MASTER THESIS

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Engineering at the University of Applied Sciences Technikum Wien
Degree Program Environmental Management and Ecotoxicology

Oxidation of per- and polyfluoroalkyl substances and evaluation of evolved perfluoroalkyl acids

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FH University of Applied Sciences TECHNIKUM WIEN

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Der Fortschritt in Abwasseraufbereitungsanlagen ist in den letzten Jahrzehnten stetig gestiegen. Durch die Verbesserungen in der Analytik konnten Spurenstoffe, wie z.B. Arzneimittelrückstände und per- und polyfluorierte Alkylsubstanzen (PFAS) in Abläufen detektiert werden, und neue Herausforderungen haben sich dadurch ergeben, weil ein Großteil dieser Spurenstoffe nicht mit dem aktuellen Stand der Technik in Kläranlagen entfernt wird. Besonders der Einsatz von Ozon oder Aktivkohle als zusätzliche Reinigungsstufe erscheint vielversprechend, um Spurenstoffe effizient zu entfernen. Etwas komplexer und herausfordernder ist jedoch die Entfernung von PFAS. Erstens, weil die bekanntesten PFAS weder mit Ozon noch mit dem Einsatz von Aktivkohle ausreichend und effektiv entfernt werden können, und zweitens, weil es zu viele PFAS gibt, um sie simultan im Ablauf zu bestimmen. Der Fokus der präsentierten Studie lag deshalb in der Erforschung der effizienten Entfernung von 31 PFAS mit Ozon und in der Evaluierung des gesamt oxidierbaren Vorläufer (TOP) Assays mit Ozon als Oxidationsmittel zur Bestimmung des gesamten PFAS-Gehalts in einer Probe. Die Ergebnisse zeigten, dass Ozon allein nicht in der Lage war die untersuchten 31 PFAS zu entfernen, auch nicht beim Einsatz hoher Ozondosen (15 mg O_3/L). Der TOP Assay mit Ozon als Oxidationsmittel erzielte eine molare Übereinstimmung von 95.2% (bei hohen Ozondosen) und 99.1% (bei niedrigen Ozondosen (6 mg O_3/L)) zwischen gespikten unbehandelten und gespikten ozonierten Leitungswasserproben. Der TOP Assay wurde für eine Ablaufprobe angewendet und der geschätzte unbekannte PFAS-Gehalt entsprach 91%.

Schlagwörter: PFAS, Ozon, TOP-Assay, Wasseraufbereitung, Abwasser

Abstract

The advances in wastewater treatment techniques steadily increased during the last decades. With improved analytical instruments micropollutants such as pharmaceutical residues and per- and polyfluoroalkyl substances (PFAS) new challenges emerged since a lot of those compounds are not removed during the common state-of-the-art wastewater treatment processes. Especially ozonation and activated carbon are considered most promising as an additional wastewater treatment step to efficiently remove micropollutants. More complex and challenging though is the removal of PFAS. First, because the most commonly known PFAS are not efficiently enough removed by ozonation or activated carbon alone and secondly too many PFAS exist to identify them individually in the effluent. Therefore, the focus of the present study was to investigate the removal efficiencies of 31 PFAS with ozone and to evaluate the total oxidizable precursor (TOP) assay with ozone as oxidizing agent for the determination of the total PFAS content in a sample. The results did show that ozone alone is incapable to remove any of the 31 observed PFAS even at high ozone doses (15 mg O_3/L). The TOP assay with ozone as oxidizing agent obtained an accordance in molarity of 95.2% (high ozone dose) and 99.1% (low ozone dose (6 mg O_3/L)) between spiked untreated and spiked ozone treated tap water samples. The TOP assay was also applied for one effluent sample, based on the results the unknown PFAS content was estimated to be 91%.

Keywords: perfluoro, ozone, TOP assay, water treatment, wastewater

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Dipl. Ing. Andreas-Marius Kaiser

Table of Contents

1 Introduction

In the last decades myriads of anthropogenic substances were produced in tons and put on the market for a diversity of applications. Substances such as pesticides to protect crops and increase agricultural yields, substances to laminate and coat furniture, carpets and curtains for fire safety, coatings for weatherproof textiles, substances used as flame retardants and substances used as additives in a lot of materials to improve mechanical, technical, physical or/and chemical properties. One group of substances which covers all the above-mentioned applications is the group of the per- and polyfluoroalkyl substances (PFAS) (Kissa, 2001; Prevedouros et al., 2006). The production of PFAS started around the 1950s, and to date around 4730 PFAS are registered on the global market (OECD, 2018) but some of them do not even have a CAS number. Today, thousands of published papers describe their worldwide detection in various environmental media, wildlife animals and human populations, as well as their ecological and human health risks (Lau, 2015). The toxicity of PFAS has extensively been studied in animals (Lau et al., 2007). The potential adverse health effects that have been identified for PFAS are hepatotoxicity, developmental toxicity, immunotoxicity (ATSDR, 2018; EFSA, 2018), endocrine disrupting effects and carcinogenicity (Kennedy et al., 2004; Lau et al., 2007). This substance group which can be detected in almost all environmental medias, biota and human populations is therefore of major concern. Although some perfluoroalkyl acids (PFAAs) are already phased out of production or banned from the market in western countries such as perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfonic acid (PFOS), they are still detected in the environment and in humans due to their high stability (BMLFUW, 2017; Poothong et al., 2020). Unpleasantly, although PFAAs are banned in western countries the production and application of long-chain PFAAs shifted to China (Wang et al., 2014). Furthermore, the industry is developing and producing substitutes for phased-out PFAAs using shorter carbon-chain lengths or designs with an inserted oxygen atom in the fluorinated carbon chain (Fromme et al., 2017; Lim, 2019). The essential chemical structure of these substitutes is equal to the chemical structure of already known toxic, bioaccumulative, and persistent PFAS. It is therefore more than plausible and imaginable that these new substitutes won´t solve the problem but moreover they will add an additional complexity in this thematic field. Young and Mabury (2010) reported that precursor compounds can degrade to PFAAs through biotransformation by microorganisms and atmospheric oxidation. Therefore, PFAA-precursors and new per- and polyfluoroalkyl ethers might be transformed to PFAAs or other unknown perfluoro-transformation-products in wastewater treatment plants and the environment; and furthermore, the exposure to precursor chemicals can probably result in an accumulation of PFAAs within the body (Kudo, 2015). It is therefore of great interest to investigate the transformation processes that can be incited in wastewater treatment plants by ozonation. Moreover, the total oxidizable precursor (TOP) assay, a method first published by Houtz and Sedlak (2012) to estimate the total PFAS content in a sample via the determination of PFAAs before and after oxidation of the sample, might by applied as well

using ozone. The TOP assay with ozone might be an alternative to the generally used persulfate-thermolysis and could be easily applied in laboratories equipped with an ozone generator. In best of my knowledge this is the first study which used ozonation for the application of the TOP assay. Additionally, the ozonation of clean water spiked with PFAS might provide an enhanced understanding of transformation processes of PFAA-precursors to PFAAs. Furthermore, the environmental behavior of poly- and perfluoro ethers under oxidative conditions can be estimated which undoubtedly will contribute to the ever-growing knowledge of PFAS.

1.1 Historical background, manufacture and use of PFAS

The first processes to commercially produce PFAS were developed in the 1940s and since the 1950s various PFAS (including PFOA and PFOS) were manufactured in large scales for product applications (3M, 2020). In general, two principal manufacturing processes exist to produce perfluoro- and polyfluoroalkyl substances: the electrochemical fluorination (ECF) process and the telomerization process.

Electrochemical fluorination. ECF is a technology where all hydrogen atoms are replaced by fluorine atoms via electrolysis of organic raw material (e.g. octane sulfonyl fluoride ($C_8H_{17}SO_2F$)) in anhydrous hydrogen fluorine (Banks et al., 1994). This process leads to a carbon chain rearrangement and results in a mixture of linear and branched perfluorinated isomers with a varying ratio of linear and branched perfluorinated isomers (Banks et al., 1994). Approximately 70% linear and 30% branched isomers are the products of this manufacturing process (Benskin et al., 2010). Perfluorooctane sulfonyl fluoride (POSF, $C_8F_{17}SO_2F$), which is the main raw material to manufacture PFOS is the yield of the electrochemical fluorination of octane sulfonyl fluoride (Lehmler, 2005). Furthermore, a series of raw materials such as sulfonamides, sulfonamido alcohols and other POSF-derivatives are derived therefrom (Lehmler, 2005). Similarly, the manufacture of perfluorooctanoyl fluoride ($C_7F_{15}COF$), which is further reacted to produce PFOA, is made by the electrochemical fluorination of octanoyl fluoride ($C_7H_{15}CO_F$) (Kissa, 1994). Perfluorosulfonic acid (PFSA) and perfluoroalkane sulfonyl fluoride (PASF)-based product production[1] started in the 1960s commonly manufactured by the ECF process in parallel with perfluoro carboxylic acids (PFCAs) in the United States, Europe and Japan (Prevedouros et al., 2006). Perfluorononanoic acid (PFNA) for example was manufactured and used from 1975 onwards as a surfactant and for the production of polyvinylidene fluoride (Prevedouros et al., 2006). In the early 1970s began the manufacture of fluorotelomer-based products which are used in many of the same industrial and consumer products as PFAAs (Kissa, 2001). The ECF process was used worldwide for the major production (80-90%) of PFAAs

[1] Mostly in form of perfluorooctyl sulfonyl (POSF) which is used as base material for PFSAs.

till the millennium changeover (Prevedouros et al., 2006). For more than fifty years PFAAs were mainly produced in large production sites in the United States, Belgium, Italy, Germany and Japan (Prevedouros et al., 2006) to be used as processing aids in the manufacture of fluoropolymers[2] (e.g. PFCAs) or for hundreds of various product applications (Scheirs, 1997).

Telomerization. In the telomerization manufacturing process - applied since the 1970s - a perfluoroalkyl iodide (e.g. pentafluoroethyl iodide) is reacted with tetrafluoroethylene to yield a mixture of perfluoroalkyl iodides with longer perfluorinated chain lengths and after applying a few further process steps create fluorotelomer-based products (Buck et al., 2011). The advantage of the telomerization process is that it yields into more linear isomers and less impurities compared to the ECF that produces mixtures of linear and branched isomers (Buck et al., 2011).

Uses. In general, more than 200 industrial and consumer applications for PFAS are known, ranging from water- and stain-resistant coatings for textiles and furniture, electroplating, photographic emulsifier, aviation hydraulic fluids, and fire-fighting foams to grease-proof paper products for food contact (Renner, 2001). Materials which were coated with a thin (fully) fluorocarbon layer, possess the additional property of having a very low surface energy, so that water and oil does not soak into the fabric (Sandford, 2000). Water and oil remains as droplets on the surface and can then be easily removed by wiping (Sandford, 2000). Therefore, perfluorinated compounds were/are used to make furniture, carpets and textiles water and oil repellent. Fluorocarbon-based paints are similarly used for a weatherproof coating of external surfaces (Sandford, 2000). They are used in fire-fighting foams because PFAS as an active ingredient in the foam gives them the capacity to cover the surface of the (liquid hydrogen carbon) fuel cutting off the oxygen to the flame (IPAN, 2018). Many pharmaceuticals have fluorine atoms in their structures and are used as antibiotic, antifungal and anticancer agents (Sandford, 2000). Large tons of agrochemicals used as herbicides, insecticides and fungicides contain fluorine atoms (Sandford, 2000). N-ethyl perfluorooctane sulfonamide (N-EtFOSA) used as a pesticide is still commonly utilized for example in South America (IPEN, 2019). This is problematic especially since N-EtFOSA can degrade to PFOS, a compound which was added to the list of persistent organic pollutants (POPs) in 2009 and therefore meeting the criteria of being persistent, bioaccumulative, toxic and able of a long-range environmental transport (IPEN, 2019).

