield) of all experiments.

The coefficients are adjustments around that average based on the factor settings. When all factors/parameters are perfectly orthogonal, all the variation inflation factors[4] (VIFs) are = 1. The software used to statistically evaluate TOP-optimization experiments (Design Expert 12) recommended to discard any evaluation if VIFs exceeded 10.

When evaluating the optimization experiments with respect to yet another response variable, the formation of PFOA, the factor/parameter 'concentration of persulfate' was significant at the 95 % confidence level (data not shown here) and hence it was decided to keep the factor in the model also with respect to the response variable $\sum PFAS_{11}$.

[4] Rawlings, John O.; Pantula, Sastry G.; Dickey, [redacted] A. (1998). "Applied regression analysis : a research tool" (2 ed.). New York: Springer. pp. 372, 373. ISBN 0387227539.

In order to assess the variance contributions on the response variable $\sum PFAS_{11}$, an ANOVA analysis was conducted (see Table 3).

Table 3. Results of an ANOVA of the aliased model.

Source	Sum of Squares	Degress of freedom (df)	Mean Square	F-value	p-value	Significant?
model	11,36	8	1,42	596,06	0,0001	Significant!
A-Persulfate	0,0059	1	0,0059	2,47	0,2143	
B- hydroxide	0,9057	1	0,9057	380,14	0,0003	
C-Temperature	0,1487	1	0,1487	62,43	0,0042	
D-Time	0,7011	1	0,7011	294,26	0,0004	
AB	4,53	1	4,53	1902,58	<0,0001	
AC	1,3	1	1,30	544,04	0,0002	
AD	1,33	1	1,33	559,29	0,0002	
BC	0	0				
BD	0	0				
CD	1,59	1	1,59	669,16	0,0001	
Pure error	0,0071	3	0,0024			
Corr. tot	11,37	11				

The model F-value from the ANOVA analysis of 596,06 implies that the model is in fact significant. There is a 0,01 % chance that an F-value this large could occur by chance

P-values < 0,05 indicate that the model terms are in fact significant. In this case factors B (concentration of sodium hydroxide), C (temperature), D (oxidation time), AB (interaction between concentration of persulfate and concentration of sodium hydroxide), AC (interaction between the concentration of persulfate and the temperature), AD (interaction between the concentration of persulfate and the oxidation time) as well as CD (the interaction between the temperature and the oxidation time).

The overall prediction model for ∑PFAS11-concentration as a function of TOP-settings

An overall coefficient of correlation for the model;

$\sum PFAS_{11}$ = 2,41- 0,0383*[Persulfate] + 0,4758*[sodium hydroxide]- 0,2727*[temperature] + 0,4187*[oxidation time] + 1,06*[Persulfate]*[sodium hydroxide] + 0,805*[persulfate]*[temperature] - 0,8162*[persulfate]*[oxidation time] + 0,8928*[temperature]*[oxidation time], was calculated (see figure 4 below).

Table 4. Overall model correlation coefficient of 0,994. '*Adeq precision*' measures the signal-to-noise ratio in the model. A ratio > 4 is recommended by the evaluation software and a ratio of 79,255 indicates an 'adequate signal'.

Standard deviation	0,0488	R^2	0,9994
Mean	2,97	**Adjusted R^2**	0,9977
C.V (%)	1,64	**Predicted R^2**	N/A
		Adeq. precision	79,2552

The corresponding figure/plot of the predicted concentration $\sum PFAS_{11}$ (in g/l) compared to the actually measured concentration from the TOP-experiments (see figure 1 below)