Recent trends. In recent years perfluoroalkyl substances with chain-length shorter than 6 C (especially PFBS) were introduced as alternatives to longer-chain PFAS, because they did show a lower bioaccumulation potential in multiple tested organisms (Olsen et al., 2009). A

[2] E.g. polytetrafluoroethylene (PTFE) also known as Teflon, and polyvinylidene fluoride (PVDF).

large production plant in Trissino (Italy) which produced large quantities of PFOA and PFOS since the late 1960s for example manufactured mostly short-chain PFAS congeners from 2013-2016 (Pitter et al., 2020). Moreover, substitutes which contain shorter chain lengths (usually C2 and linear or branched C3-chains) which are linked together by ether-bonds (e.g. GenX, ADONA and F-53B) are uprising. Such perfluoro- and polyfluoroethers are synthesized by other manufacturing processes than ECF and telomerization, for example by photoinduced polymerization of hexafluoropropene or tetrafluoroethylene (Kissa, 2001). Moreover, ultra-chain perfluoroalkyl acids with C2-C3 carbon backbones have started to attract increased attention recently (Janda et al., 2019; Wang et al., 2020).

1.2 Terminology and characteristics of PFAS

The term perfluoroalkyl substances defines an aliphatic substance group for which all of the hydrogen atoms attached to a carbon atom were replaced by a fluorine atom, except for the hydrogen atoms of the respective functional group (Banks et al., 1994). In comparison, polyfluoroalkyl substances are defined as aliphatic substances were at least one hydrogen atom attached to a carbon atom is replaced by a fluorine atom (Banks et al., 1994). Figure 1 shows the difference between perfluoroalkyl substances and polyfluoroalkyl substances. If all hydrogen atoms are substituted by a fluorine atom so that the molecule contains only carbon and fluorine atoms, and the functional group is absent (e.g. hexafluoroethane (C_2F_6)), the term perfluorocarbon (PFC) is used, as recommended by Buck et al. (2011). Although, mostly considering the definitions perfluoroalkyl substances and polyfluoroalkyl substances are addressed in discussions, all three perfluoro-groups are members of the PFAS family.

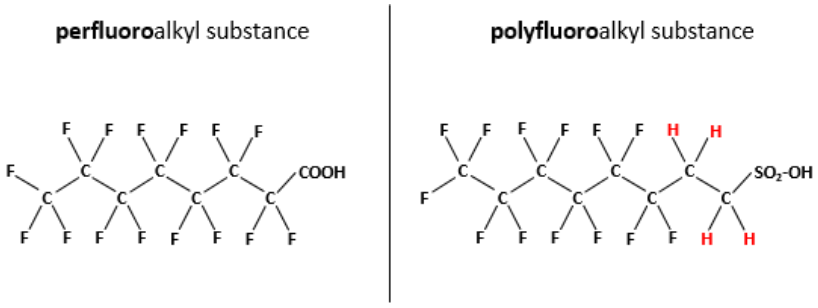


Figure 1: Example of one perfluoroalkyl substance (left: PFOA) and one polyfluoroalkyl substance (right: 6:2 FTS)

The two major sub-categories of (ionic) PFAS are the perfluoroalkyl carboxylic acids (PFCAs) and the perfluoroalkyl sulfonic acids (PFSAs). The most well-known representatives of these two perfluoroalkyl acid (PFAA) sub-categories are the perfluorooctanoic carboxylate (PFOA) and the perfluorooctanoic sulfonic acid (PFOS). Furthermore, due to branching of the main C backbone PFAS exist of various isomers as well (Banks et al., 1994). In many environmental samples for example mixtures of linear and

up to 10 branched isomers for PFOS are present (Riddell et al., 2009). According to Rayne et al. (2008) 89 congeners are theoretically possible for PFOS alone. Due to the enormous quantity of isomers their analytical determination is quite challenging and therefore mostly the linear PFAS are quantified in environmental samples. Nevertheless, the study of branched isomers is useful and important as well and therefore shouldn´t be totally neglected. PFAS (linear and branched isomers) in general, are chemically very stable substances which might ultimately be deposited to the earth´s surface but it is very uncertain if they degrade in the environment at all[3] (Buck et al., 2011). Figure 2 shows the PFAS family tree with the sub-groups that were addressed in this study colored in green.

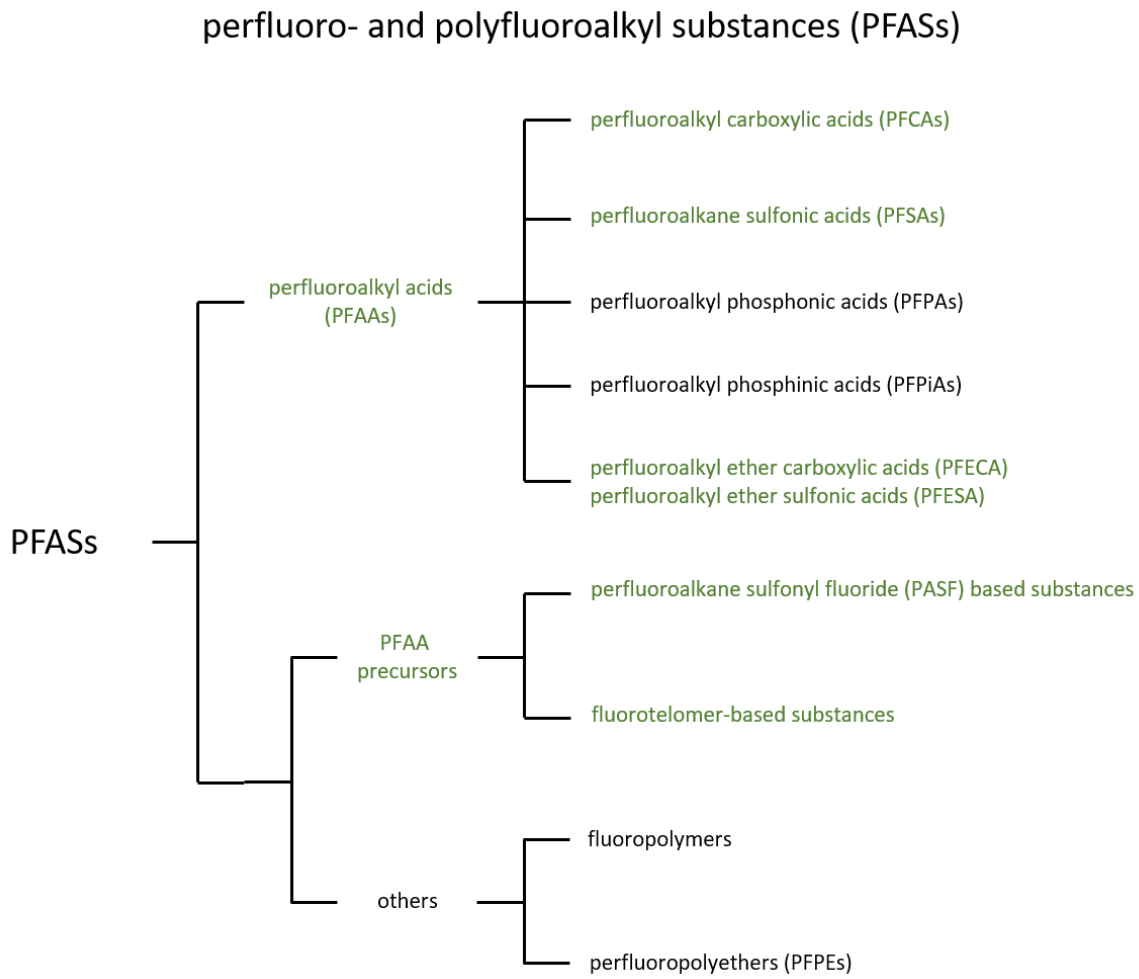


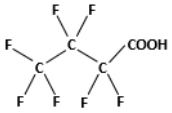
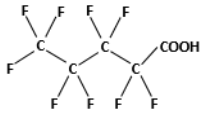
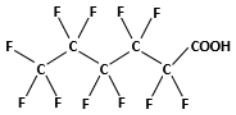
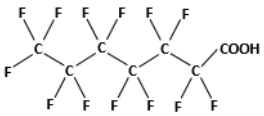
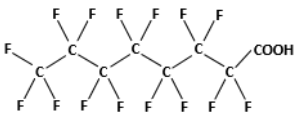
Figure 2: Own visualization of the PFAS family tree (Buck et al., 2011; OECD, 2015) - in green the PFAS sub-groups that were addressed in the current study.

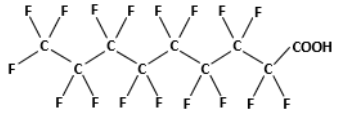
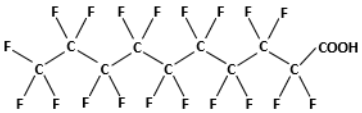
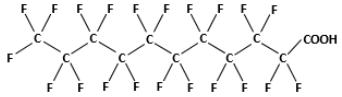
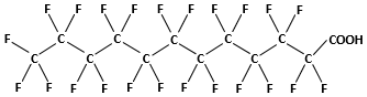
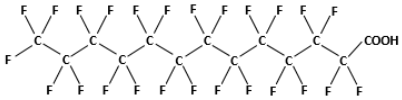
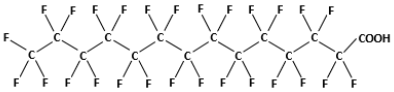
[3] This accounts mostly for PFAAs, since it is known that precursors can degrade to PFAAs in the environment (Young and Mabury, 2010) but recently it was reported as well that some bacteria are able to degrade PFOA (Yi et al., 2016).

1.2.1 Perfluoroalkyl carboxylic acids (PFCAs)

The perfluoroalkyl carboxylic acids (PFCAs) belong to the family of perfluoroalkyl acids (PFAAs), which are or were used in a wide variety of industrial and consumer applications. They are very persistent, they can directly be emitted to the environment or they can be formed indirectly from environmental degradation or metabolism of precursor substances (Buck et al., 2011). Their carbon backbone can vary in their length but typically consists of four to fourteen carbon atoms. More recently ultra-short chain perfluoroalkyl carboxylic acids such as trifluoroacetic acid (TFA, C2) and perfluoropropionic acid (PFPrA, C3) are included in studies and discussions as well. PFCAs have a carboxylic functional group attached to the perfluoroalkyl chain. Their general chemical formula is $C_nF_{2n+1}COOH$. The most frequently discussed PFCA is PFOA, which was used for decades as an emulsifier in the production of polytetrafluoroethylene (Kissa, 1994). Table 1 shows the PFCAs that were included in the present master thesis.

Table 1: Characteristics of the PFAS sub-group perfluoroalkyl carboxylic acids (PFCA)

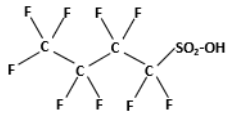
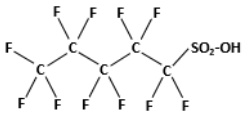
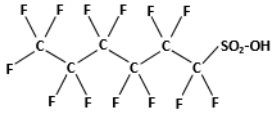
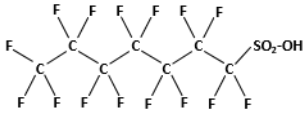
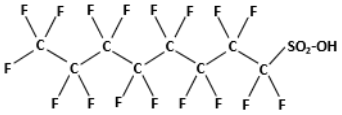
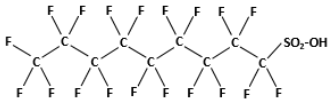
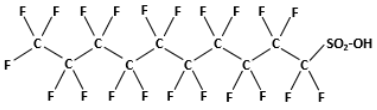
perfluoroalkyl carboxylic acids (PFCAs)			
substance (acronym)	molecular weight [g/mol]	CAS	chemical structure
perfluoro-n-butanoic acid (PFBA)	214.04	375-22-4	
perfluoro-n-pentanoic acid (PFPeA)	264.05	2706-90-3	
perfluoro-n-hexanoic acid (PFHxA)	314.05	307-24-4	
perfluoro-n-heptanoic acid (PFHpA)	364.06	375-85-9	
perfluorooctanoic acid (PFOA)	414.07	335-67-1	

perfluoro-n-nonanoic acid (PFNA)	464.08	375-95-1	
perfluoro-n-decanoic acid (PFDA)	514.08	335-76-2	
perfluoro-n-undecanoic acid (PFUnDA)	564.09	2058-94-8	
perfluoro-n-dodecanoic acid (PFDoDA)	614.10	307-55-1	
perfluoro-n-tridecanoic acid (PFTrDA)	664.11	72629-94-8	
perfluoro-n-tetradecanoic acid (PFTeDA)	714.11	376-06-7	

1.2.2 Perfluoroalkane sulfonic acids (PFSAs)

Perfluoroalkane sulfonic acids (PFSAs) belong to the PFAA-group and diver in their structure compared to PFCAs that they have a sulfonic acid functional group instead of a carboxylic one. Like PFCAs they have a fully fluorinated alkyl chain (hydrophobic part), and a functional head group which is hydrophilic (Trojanowicz et al., 2018). The polarity and aqueous solubility of PFAAs in general increases with decreasing carbon chain length (Eschauzier et al., 2012). Their general chemical formula is $C_nF_{2n+1}SO_2OH$. Table 2 shows the PFSAs and their structures which were included in the laboratory experiments.

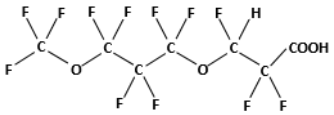
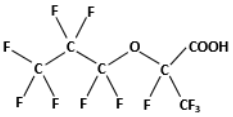
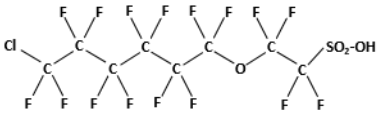
Table 2: Characteristics of the PFAS sub-group perfluoroalkane sulfonic acids (PFSAs)

perfluoroalkane sulfonic acids (PFSAs)			
substance (acronym)	molecular weight [g/mol]	CAS	chemical structure
perfluoro-1-butane sulfonate (PFBS)	300.10	375-73-5	
perfluoro-1-pentane sulfonate (PFPeS)	349.10	630402-22-1	
perfluoro-1-hexane sulfonate (PFHxS)	400.12	355-46-4	
perfluoro-1-heptane sulfonate (PFHpS)	450.12	375-92-8	
perfluorooctane sulfonate (PFOS)	500.13	1763-23-1	
perfluoro-1-nonane sulfonate (PFNS)	549.13	98789-57-2	
perfluoro-1-decane sulfonate (PFDS)	600.15	335-77-3	

1.2.3 Per – and polyfluoroalkyl ether acids (PFEAs)

Per- and polyfluoroalkyl ether acids (PFEAs) were generally produced to replace PFOS and PFOA. ADONA for example was developed to replace PFOA as an emulsifier in the manufacture of fluoropolymers (Gordon, 2011). The 6:2 chlorinated polyfluoroalkyl ether sulfonic acid (6:2 Cl-PFESA, trade name F-53B) is used as an alternative to PFOS. The perfluoroether carboxylic acid with the trade name “GenX” is used in fluoropolymer manufacturing processes since 2010 as a replacement of PFOA (Pan et al., 2018). Table 3 shows examples for one polyfluoroalkyl ether carboxylic acid (ADONA), one perfluoroether carboxylic acid (GenX) and one chlorinated polyfluorinated ether sulfonate (F-53B).

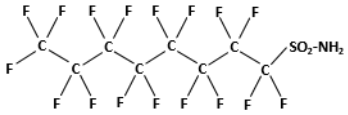
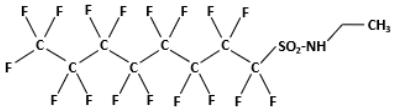
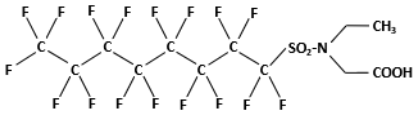
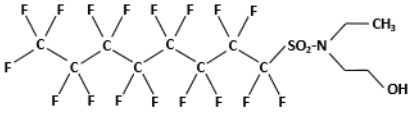
Table 3: Characteristics of per- and polyfluoroalkyl ether acids

per – and polyfluoroalkyl ether acids (PFEAs)			
substance (acronym)	molecular weight [g/mol]	CAS	chemical structure
polyfluoroalkyl ether carboxylic acid (ADONA)	376.96	958445-44-8	
perfluoroether carboxylic acid (HFPO-DA; GenX)	330.05	13252-13-6	
6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFESA; F-53B)	531.57	73606-19-6	

1.2.4 Perfluoroalkane sulfonyl fluoride (PASF) based substances

Characteristic for this sub-class is that these substances contain an amino head group or an amino group with one or various methyl, ethyl, ethyl alcohol or methyl carboxyl acid extensions. Four perfluoroalkane sulfonyl fluoride (PASF) based substances were included in the present master thesis (see Table 4).

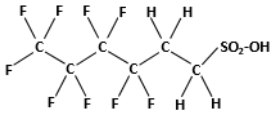
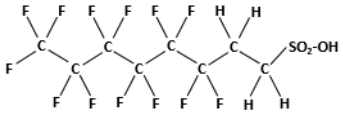
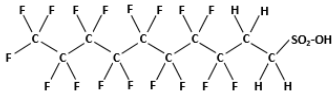
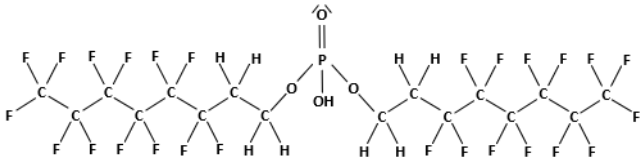
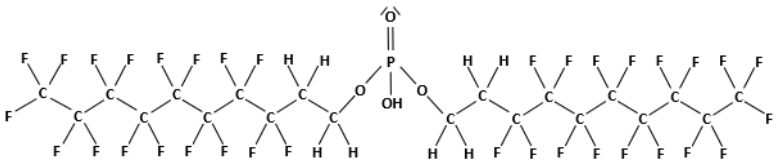
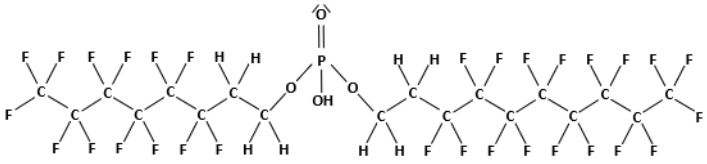
Table 4: Characteristics of perfluoroalkane sulfonyl fluoride (PASF) based substances

perfluoroalkane sulfonyl fluoride (PASF) based substances			
substance (acronym)	molecular weight [g/mol]	CAS	chemical structure
perfluoro-1-octanesulfonamide (PFOSA)	499.15	754-91-6	
N-ethylperfluoro-1-octanesulfonamide (N-EtFOSA)	527.20	4151-50-2	
N-ethyl-perfluoro-1-octanesulfonamido acetic acid (N-EtFOSAA)	585.24	2991-50-6	
N-ethylperfluoro-1-octanesulfonamido-ethanol (N-EtFOSE)	571.25	1691-99-2	

1.2.5 Fluorotelomer-based substances

Polyfluoroalkyl phosphate diesters (diPAPs) are used for example in food contact materials (D'eon et al., 2009). Table 5 shows the structure of six fluorotelomer-based substances.

Table 5: Characteristics of fluorotelomer-based substances

fluorotelomer-based substances			
substance (acronym)	molecular weight [g/mol]	CAS	chemical structure
4:2 fluorotelomer sulfonate (4:2 FTS)	328.15	757124-72-4	
6:2 fluorotelomer sulfonate (6:2 FTS)	428.17	27619-97-2	
8:2 fluorotelomer sulfonate (8:2 FTS)	528.18	39108-34-4	
6:2 polyfluoroalkyl phosphoric acid diesters (6:2 diPAP)	790.17	57677-95-9	
			
8:2 polyfluoroalkyl phosphoric acid diesters (8:2 diPAP)	989.19	678-41-1	
			
6:2/8:2 polyfluoroalkyl phosphoric acid diesters (6:2/8:2 diPAP)	890.20	943913-15-3	
			

1.3 PFAS sources, exposure, and adverse effects

Direct and indirect sources of PFAS emission to the environment exist. While emissions from manufacturing processes and the use of PFAS are direct sources, indirect sources are those were PFAS (e.g. chemical reaction impurities or precursors) degrade in the environment to PFAAs (Prevedouros et al., 2006).

Direct PFAS sources. Prevedouros et al. (2006) summarized in their study past direct releases from PFCAs to soils and water via aqueous fire-fighting foams (AFFFs) which contained PFCAs in their formulations. AFFFs which are used to extinguish especially hydrocarbon and solvent based fires contain often a mixture of various PFAS (D'Agostino and Mabury, 2014). When AFFFs are applied to extinguish fires at airports and military sites during firefighting training exercises they can pose a potential threat to drinking water supplies. In Ronneby (Sweden) for example around 28,000 inhabitants were exposed to contaminated drinking water with PFAS because in a nearby airfield firefighting foams which contained PFAS were used (Li et al., 2018). Groundwater contamination with PFAS can also be caused by manufacturing plants. Besides known larger incidents in the United States a PFAS production plant in Trissino (Italy) contaminated the groundwater probably from the late 1960s till autumn 2013 which affected the drinking water supply of around 140,000 inhabitants (Pitter et al., 2020) and also the plants grown in the region. In Germany around 40,000 inhabitants were exposed to contaminated drinking water due to the usage of PFAS contaminated sludge that was used as conditioner in agricultural fields (Hölzer et al., 2008). Other direct groundwater contaminations can derive via the leachate of waste disposals (Wang et al., 2020) and the application of sewage sludge as fertilizer (Hölzer et al., 2008). Furthermore, PFAAs can be directly released to the environment through consumer products such as floor polishes, cleaning formulations, hair care products, inks, medical inhalers, air fresheners, paper, leather, textile treatments (Prevedouros et al., 2006) and ski-waxes (Freberg et al., 2010; Nilsson et al., 2013). In general, PFAS which enter the environment through one direct source can stay stable during a certain amount of time or depending on the individual structure and environmental conditions transform to more stable molecules.

Indirect PFAS sources. Indirect sources of PFAS are those when less stable polyfluorinated substances degrade partly to form more stable perfluorinated substances. For example perfluoroalkyl sulfonamides can degrade under atmospheric oxidative conditions to PFCAs such as PFOA (Martin et al., 2006) and probably as well to PFSAs considering the sulfonic functional group in there structures. The presence of N-EtFOSE and N-MeFOSE was reported in outdoor and indoor air (Shoeib et al., 2004), deriving probably from one direct source, if these substances are then transformed into more stable PFCAs, the resulting PFCAs are considered to derive from an indirect source. Polyfluorinated substances which can be transformed to PFAAs are called precursors (or PFAA-precursors). Mostly PASFs and fluorotelomer-based substances are considered as precursors. But it also

shall be mentioned that polyfluorinated ether acids have the potential to be transformed under environmental conditions to other perfluorinated ether acids (Zhang et al., 2019). Even the perfluorinated backbone of a long-chain PFAA could be partially zipped open under oxidative conditions to form ultra-short chain PFAAs (chain length <C4) which were demonstrated in laboratory experiments (Janda et al., 2019). Zheng et al. (2020) reported that dust and childcare maps in indoor childcare facilities in the United States contained 25 and 21 PFAS, including PFCAs, PFSAs and PFAA-precursors such as FTS, N-EtFOSE and N-EtFOSA. Polyfluoroalkyl phosphoric acid monoesters (monoPAPs) and polyfluoroalkyl phosphoric acid diesters (diPAPs) which are considered as well as PFAA-precursors were and are used for grease-proofing agents for food contact paper (Lee et al., 2010). A further specific use of monoPAPs and diPAPs was (or is) their addition in pesticide formulations as an approved defoaming adjuvant (Buck et al., 2011). Fluorotelomer-based substances were (and/or are) used for example in medicinal applications or in ski waxes (Kirchhof et al., 2002; Rogowski et al., 2007). Many precursors probably are the cause of the long-range transports of already banned PFAS. Trojanowicz et al. (2018a) states for example that PFOS is essentially nonvolatile and its world-wide distribution is probably not attributed to its transport in the atmosphere but rather its volatile precursors undergo long range atmospheric transports prior to degrading to PFOS. The same can very much be expected for other long and short chain-length PFAAs which are globally detected.