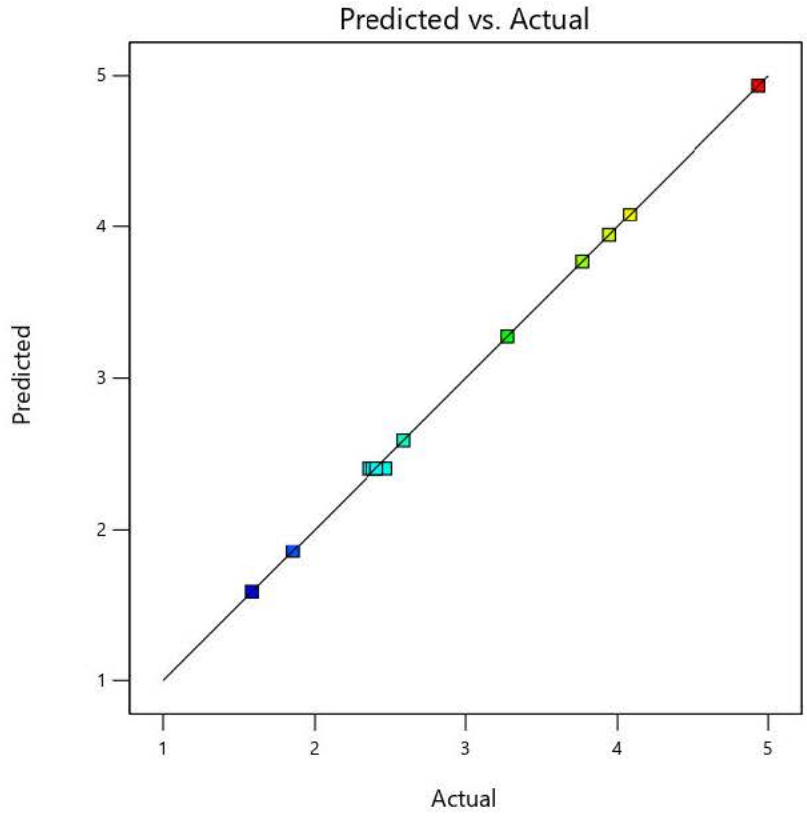

Figure 1. Predicted $\sum PFAS_{11}$-concentration (using varying experimental TOP-settings) vs. measured $\sum PFAS_{11}$-concentrations in the AFFF-concentrate.

A very versatile tool in order to get some clue as how the different parameters/factors of the experimental design, affects the response variable (the $\sum PFAS_{11}$-concentration) is to plot the response surface of the response variable as a function of two factor at the time. As can be seen in figure 2 and 3 below some intrinsic properties of the relation between the response variable and the investigated factors/parameters are revealed.