1.3.1 PFAS detection in the environment and in humans

Giesy and Kannan (2001) were the first that demonstrated the global contamination of PFOS in wildlife. In the same year Hansen et al. (2001) discovered the presence of PFOS, PFOA, and other PFAS in numerous human blood samples. Since then the scientific literature on environmental and toxicological aspects of PFAS steadily and rapidly increased (Buck et al., 2011). Contemporary multiple PFAAs are typically detected in all environmental media, humans and wildlife (Lau, 2015). Today the existing body of literature, including governmental reports consists of thousands of scientific studies on PFAS, while PFOS and PFOA by far are the most reported and discussed PFAS but other compounds of the PFAS family are gaining more attention as well. Fluorotelomer sulfonates were detected in groundwater, wastewater effluents and human serum (Kannan et al., 2004; Schultz et al., 2004; Ahrens et al., 2009; Lee and Mabury, 2011). Long-chain and short-chain PFAAs (C2-C4) were detected in rainwater (Taniyasu et al., 2008). DiPAPs as well were already detected in wastewater treatment plant sludge and in human serum (D'eon et al., 2009). ADONA was detected in human plasma samples in Germany (Fromme et al., 2017). Considering the detection of different PFAS in human serum tap water probably is one of the main exposure pathway for the intake of PFAS (Vestergren and Cousins, 2009; Eschauzier et al., 2013). PFAS for example were detected in tap water and in tap water-based beverages (e.g. coffee and cola) in Amsterdam (Eschauzier et al., 2013). According to Dagnino (2015) tap water produced from ground water is often PFAS free, but it often has

a PFAS background contamination when it is produced from surface waters. Wastewater treatment plants which are unable to completely eliminate PFAS are one-point sources for PFAS contamination of surface waters. In the case of Amsterdam the tap water is originated from the river Rhine and PFAS are hardly removed during the water treatment, especially short chain compounds such as PFBA and PFBS (Eschauzier et al., 2012). The drinking water in Amsterdam[4] is sourced by the Lek canal which is fed by the river Rhine, and the river Rhine is known to have some industrial point sources which attribute to the PFAS contamination (Eschauzier et al., 2012). Besides the exposure via tap water alone further exposure to additional PFAS might derive from other sources during preparation processes, e.g. leaching/contamination from tubes containing fluoropolymers in automatic beverage dispensers (Eschauzier et al., 2013). Pitter et al. (2020) for example observed in a large cohort study in Italy that alcohol intake showed a significant positive association with PFOA, PFOS and PFHxS concentrations in human serum. It can´t be completely excluded that PFAS in alcoholic beverages might derive from leaching of tubes as well, or that they might enter the beverage during any other specific processing step. A potential source might also be paper cups, containing fluorinated polymers to provide water repellency, which are used for hot beverages and take-away coffees (Eschauzier et al., 2013). In contrast a Norwegian study reported that food can be considered as the major source of PFAS in humans, particularly seafood (Haug et al., 2010). A recently published study reported the detection of PFOA, PFHxS and PFOS in Swedish milk, butter, beef, fish and chicken (Sadia et al., 2020). The same study reported the detection of PFOA, PFHxS and PFOA in food in Asian, American and African countries, while PFOA and PFOS were detected as well in chicken eggs PFHxS was not detected in any egg sample of the observed countries (Sadia et al., 2020). This results would be in accordance with the observations from a recently published Italian study, that fish consumption is associated with increasing PFHxS levels in the human body (Pitter et al., 2020). Furthermore, the consumption of vegetables which were grown in a contaminated area were associated with higher PFAS concentrations in the human bodies as well (Pitter et al., 2020). In general, probably the main pathway of exposure to PFAS are diet, drinking water and to a leaser content indoor dust (Kato et al., 2015). Table 6 shows some examples of PFAS detections in the environment.

[4] Treating water steps applied for Amsterdam's tap water: 1) rapid sand filtration, 2) softening (NaOH), 3) ozonation, 4) GAC filtration and 5) slow sand filtration.

Table 6: Examples for environmental and human exposure to PFAS

medium	compounds	concentration ∑PFAS in [ng/L]	country	reference
effluent	21 PFAS (PFSAs C4-C8, PFCAs C5-C13, 6:2 FTSA, PFOSi, PFHxSi, PFOSA, MeFBSA, MeFBSE and FHUEA)	30.5 – 266.3	Germany	Ahrens et al. (2009)
river (Elbe)	17 PFAS (PFSAs C4-C8, PFCAs C5-C12, 6:2 FTSA, PFOSi, PFOSA, FOUEA and FDUEA)	7.6 – 26.4	Germany	Ahrens et al. (2009)
tap water and tap water-based beverages	tap-water: PFCAs (C4-C9), PFBS, PFHxS and PFOS	44	Netherlands	Eschauzier et al. (2013)
	cola: PFCAs (C4-C7), PFBS and PFOS	24		
	coffee: PFCAs (C7-C8, and C10), PFBS and PFOS	11		
drinking water/tap water	6 PFCAs: PFBA, PFPeA, PFHxA, PFOA, PFBS and PFHxS	60	Netherlands	Eschauzier et al. (2012)
food (milk, butter, beef, fish, chicken)	PFOA, PFHxS and PFOS	range: 1.4 – 50.1 pg/g	Sweden	Sadia et al. (2020)

1.3.2 Effects on ecosystems and humans

The effects on ecosystems and on humans vary widely depending on the specific PFAS (i.e. based on chain length and functional groups), on sex and on the type of species (Lau et al., 2007; Lau, 2015). For example the half-lives of long-chain PFCAs are hours to days in rodents, days to month in monkeys and month to several years in humans, the half-lives in females are slightly shorter than in males while short-chain length PFCAs (<C6) in general are less persistent in bodies (Lau, 2015). Estimated mean half-lives in humans for PFHxS, PFOA and PFOS are 5.3-8.5 years, 2.7-3.8 years and 3.4-5.4 years (Olsen et al., 2007; Li et al., 2018). Although the estimated mean half-lives of studies might (slightly) differ they have in commune that the half-lives in general for PFHxS, PFOA and PFOS are a couple of years in humans. Moreover, the reported half-lives of different studies show the same order PFHxS > PFOS > PFOA. Rappazzo et al. (2017) reported in their systematic review of epidemiologic literature six categories of health outcomes: immunity/infection/asthmas, cardio-metabolic, neurodevelopmental/attention, thyroid, renal and puberty onset. A large US cohort study from a community which was exposed to PFAS contaminated drinking water found that PFOA was associated to kidney and testicular cancer (Barry et al., 2013). Wolf et

al. (2008) reported that PFAAs induced an increasing activity of peroxisome proliferator-activated receptor-alpha (PPARα) in mouse and humans, while the human PPARα appeared to be less sensitive than the mouse PPARα and PFCAs were stronger activators than PFSAs. Links between blood serum levels and a low birth weight were reported (Fei et al., 2007). PFAS exposure might lead to an early menopause in women (Knox et al., 2011). Studies reported that some PFAS can increase impulsivity and initiate a delayed the puberty in children (Gump et al., 2011; Lopez-Espinosa et al., 2011). Low semen quality in young men was observed as well (Joensen et al., 2009). Melzer et al. (2010) reported associations between thyroid diseases and PFAS exposure in a general adult population. Considering the presence of multiple PFAS in organisms, cumulative risks, potential interactions and combined effects must be taken into account as well (Lau, 2015). Table 7 provides a summary of some reported adverse health effects on humans and ecosystems.

Table 7: Adverse health effects on humans and ecosystems

	adverse health effects	**reference**
HUMANS	low birth weight	Fei et al. (2007)
	early menopause in women	Knox et al. (2011)
	increased impulsivity in children	Gump et al. (2011)
	delayed puberty in children	Lopez-Espinosa et al. (2011)
	low semen quality in young men	Joensen et al. (2009)
	thyroid disease in general adult population	Melzer et al. (2010)
	liver hypertrophy, elevated cholesterol levels	Lau (2015)
H&A	increased activity of PPARα	Wolf et al. (2008)
ANIMALS	growth deficits and developmental delays	Lau (2015); Lee et al. (2020)
	induce tumors	Lau (2015); Lee et al. (2020)
	endocrine disrupting	Lau (2015); Du et al. (2013)
	neurotoxic effects	Lau (2015); Lee et al. (2020)

1.4 Ozone and its application

Ozone (O_3) is the allotropic form of oxygen with a redox potential of + 2.07 V (Gutmann and Hengge, 1988). It is a strong oxidizing agent. The ozone molecule was first discovered in 1839 by Christian Friedrich Schönbein and a few years later Werner von Siemens invented the generation of ozone via silent electrical discharge which is still applied today (Roeske, 2007). Nowadays, ozone is sparsely used for drinking water treatment purposes, for example for water disinfection. In wastewater treatment plants ozone is increasingly applied if the removal of micropollutants is required (e.g. in Switzerland).

1.4.1 Ozone for (waste)water treatment

The induced elimination of micropollutants by ozone in water is based on two mechanisms. First, ozone can react directly with other substances or secondly ozone indirectly reacts with compounds via highly reactive generated hydroxyl radicals (·OH) (Gottschalk et al., 2010). Both mechanisms occur simultaneously but depending on the water matrix one of both dominates. In general, ozone is more selective than hydroxyl radicals and it prefers compounds with electron-rich moieties such as phenols, amines and olefins at higher pH levels (Nöthe, 2009). Hydroxyl radicals in comparison are less selective and reduce therefore ozone refractory compounds significantly better (Nöthe, 2009). In general, ·OH oxidize compounds more quickly (Hoigne and Bader, 1977).

PFAS with their fluorine-carbon backbones are very persistent towards both reaction mechanisms of ozone, the direct and the indirect one via hydroxyl radicals. Fluorine is the strongest inorganic oxidant with a reduction potential of $E°$ = 3.6 (Wardman, 1989), it is the most electronegative element and it resists oxidation to keep its electrons (Sun et al., 2018). It is therefore thermodynamically unfavorable to oxidize fluorine with any other one-electron oxidant to its elemental state of F_2 (Vecitis et al., 2009). The removal of perfluoroalkyl substances in water with ozone alone is therefore not promising, even at high ozone doses and relatively long contact times (Sun et al., 2018), but the usage of additional catalyzed material or further downstream clean-up steps (e.g. filters) might improve the elimination efficiencies (Sun et al., 2018; Franke et al., 2019). In comparison, granular activated carbon (GAC) filters are capable of removing PFAS if they are freshly regenerated or newly replaced (Merino et al., 2016). GAC is less efficient though for short-chain PFAS (< C5) and the activated carbon needs to be changed often which makes them a costly interim solution (Eschauzier et al., 2012; Dai et al., 2019; Franke et al., 2019). Merino et al. (2016) reviewed studies which tested 12 different sorbents in total for PFAS removal besides GAC, and it seems that electrocoagulation[5] is the most favorable technology from those 12 tested

[5] Metal ions are generated by electrolytic oxidation, which than undergo hydrolysis in water forming various coagulant species including hydroxide precipitates which are able to remove micropollutants by adsorption (Hakizimana et al., 2017).

sorbents (Lin et al., 2015). Although this method might be a cost-effective option (Merino et al., 2016) a disadvantage of the electrocoagulation is that it can result in formation of chlorinated organic compounds (Mollah et al., 2004). Other effective removal techniques which use high temperature (e.g. thermal destruction) and/or pressure are very costly as well (Merino et al., 2016). Reverse osmosis (RO) and nanofiltration (NF) for example can effectively remove PFAS, but these two methods also produce a PFAS concentrated stream (approximately 10% of the treated water volume) that needs further treatment (Dai et al., 2019). Therefore, a proper disposal for the remained PFAS-enriched concentrate needs to be carefully considered (Rahman et al., 2014). To enable a more cost-efficiently and safe removal several different treatment methods need to be combined (Merino et al., 2016). For example, it is suggested that the performance of GAC for short-chain PFAS can be effectively improved with a pre-ozonation step, which leads to the oxidation of otherwise competitive organic compounds (Sun et al., 2018).

1.4.2 Advanced oxidation process (AOP)

Advanced oxidation processes (AOPs) describe a combination of treatment techniques where additional chemicals are purposefully used to generate higher quantities of (nonselective) radicals. Generated radicals can then react with compounds which are poorly eliminated by ozone molecules alone (Hübner et al., 2015). One AOP can be applied by the addition of hydrogen peroxide (H_2O_2) to ozone. This process leads to a greater formation of ·OH-radicals (16-57%) compared to O_3-processes under the same ozone doses in natural waters (Anumol et al., 2016). Another example of an AOP is based on a heterogeneously catalyzed ozonation by using an additional heterogeneous iron-oxide catalyst and/or persulfate (Franke et al., 2019). While the addition of H_2O_2 doesn´t seem to improve the elimination of PFAS in water samples (Anumol et al., 2016), the PFAS elimination can be improved by the addition of a heterogeneous iron-oxide based catalyst or/and persulfate (Hori et al., 2007; Wang et al., 2008; Wu et al., 2018; Franke et al., 2019). Persulfate ($S_2O_8^{2-}$) is a strong oxidant ($E°$ = 2.1 V) and is highly soluble; it can generate free sulfate radicals ($\cdot SO_4^-$) under oxidative conditions which then successfully can decompose PFCAs (Tsitonaki et al., 2010). The oxidation capabilities can be increased if various radicals (e.g. ·OH and $\cdot SO_4^-$) are available to interact with PFAS. Moreover, catalyzing agents (e.g. iron or other divalent minerals) in addition with oxidants can generate free radical species with greater oxidation potential and faster reaction kinetics (Huling and Pivetz, 2007). Merino et al. (2016) reported that five studies demonstrated the decomposition of PFOA, PFDA and 4:2 FTUCA to shorter-chain PFCAs and elemental components, such as F^-, with persulfate. This decomposition characteristics might also be similar for other PFAS. It is very important to note that different intermediate oxidation products (from ozonation and AOP) could be as toxic as or even more toxic than the initial compound and this must always be investigated in detail before large scale applications (Munter, 2001). This indicates that further downstream clean-up steps (e.g. filter) are suggested after ozonation (or AOP) and that

(eco)toxicological test should always be applied before large scale operations. Furthermore, it is recommended to perform (eco)toxicological tests on a random basis afterwards.

1.4.3 Ozone applied for other experimental purposes

Beside the usage of ozone as a water treatment tool, ozone can also be used to imitate oxidative processes that occur in the environment in laboratory scale experiments. The idea behind that is to imitate natural transformation processes initiated by ozone in the atmosphere or on surfaces. In context of this point of view, according to Houtz and Sedlak (2012) oxidation methods may be used to characterize the fate of difficult-to-measure PFAA-precursors in environmental samples.

Additionally, ozone can be used for the total oxidizable precursor (TOP) assay. The TOP assay is a mass balance approach, where a strong oxidizing agent (e.g. ozone) is added to the sample and the levels of PFAAs are measured before and after the oxidative pre-treatment (NCM, 2019). This assay was first introduced by Houtz and Sedlak (2012). They used hydroxyl radicals generated by thermolysis of persulfate ($S_2O_8^{2-}$) under basic pH (≥12) conditions, but the assay should work with other oxidizing agents as well. PFAAs are very stable and persistent compounds, and are hardly oxidized, so the differences in PFAA levels before and after the oxidation are expected to account only for the degradation of precursor compounds (Houtz and Sedlak, 2012; NCM, 2019). The TOP assay is therefore a suitable approach to estimate the presence of non-targeted precursor compounds and assess the total PFAS content in a sample (Houtz and Sedlak, 2012).

1.5 Aims and research questions

One environmental exposure route, under many, is the PFAS emission via wastewater as (most) PFAS do not degrade in current state-of-the-art waste water treatment plants with carbon, nitrogen and phosphorous removal (BMLFUW, 2017). One goal of the present master thesis is to provide an overview of the historical discoveries and evaluate the contemporary progress concerning the knowledge of PFAS. A second goal is to implement a laboratory experiment to test the total oxidizable precursor (TOP) assay with ozone as its oxidizing agent and in parallel observe the PFAS elimination rates. For the laboratory experiment spiked water samples with PFAS will be ozonated and their elimination efficiencies and degradation to terminal end products will be evaluated. Recent studies did demonstrate that PFAAs are hardly eliminated in water via ozonation (Schröder and Meesters, 2005; Eschauzier et al., 2012; Yang et al., 2014; Sun et al., 2018). The present study therefore is not putting the focus on the elimination of PFAAs alone, but much more investigating the transformation of PFAA-precursors (e.g. fluorotelomer-based substances) and per- and polyfluoroalkyl ethers to PFAAs. The aim is to gain a better understanding of oxidative processes concerning PFAS. It is known for example that fluorotelomer-based substances can be transformed in biota and the environment to perfluoroalkyl acids (PFAAs)

such as perfluorooctanoic acid (PFOA) and perfluorooctanoic sulfonic acid (PFOS), two of the most frequent detected PFAS (Poothong et al., 2020). However, although the insufficient degradation of some PFAAs via ozonation alone is known, the elimination efficiencies of other PFAS is still of interest, especially the transformation processes. Therefore, a reasonable ozone concentration which is applied in wastewater treatment plants (e.g. Switzerland) as well as unrealistically high ozone concentrations were used for the study experiments. Clean water was spiked with a mixture containing 31 PFAS and subsequently was ozonated with different ozone dosages. The aim of the study is (i) to provide an overview of historical discoveries and evaluate the contemporary progress concerning the knowledge of PFAS, (ii) the oxidation of PFAS in clean (matrix free) water with ozone to investigate the elimination efficiencies, (iii) the determination of transformed PFAA-precursors and per- and polyfluoroalkyl ethers to PFAAs with high-performance liquid chromatography tandem-mass spectrometry (HPLC-MS/MS), (iv) and the evaluation of the usefulness of the TOP assay with ozone as oxidizing agent.

The following research questions shall be answered in this study:

1. To which content can specific PFAS effectively be eliminated in water with ozone at justifiable ozone concentrations?
2. Which specific PFAA precursors and per- and polyfluoroalkyl ether acids are transformed to PFAAs and to which content?
3. Is the TOP assay with ozone as oxidizing agent a useful approach to estimate the total PFAS content in a sample?

2 Methods

An overview of the historical discoveries and the evaluation of the present knowledge of PFAS was reported by browsing through the web of science using the keyword-combinations “perfluoro* ozon*”, “perfluoro* TOP* assay*” and “perfluoro* biotransformation*”. Articles were collected first subjectively if the title of an article seemed to match to the current master thesis and secondly if a quick overview (e.g. reading the abstract or looking at the figures and tables) convinced with a high relevance for the current study. To achieve a well-organized and structured free available digital library the citation software Zotero was used. Furthermore, literature on PFAS which was collected and stored in Zotero during other studies and projects was used as well for the present master thesis. Moreover, additional literature and norms (e.g. DIN and ISO) used for the current master thesis were suggested or/and provided by the supervisors.

2.1 Ozonation

2.1.1 Materials, reagents and chemicals

The used ozone generator was an OZ500/5 manufactured by Fischer Technology with a production capacity of 5 g O_3/h. For the residue ozone destruction 100 g potassium iodide were dissolved in one-liter tap water. Furthermore, oxygen (O_2) was used as feed gas and an ozone alarm device (GIG) which alarmed at an ozone concertation of 0.1 ppm. The indigo stock solution was prepared according to the DIN 38408-3 (2011). For the indigo reagents solution a modification suggested by Zappatini and Götz (2015) was implemented by using an additional acidic phosphate solution (see Table 8). The spectrophotometer used to determine the ozone concentration was a UV/VIS-spectrometer Dr. Lange – Cadas 100. Moreover, a spectrophotometer with a UV-detector L-7400 LaChrom and a pump L-7100 from Merck-Hitachi were connected to the ozone reactor. The O_3-stock solution was pumped continuously in circles (flow rate 1.5 mL/min) between the ozone reactor and spectrophotometer (see Figure 3). With this setup the absorption of the O_3-stock solution could be measured directly at 258 nm and the ozone concentration was calculated with the formula: extinction (ε) x 22.2 (for a 0.73 cm cuvette) = mg O_3/L. Although only the values from the O_3-stock solution concentration from the indigo-method were used, this second measurement with the directly connected spectrophotometer allowed to double check the O_3-concentrations (see Appendix B). Table 8 shows the compositions of the indigo reagent solution (IRS) which was used to determine the O_3-concentration according to DIN 38408-3 (2011).

Table 8: Composition of the reagents used for the determination of the ozone concentration (DIN 38408-3, 2011; Zappatini and Götz, 2015)

name of the reagent	composition
indigo stock solution (ISS)	mixture of 0.5 mL phosphoric acid (H_3PO_4) with 385 mg potassium indigo tri-sulfate ($C_{16}H_7K_3N_2O_{11}S_3$) and deionized water in a 500 mL volume flask
acid stock solution (ASS)	mixture of 10 g di-sodium hydrogen phosphate di-hydrate (NaH_2PO_4*$2H_2O$) with 7 mL H_3PO_4 and deionized water in a 1000 mL volume flask
indigo reagent solution (IRS)	mixture of 100 mL ISS and 900 mL ASS in a 1000 mL volume flask

2.1.2 Generation of ozone (O_3)

Ozone (O_3) is very instable compared to oxygen (O_2) and it was therefore necessary to produce ozone freshly at the day of the experiment. For the current master thesis, a laboratory setup under a fume hood was used to generate ozone. Figure 3 shows the laboratory setup for the ozone generation. Oxygen, with a flowrate of 10 mL/min, was led to the ozone generator where ozone was generated by silent electric discharge (35 W). The generated ozone was led to a 2000 mL glass bottle filled with deionized water where it was dissolved via fine-bubble aeration (aquarium aerator stone), collected and concentrated (ozone reactor). The ozone reactor was connected to a bottle containing dissolved potassium iodide in water which was used to eliminate the residues of the outgassing ozone. Since the solubility from ozone is temperature dependent a constant cooling was necessary which was implemented by placing the ozone reactor in a pocket filled with ice. Ozone was led in a continuous flow to the ozone reactor during the whole experiment to gain a constant ozone concentration. An equilibrium concentration of ozone was reached after approximately two hours. After the O_3-concentration in the reactor was equilibrated specific volumes from the stock solution were extracted with a glass syringe for the determination of the ozone concentration or for experimental purposes. The tubes and seals used were made of polytetrafluoroethylene (Teflon) and silicon. Polytetrafluoroethylene might be a potential source of contamination for this type of experiment, but it was used because of its robustness towards ozone compared to other materials. In the water blank from the O_3-reactor no PFAS were detected except for PFBA and PFHxA which were detected below the limit of quantification (LOQ).

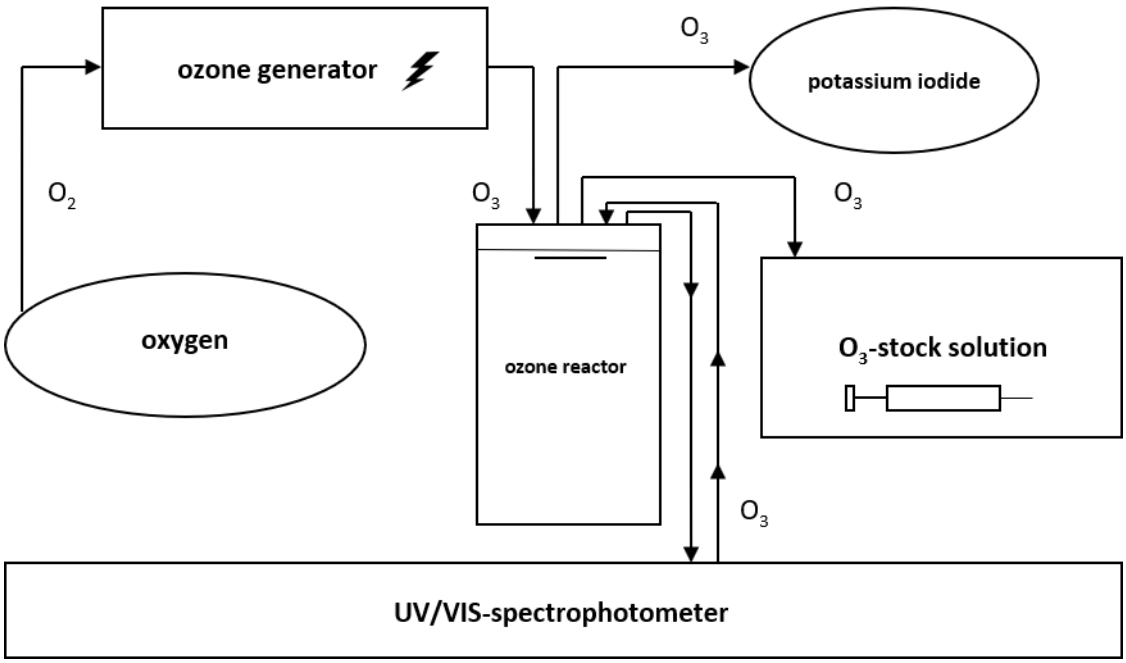


Figure 3: Setup for the generation of ozone

2.1.3 Determination of the ozone concentration

The ozone concentration in the ozone reactor was determined according to the DIN 38408-3 (2011) - photometric determination via indigo. Therefore 10 mL of the indigo reagent solution (IRS, see Table 8) were placed in a 50 mL glass beaker. The glass beaker was then filled up with deionized water to approximately 50 mL. The glass beaker was placed on a table next to the fume hood, where it was easy to reach while working under the fume hood, to avoid a potential pre-exposure to ozone. Then first, approximately 15-20 mL were extracted with a glass syringe and put in the waste. This step was performed to empty the reactor tubes from a O_3-stock solution where the ozone concentration is potentially lower compared to the O_3-stock solution in the reactor. Secondly, a 1 mL glass syringe was once rinsed with the O_3-stock solution and then slightly more than 0.5 mL were extracted. A needle of stainless steel was placed on the syringe and the volume was adjusted to 0.5 mL while simultaneously the air bubbles were removed. Thirdly, the glass beaker was put under the fume hood, the needle was placed under the surface, the 0.5 mL of the O_3-stock solution were injected into the IRS (mixed with deionized water) and the solution was stirred with the needle for 3-5 seconds. Fifthly, the IRS treated with the O_3-stock solution was replaced into a 100 mL volume flask and the volume flask was further adjusted to 100 mL with deionized water. After the volume flask was shaken a 4 cm polystyrene cuvette was first two times rinsed with the content of the volume flask, then filled up again and placed in the spectrophotometer to measure the extinction at 600 nm. The concentration was calculated with the formula (2-1) according to DIN 38408-3 (2011).