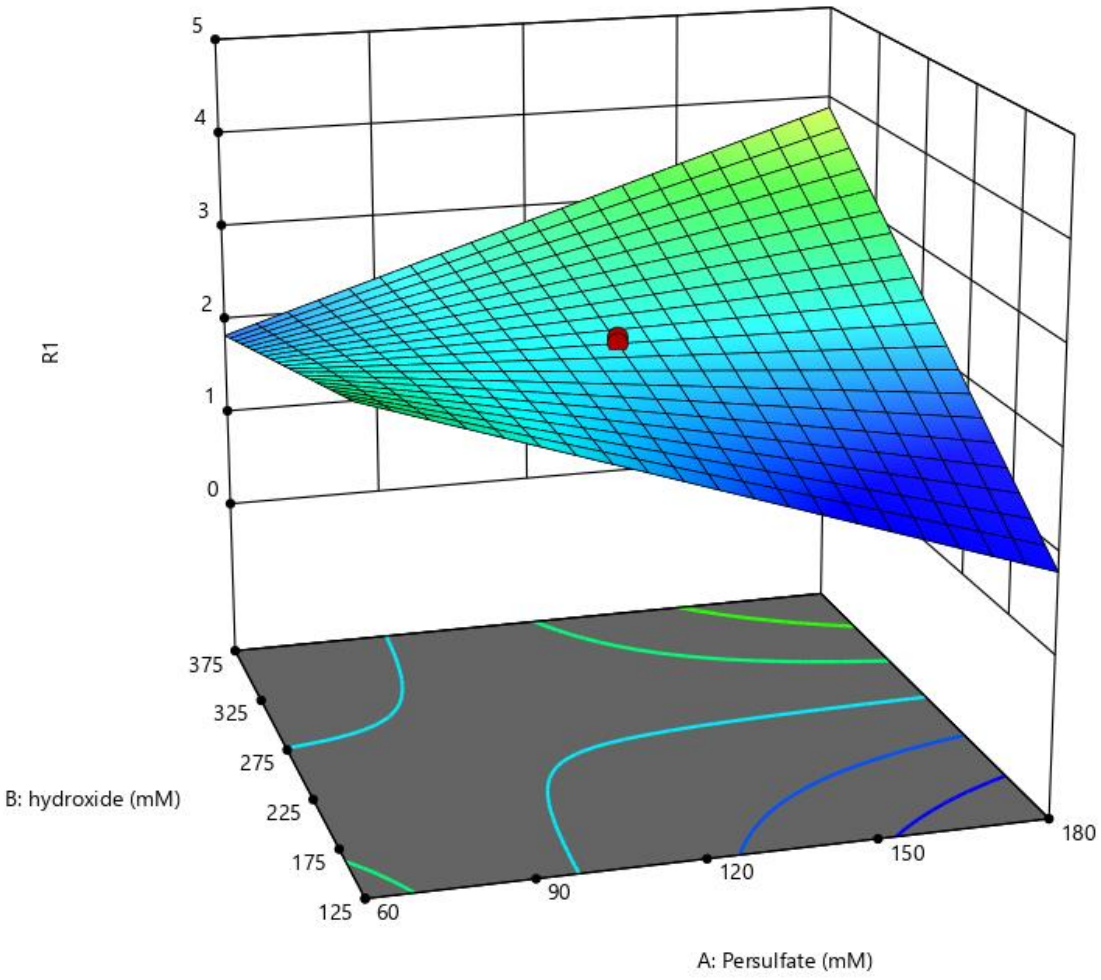

Figure 2. The response surface of the response variable $\sum PFAS_{11}$ as a function of the concentration of persulfate and the concentration of sodium hydroxide in the TOP-mixture, when the oxidation temperature is fixed at 85 ºC and the oxidation time is kept constant at 8 h. The fact that the response surface is skewed and not a plane indicate that interaction factors (such as [Persulfate]*[sodium hydroxide], [persulfate]*[temperature] or [temperature]*[oxidation time]) strongly influences the response function.

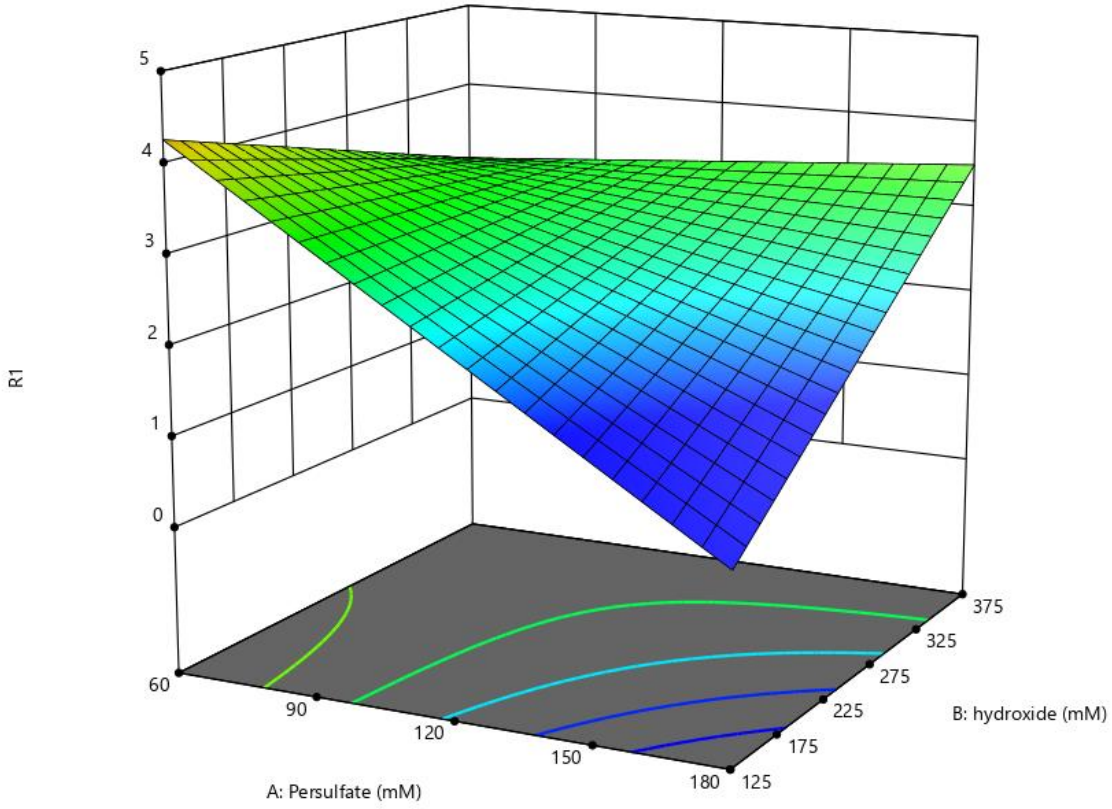

Figure 3. The response surface of the response variable R1 (the concentration of $\sum PFAS_{11}$) as a function of concentration of persulfate and the concentration of sodium hydroxide, at 85°C and 6 h of oxidation time. It seems as if maximum yield of $\sum PFAS_{11}$ is reached either at this temperature and oxidation time either when the TOP-assay is executed using a diluted persulfate (60 mM) and a diluted sodium hydroxide (125 mM), or a very concentrated sodium hydroxide (375 mM) in combination with a concentrated persulfate (180 mM).

By plotting a vast number of response surfaces (parameter combinations) and comparing them qualitatively, it became clear that the response (the $\sum PFAS_{11}$-concentration) has two local maximas, indicating that in this experimental domain, the interaction factors are perhaps more important than the main factors/parameters. Under these circumstances, an option would off course to have repeated the optimization experiments as a full 2^4-experiment which would have provided the freedom to assess all 2-factor interactions independently.

However, the project budget did not allow for such in-depth study and the response function (shown in its entirety on page 8 of this appendix) was used to calculate/predict the $\sum PFAS_{11}$-concentration in the two maximas referred to.

Calculating the $\sum PFAS_{11}$-concentration for all values of the persulfate concentration between 60-180 mM, all values of the sodium hydroxide concentration between 125-375 mM, all temperatures in the interval 80 – 90 °C and for all oxidation times in the time window of 6-10 hours, revealed that using the settings (low, low, low, high) corresponding to (persulfate concentration, sodium hydroxide concentration, the temperature and the oxidation time correspondingly), yielded a $\sum PFAS_{11}$-response of 4,45 g/l with a 95 %-double sided confidence interval of 4,183 – 4,721 g/l.

The corresponding calculation of the $\sum PFAS_{11}$-response at the TOP-settings (high, high, high high) revealed an even higher concentration of 4,935 g/l and a 95% confidence interval of 4,778 – 5,09 g/l.

However, when evaluating the factorial design experiment using another response variable, the formation of PFOA[5] in exactly the same way as described for the response variable $\sum PFAS_{11}$, the TOP-settings (low, low, low, high) provided a response of 10 505 (chromatographic area units) with a 95 % double-sided confidence interval of 10 168,8 – 10 841,2 area units, which was considerably higher than the PFOA-formation at the TOP-settings (high, high, high, high) which provided 4 014 area units (3 819,9– 4208,1).

Another point considered upon choosing the optimized TOP-parameters was that it seemed more stable and less error-prone not to use the extreme conditions of the settings (high, high, high, high) but to select the TOP-settings of (low, low, low, high).

Conclusions

The TOP-assay parameters of persulfate, sodium hydroxide, temperature and oxidation time was evaluated with respect to a AFFF-concentrate, despite the fact that the assay settings were then used to evaluate and validate the TOP-method with respect to F3-concentrates.

The optimization of the TOP-settings was conducted using a factorial design study, a 2^{4-1}-experiment with central composite design using four replicates in a centrum point.

The results showed that two different points (combination of parameter settings) provided maximal PFAS-yield and maximal PFOA-formation (data not shown), and for practical reasons mainly, it was chosen that the optimal setting of the TOP-assay would be (low, low, low, high) reflecting the following parameters;

Concentration of potassium persulfate;	60 mM
Concentration of sodium hydroxide;	125 mM
Temperature of oxidation;	80 °C
Oxidation time;	10 hr.

[5] PFOA was not detected in the AFFF-concentrate prior to the TOP-assay.