$$p = \frac{(A_A - A_B) \cdot f \cdot Vmax}{s \cdot V_p} \quad (2\text{-}1)$$

p	O_3-concentration of the O_3-stock solution [mg/L]
A_A	extinction of the reference [1/cm]
A_B	extinction of the sample treated with the O_3-stock solution [1/cm]
f	calibration factor: $f = 2{,}4\ mg \cdot cm/L$
$Vmax$	volume of the reference and the sample [mL]
s	thickness of the cuvette [cm]
V_p	sample volume [mL]

The reference and the O_3-concentration were always measured in duplicates. Furthermore, the O_3-concentration in duplicates were measured before the experiment, during the experiment and after the experiment. The value of the O_3-concentration that was used for further calculations was the mean of all three duplicate results. Moreover, since methanol reacts with ozone[6], and the PFAS standards were dissolved in methanol, this interaction and its potential to influence the results was tested as well. Therefore, a 100 mL volume flask was filled with approximately 80 mL deionized water and 20 µL methanol were injected. The volume flask was shaken, and 0.5 mL of the O_3-stock solution - the procedure was similar as explained above - was added to the volume flask. The volume flask was shaken for approximately 10 seconds and then the 10 mL IRS was added. The volume flask was shaken again, the volume was adjusted to 100 mL and the extinction was determined as explained above.

2.2 Analysis of per- and polyfluoroalkyl substances

2.2.1 Materials, reagents and chemicals

The simultaneous determination of the 31 PFAS (selected according to their relevance reported in literature) and 14 related isotope-labeled PFAS was performed by high-performance liquid chromatography tandem-mass spectrometry (HPLC-MS/MS). Therefore, a PFAS-standard mixture with a concentration of 0.5 µg/mL (each compound) was prepared containing perfluoro-n-butanoate (PFBA), perfluoro-n-pentane carboxylate (PFPeA),

[6] The reaction rate constant from methanol with ozone is very low (Hoigné and Bader, 1983) and therefore no effect would be expected, but for quality control reasons the effect of methanol was tested as well.

perfluoro-n-hexane carboxylate (PFHxA), perfluoro-n-heptane carboxylate (PFHpA), perfluoro-n-octane carboxylate (PFOA), perfluoro-nonane carboxylate (PFNA), perfluoro-n-decanoate (PFDA), perfluoro-n-undecane carboxylate (PFUnDA), perfluoro-n-dodecane carboxylate (PFDoDA), perfluoro-n-tridecane carboxylate (PFTrDA), perfluoro-n-tetradecane carboxylate (PFTeDA), perfluoro-n-butane sulfonate (PFBS), perfluoro-n-pentane sulfonate (PFPeS), perfluoro-n-hexane sulfonate (PFHxS), perfluoro-n-heptane sulfonate (PFHpS), perfluoro-n-octane sulfonate (PFOS), perfluoro-n-nonane sulfonate (PFNS), perfluoro-decane sulfonate (PFDS), dodecalfluoro-3H-4,8-dioxanonanoate (ADONA), perfluoroether carboxylic acid (GenX), 6:2 chlorinated polyfluorinated ether sulfonate (F-53B), perfluoro-n-octane sulfonamide (PFOSA), N-ethyl-perfluoro-n-octane sulfonamide (N-EtFOSA), N-ethyl-perfluoro-n- octane sulfonamido acidic acid (N-EtFOSAA), N-ethyl-perfluoro-n-octane sulfonamido ethanol (N-EtFOSE), 4:2 fluorotelomer sulfonate (4:2 FTS), 6:2 fluorotelomer sulfonate (6:2 FTS), 8:2 fluorotelomer sulfonate (8:2 FTS), 6:2 polyfluoroalkyl phosphate diester (6:2 diPAP), 8:2 polyfluoroalkyl phosphate diester (8:2 diPAP) and 6:2/8:2 polyfluoroalkyl phosphate diester (6:2/8:2 diPAP) in methanol – see Table 1 to Table 5. The 14 isotope-labeled or internal standards (IS) were prepared in a methanol solution with a concentration of 0.5 µg/mL (each compound) containing perfluoro-n-[$^{13}C_4$]-butanoic acid (IS-PFBA), perfluoro-n-[1,2-$^{13}C_2$]-hexanoic acid (IS-PFHxA), perfluoro-n-[1,2,3,4-$^{13}C_4$]-octanoic acid (IS-PFOA), perfluoro-n-[1,2,3,4,5-$^{13}C_5$]-nonanoic acid (IS-PFNA), perfluoro-n-[1,2-$^{13}C_2$]-decanoic acid (IS-PFDA), perfluoro-n-[1,2-$^{13}C_2$]-undecanoic acid (IS-PFUnDA), perfluoro-n-[1,2-$^{13}C_2$]-dodecanoic acid (IS-PFDoDA), perfluoro-n-hexane-[$^{18}O_2$]-sulfonate (IS-PFHxS), perfluoro-n-[1,2,3,4-$^{13}C_4$]-octane sulfonate (IS-PFOS), N-ethyl-d5-perfluoro-n-octane sulfonamide (IS-N-EtFOSA), N-ethyl-d5-perfluoro-1-octane sulfonamido acetic acid (IS-N-EtFOSAA), N-ethyl-d9-perfluoro-n-octane sulfonamido ethanol (IS-N-EtFOSE), 1H,1H,2H,2H-perfluoro-[1,2-$^{13}C_2$]-octane sulfonate (IS-6:2 FTS) and (bis-)1H,1H,2H,2H-[1,2-$^{13}C_2$]-perfluorodecyl-phosphate (IS-8:2 diPAP). All PFAS-standards and IS-PFAS-standards were purchased from Wellington Laboratories (Ontario, Canada). For the analytics by HPLC-MS/MS an Agilent Technologies 1290 Infinity Series (Agilent Technologies, Santa Clara, CA, USA) HPLC and a SCIEX 4000 QTRAP mass spectrometer (AB Sciex Technologies, Framingham, MA, USA) in electrospray ionization (ESI) negative mode were used. A Luna 5 µm C18(2), 100 x 2 mm (Phenomenex, California, USA) was used as analytical column. Eluent-(a) was LC-MS-grade methanol and eluent-(b) was LC-MS-grade water containing 10 mM ammonium acetate. Further analytical settings were an analyzing time of 23 min, a flow rate of 0.3 mL/min, an injection volume of 10 µL and a column temperature of 40 °C. A summary of all reagents and materials used for the PFAS analysis is presented in Table 9 and the reagents compositions for the solid-phase extraction (SPE) procedure is provided in Table 10.

Table 9: Reagents and material for the PFAS analysis

reagents	grade and supplier
methanol	LC-MS-grade (Merck, Darmstadt, Germany)
ammonium acetate	for mass spectrometry (Sigma-Aldrich®, St. Louis, USA)
formic acid	98-100% (Merck, Darmstadt, Germany)
ammonia solution	25% (Fisher Scientific, Loughborough, UK)
PFAS-standards and isotope-labeled-PFAS-standards	50 µg/mL or 2 µg/mL (Wellington Laboratories, Ontario, Canada)
cartridge WAX	Oasis® WAX-column (6 mL, 150 mg; Waters Corporation, Milford, MA, USA)
cartridge HLB	Oasis® HLB-column (6 mL, 500 mg; Waters Corporation, Milford, MA, USA)

Table 10: Compositions for the solid-phase extraction (SPE) procedure (ISO 21675, 2019).

name of the reagent	composition
acetate buffer 0.025 mol/L, pH 4	(a) 96.7 mg ammonium acetate in 50 mL filtered tap water (b) 71.5 µL acetic acid in 50 mL filtered tap water Final: 10 mL of the acetate buffer (a) 40 mL acetic acid solution (b)
0.1% ammonia/methanol	200 µL ammonia solution (25%) in 50 mL methanol

2.2.2 Experiment I – TOP assay

For the first experiment (exp-I) 7 samples were prepared including one blank (O_3-stock solution). First, six 50 mL polypropylene (PP) tubes were filled with 40 mL of tap water that was pre-filtered with Oasis® HLB-column (6 mL, 500 mg) cartridges a couple of months before. All six samples were spiked with 10 µL of a PFAS-standard mix containing 31 PFAS with a concentration of 0.5 µg/mL (each compound), see Table 1-5. The samples were shortly shaken and vortexed. After one hour the temperature and the pH was measured for three of the samples with a standard-pH-meter PHM 210 from Radiometer Copenhagen. Only three samples were checked for the pH to have the possibility to encounter potential recovery loses by an adsorption of PFAS on the pH glass electrode (one blank and two samples). After the O_3-stock solution reached its equilibrium of approximately 40 mg O_3/L, 4 mL of the O_3-stock solution were extracted with a glass syringe (as explained above) and

injected into the sample A (approximately 1-2 cm below the surface two avoid cross contamination and possible losses throw adsorption). The sample than was closed and shaken for 15 seconds. This procedure was repeated three times (for B, C and D) to have four PFAS spiked and ozonated samples. The two blanks (I and II) were filled up to 44 mL with ozone free deionized water. One blank, which was an empty 50 mL PP-tube was filled up to 44 mL with the pure O_3-stock solution; this blank was kept open under the fume hood. The last blank was used to check for possible contaminations from the ozone reactor, especially concerning the polytetrafluoroethylene tubes. After 30 min the samples were checked for ozone residues with a potassium iodide paper. Subsequently the pH was measured again for the samples for which the pH was determined before the ozonation as well. The samples were stored at room temperature for approximately 3-4 hours and then all seven samples were spiked with 20 µL of a mass-labeled standard mix containing 0.5 µg/mL of each of the 14 surrogate compounds (see chapter 2.2.1). Subsequently the samples were shaken and vortexed for 10 seconds. Although the potassium iodide paper did not detect the presents of ozone residues before, the samples might still have contained some ozone residues, since ozone was perceived by smell during the spiking with the isotope labeled standards. Figure 4 shows the general experimental set up and Table 11 shows the summery of the sample list.

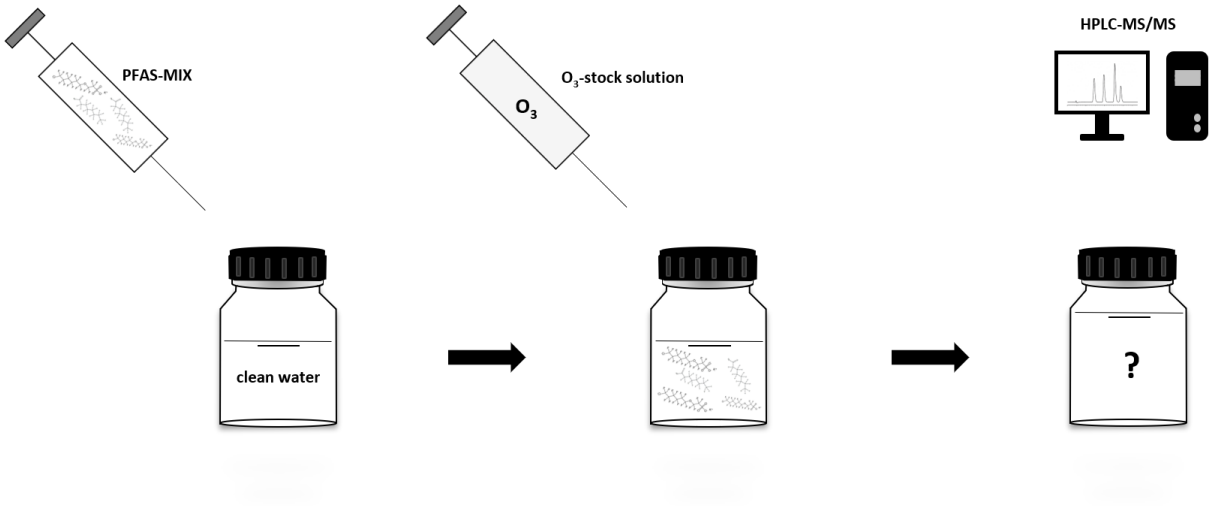


Figure 4: Methodological approach of the study experiment – from left to right: sample spiking, ozonation, sample preparation and analytical analysis.

Table 11: Sample list for experiment I

sample name	filtered tap water [mL]	PFAS-standard mix [µL]	O_3-stock solution [mL]	deionized tap water [mL]
blank I	40	10	-	4
blank II	40	10	-	4
sample A	40	10	4	-
sample B	40	10	4	-
sample C	40	10	4	-
sample D	40	10	4	-
blank O_3-stock	-	-	50	-

2.2.3 Experiment II – TOP assay

For the second experiment (exp-II) 7 samples were prepared including 2 blanks. First, five 50 mL polypropylene (PP) tubes were filled with 40 mL of tap water that was freshly pre-filtered with Oasis® HLB-column (6 mL, 500 mg) cartridges[7]. All five samples were spiked with the PFAS-standard mix similarly to experiment I (see chapter 2.2.2). The samples were then stored at 4 °C till further experimental procedures. On the day of the ozonation experiment the samples were transported in a polystyrene box to the second laboratory. The samples were stored at 4 °C meanwhile the ozone generator was started. After the O_3-stock solution reached its equilibrium of 40 mg O_3/L, 4 mL of the O_3-stock solution were extracted with a glass syringe (as explained above) and injected in the sample. The needle touched the ground of the sample bottle while the O_3-stock solution was slowly injected (to avoid a rapid outgassing of ozone). After the injection, the sample was carefully stirred with the needle for 3-5 seconds and then the sample was closed. This procedure was repeated two times to have three PFAS spiked and ozonated samples. The two blanks were adjusted to 44 mL with ozone free deionized water. One blank, which was an empty 50 mL PP-tube was filled up to 44 mL with the pure O_3-stock solution - this blank was kept open under the fume hood – and another blank (empty 50 mL tube) was filled with pure deionized water (44 mL, containing no O_3). The last two blanks were used to check for possible contaminations from the ozone reactor, especially concerning the polytetrafluoroethylene tubes. The samples were transported back in the polystyrene box to the first laboratory where they were stored at 4 °C till the next day. At the following day, the pH value was measured using pH-indicator

[7] The pH value was determined with pH-indicator strips; the pH of tap water was 7 and the pH of tap water filtered with Oasis® HLB-column (6 mL, 500 mg) cartridges was 5.

strips (Merck, (Darmstadt, Germany)). Approximately 50-100 µL of the sample were therefore extracted with a glass Pasteur pipette to cover the strips. Then all seven samples were spiked with 20 µL of an internal standard containing 0.5 µg/mL of isotope labeled PFAS (see chapter 2.2.1). Subsequently the samples were shaken for 10 seconds and they were ready for the solid-phase extraction (SPE) sample preparation. Table 12 shows the summary of the sample list for experiment II.

Table 12: Summary of the sample list for experiment II - *dw = deionized water

sample name	filtered tap water [mL]	PFAS-standard mix [µL]	O_3-stock solution [mL]	deionized tap water [mL]
blank I	40	10	-	4
blank II	40	10	-	4
sample F	40	10	4	-
sample G	40	10	4	-
sample H	40	10	4	-
blank (O_3-stock)		-	44	-
blank (dw*)		-	-	44
blank-blank	44	-	-	-

2.2.4 Experiment III – TOP assay

For third experiment (exp-III) 8 samples were prepared including one blank. Four 50 mL tubes containing 25 mL tap water were spiked with 10 µL of a 5 µg/mL PFAS-standard mix. One 50 mL tube containing 40 mL tape water was spiked with the same PFAS-standard mix and the same volume (10 µL). The blank contained only 50 mL tap water and was not spiked with the PFAS-standard mix. All samples were briefly vortexed. Additionally, two 50 mL tubes were each filled with 25 mL of wastewater treatment plant effluent. The pH values were determined by using pH-strips (20 µL of the sample volume were used). To three samples 25 mL of the O_3-stock solution were added by placing the needle almost to the ground of the tube, injecting the O_3-stock solution and simultaneously stirring carefully to enable a good mixture. After the injection, the tube was immediately closed. The needle was wiped between each injection with a clean paper to avoid cross contamination. The same procedure was repeated for one of the effluent samples while the second effluent sample was only filled up to 50 mL with deionized tap water. Sample number four was not ozonated but was filled up to 50 mL as well with deionized tap water. This sample was used as a reference for PFAS

untreated with ozone. The sample that contained 40 mL of spiked tap water was treated similarly with the O_3 as explained above but by using only 10 mL of the O_3-stock solution. Table 13 provides a summary of the sample list.

Table 13: Summary of the sample list for experiment III

sample name	tap water [mL]	PFAS-standard mix [µL]	O_3-stock solution [mL]	deionized tap water [mL]
blank I	25	10	-	25
sample I	25	10	25	-
sample J	25	10	25	-
sample K	25	10	25	-
sample a	40	10	10	-
effluent (no O_3)	25	-	-	25
effluent (O_3)	25	-	25	-
blank-blank	50	-	-	-

2.2.5 Sample preparation and extraction procedure

For the extraction of the analytes the ISO 21675 (2019) solid-phase-extraction (SPE) method was applied with slight adjustments. First, the samples were adjusted to the pH value of 3. Therefore, 10 µL of concentrated formic acid was added to the samples. Although the ISO 21675 (2019) suggests acidic acid, formic acid was used since a higher volume of acidic acid would have been needed to adjust for a pH value of 3. The sample was shortly shaken, and the pH value was determined with pH-indicator strips (as explained in chapter 2.2.4). Secondly, the samples were ultrasonicated for 15 min. Thirdly, the SPE-method was applied by four performing steps: condition, load sample, wash and elution. After reducing the sample volume to 1 mL, the sample was ready to be injected into the HLPC-MS/MS system.

2.3 Implementation of the TOP assay

For the TOP assay the measured concentrations in ng/mL for all samples were transformed to their respective molarity (M = mol/L). For example, the molecular weight of PFBA is 214.04 g/mol (see Table 1). If the concentration in one sample for PFBA was 5 ng/mL, then 5 ng/mL were divided by 214.04 g/mol resulting in 0.0233 nmol/mL which is equal to 0.0233 µM. These calculations were performed for each single substance and sample. For the experiment II, two blanks and three samples were prepared, therefore the mean of the blanks (n = 2) was compared to the mean of the ozonated samples (n = 3). For the experiment III the mean of the ozonated samples (n=3) were compared to the mean of the blank from experiment III and the two blanks from experiment II. Figure 5 shows how the samples were compared with each other for the TOP assay. Since the systematic measurement error in general can be estimated to be around 15-20% for the detailed evaluation of gains and losses of PFAS after ozonation the boundary was set at 20% (except for exp-I for which the boundary was set at 15%). This means that only PFAS which showed decreases or increases of ≥ 20% after the ozonation were included in the detailed evaluation, gains and losses of PFAS under 20% were considered as unaffected by ozone.

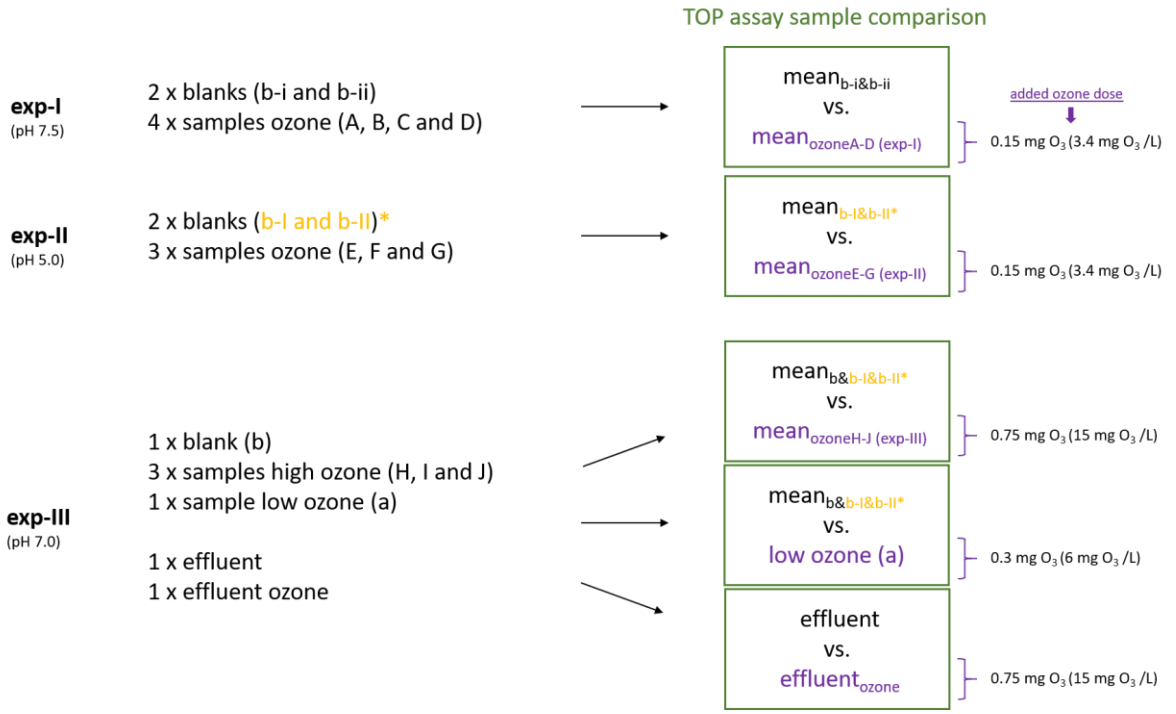


Figure 5: Comparison of treated and untreated samples with ozone – for exp-I and exp-II filtered tap water was used, but for exp-III unfiltered tap water was used

2.4 Quality control

Authentic isotope labeled standards (see chapter 2.2.1) were spiked to the samples before the extraction procedure to allow to correct for recovery losses during the sample preparation. Corrections for recovery losses are necessary to avoid errors in quantification and to make the results more accurate. The correction for the recovery loss for each compound was performed by calculating first the recovery of the authentic isotope labeled standard by comparing its peak area with the peak area of the control standard, and secondly by multiplying the related compound concentration by 100 divided by the calculated recovery of the authentic isotope labeled standard.

For the quality control (QC) a 1:1 mixture of methanol and LC-MS water containing 10 mM NH_4Ac was spiked with 10 µL of the 0.5 µg/mL PFAS-standard mixture and 20 µL of the 0.5 µg/mL isotope labeled PFAS standard mixture; for experiment III the same procedure was repeated but instead of the methanol/water mixture methanol alone was used. A second QC was prepared equally but without the PFAS-standard mixture (i.e. only the isotope labeled PFAS standard was used). A 10-point calibration curve was prepared with 1:1 mixture of methanol and LC-MS water (10 mM NH_4Ac) with the concentrations in ng/mL of 0.0, 0.05, 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0 and 25. For the experiment III a 11-point calibration curve was prepared only in methanol with the concentrations in ng/mL of 0.0, 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0, 7.5, 10.0 and 25.0. Limit of detections (LODs) and limit of quantifications (LOQs) were calculated in accordance with the DIN 32645 and at least one blank was used for each batch to check for potential contaminations.

3 Results and Discussion

3.1 Literature Review

In total 24 articles were found to be relevant for the present master thesis by browsing through the web of science (all databases, all years (1900-2020)) using the keyword-combinations “perfluoro* ozon*”, “perfluoro* TOP* assay*” and *perfluoro* biotransformation*”. When e.g. the keyword “PFAS*” was used less articles were found (2,670 articles) compared to the usage of the keyword “perfluoro” (39,839 articles). With the keyword-combination “perfluoro* ozon*” 17 articles were found to be relevant for the present master thesis, since these articles were related to PFAS degradation in (waste)water treatment plants (mostly) by ozone or other (oxidative) treatment techniques. In total 37 articles with the keyword-combination “perfluoro* TOP* assay*” were found. Out of these 7 seemed to be relevant for this study. With this keyword-combination though the first article that described the TOP assay from Houtz and Sedlak (2012) was not found. The reason for that is probably that neither the word “TOP” nor “assay” were used in the title or abstract of their article. Further keyword-combinations used were “perfluoro* environment*” and “perfluoro* human* health*; no articles were collected though by using these keyword-combinations, since a private online library contained already most of those relevant articles. Further references used for the present study were (I) picked from an own personal private online library which contained literature on PFAS which was collected during other projects and (II) literature (suggestions) from the supervisors. A review on the current knowledge of PFAS based on the literature (used reference collection shown in Figure 6) are provided in chapter 1 and are discussed as well in the sub-chapters of chapter 3.

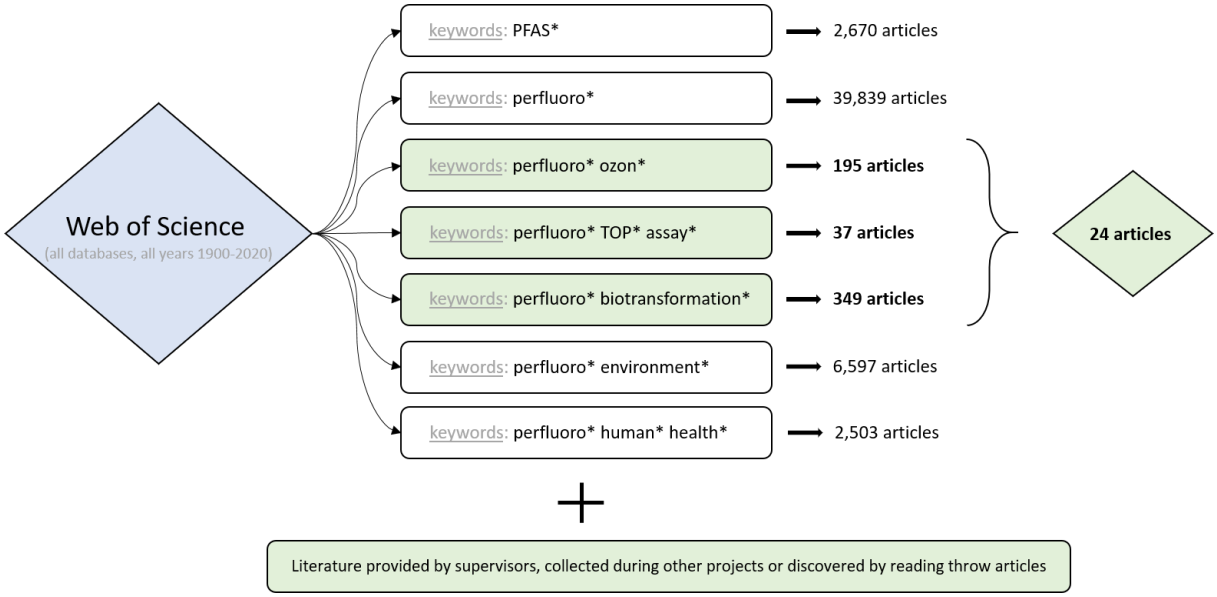


Figure 6: Sources of references (December 2019 - February 2020) – in green the most relevant information that was used

3.2 Measured ozone concentrations

The measured O_3-concentrations of the respective experimental day are shown in Table 14. The values from the direct measurement via UV-detection were quite similar compared to the resulted values from the indigo-method – results from the direct measurement via UV-detection are shown in the appendix. The mean O_3-concentration of the first two experimental days was 40 mg O_3/L. The mean O_3-concentration on the third experimental day was 32.5 mg O_3/L. The lower O_3-concentration on the third day can be explained by the usage of a different aquarium aerator stone. For reasons of simplicity 30 mg O_3/L were used for all calculations for experiment III. The low added amount of methanol (10 µL) showed no significant influence on the measured O_3-concentration (data shown in the appendix). Although, it is expected that the methanol present in one sample is a competitor for PFAS concerning the reaction with ozone molecules, the influence could be neglected for the present experiments. Since the added volume of the O_3-stock solution to the samples for the first two experiments was 4 mL, it can be estimated that the samples were treated with approximately 0.15 mg O_3. For the third experiment the samples were treated with approximately 0.75 mg O_3 (25 mL of the O_3-stock solution), and 0.3 mg O_3 (10 mL of the O_3-stock solution), respectively. The instantaneous ozone demands (IOD) which expresses the amount of ozone that is consumed within the first 5 seconds and ozone decay curves were not calculated for the present master thesis for reasons of the limited availability of the analytical instruments. But according to Xiao et al. (2018) polyfluoroalkyl amides showed a half-life of a few minutes during ozonation (around 3.2-7.9 minutes).

Table 14: Measured and calculated O_3-concentations of the stock solution (mean values of duplicates)

day	sample	O_3-concentration [mg O_3/L]	n
	before the experiment I	37	2
	during the experiment I	36	2
	after the experiment I	46	2
1)	mean I	39.7 (± 5.5)	6
	before the experiment II	35	2
	during the experiment II	41	2
	after the experiment II	42	2
2)	mean II	39.3 (± 3.7)	6

	before the experiment III	32	2
	after the experiment III	33	2
3)	mean III	32.5 (± 0.70)	4

3.3 Experiment I

For the first experiment it was assumed that for a wastewater effluent with a dissolved organic carbon (DOC) concentration of 7 mg approximately 4-5 mg O_3/L would be considered as appropriate, to account for the recommended specific ozone dose of 0.6-0.7 mg O_3 / mg DOC (Schaar, 2016). The samples were therefore treated with approximately 0.15 mg O_3 (= 4 mL of the O_3-stock solution), which would be equal to a concentration of 3.4 mg O_3/L. Since the sample water was matrix free and no competitors to the spiked PFAS were present the O_3-concentration of 3.4 mg O_3/L seemed very reasonable. Furthermore, quite similar ozone doses were used in other experiments (Anumol *et* al., 2016). The pH value of the samples was 7.5 before and after the ozonation. The recoveries were unacceptably low for 12 PFAS (recoveries < 30%, see Figure 16 in Appendix C). The average recovery for all substances was 44.1% (± 35). To enable a statement of an observed PFAS concentration increase or decrease the concentration of the treated sample should at least differ from the concentration of the untreated sample by ≥15%. The concentration of PFOSA and N-EtFOSAA decreased by 18% and the concentration of N-EtFOSE declined by 62%. PFBS and GenX were the only two substances that showed a concentration increase (25% and 52%). The concentrations of the treated samples in comparison to their corresponding untreated samples are shown in Figure 7. The present results from the first experiment would indicate that 73% of the mole fractions from PFOSA, N-EtFOSAA and N-EtFOSE are explained by a decrease of the PFBS mole fraction. The 27% of the mole fraction leftovers are unclear, although it can´t be excluded, that some molecules were transformed into GenX. Although the transformation to GenX seems rather unrealistic a potential explanation could be that “activated and highly reactive” PFBA are present as “unzipping by-products” and react with some sort of trifluoroacetic acid branched alcohol and “activated oxygen or radicals” to form the typical GenX molecule as shown in Figure 8.

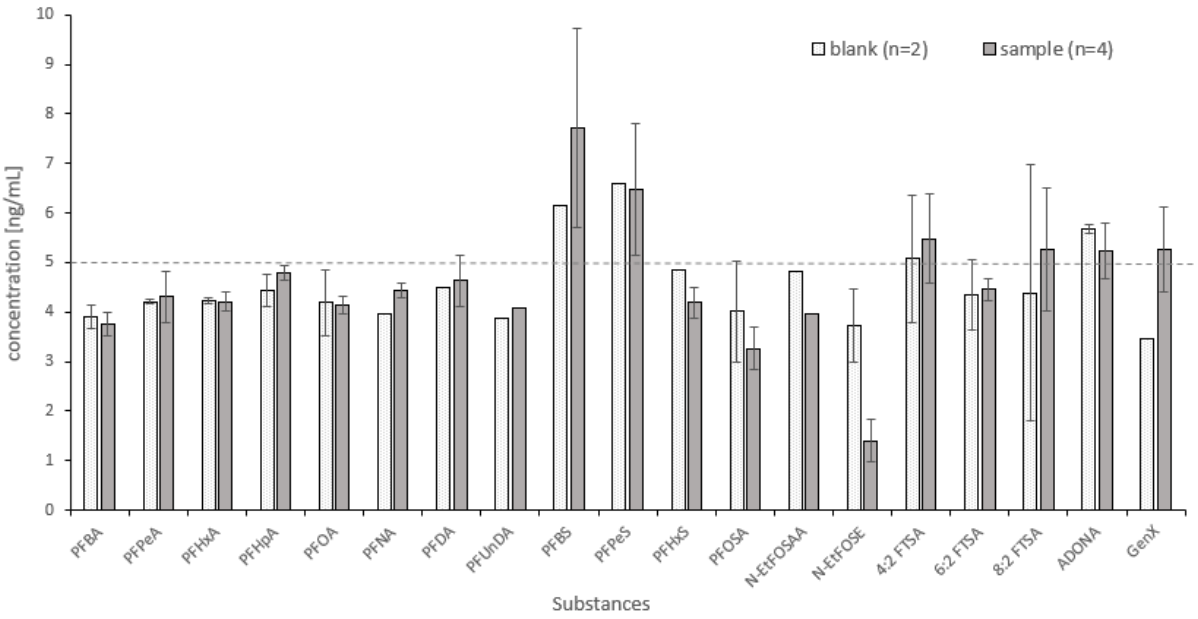


Figure 7: Samples after ozonation in comparison to their related blanks

However, the TOP assay in this form can´t explain the precursors via the determination of PFAAs, since essential PFAAs for the TOP assay such as PFOS could not be included in the present calculations. But it needs to be mentioned that several unfavorable working steps were performed, which might have had a negative effect on the results. For example, while injecting ozone the needle was only dipped 2 cm into the sample, so probably ozone gassed out, and furthermore the samples were adjusted to room temperature which probably promoted the outgassing of ozone.

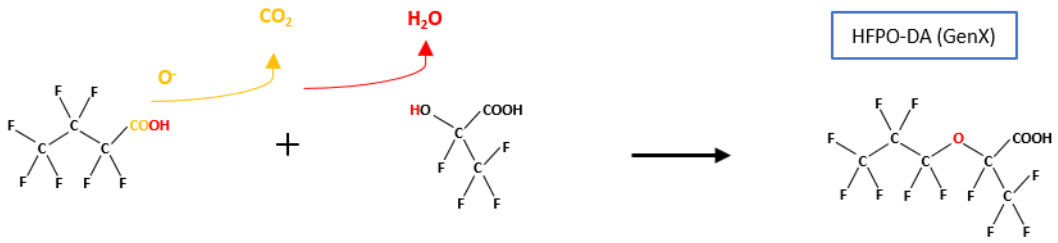


Figure 8: Potential transformation of by-products to GenX during ozonation

3.4 Experiment II

For the second experiment the recoveries improved by a factor of approximately 1.6 for all substances in contrast to the first experiment (see Figure 9). But the achieved recoveries were still improvable (average recovery: 69.6% (± 23)). The pH value before and after the ozonation was 5. Tap water filtered with HLB-cartridges decreases the pH value. Before the performance of the experiment it was assumed that filtered tap water with HLB-cartridges would reduce potential contaminations and that a slightly lower pH value would not have an impact on the experimental results. However, the results indicate that the lower pH value had a negative influence on the results, as no degradation of PFAS was observed. Additionally in literature it was reported that at lower pH values O_3 decomposes more slowly which enables a “higher” steady-state O_3 concentration (Lin et al., 2012). This probably explains why the PFAS were not degraded since PFAS by themselves have no affinity to interact with other molecules.

Figure 10 shows the concentrations of the treated samples in comparison to their related blanks. Although this time the ozone injection was performed by placing the needle of the syringe to the bottom of the sample, the results should no significant and obvious differences between the treated and untreated samples. The explanation for that is probably that the pH was too low, and that the ozone concentrations in general were too low for the performance of the TOP assay. Therefore, further interpretations of the results seemed unnecessary. For the third experiment much higher ozone concentrations were used at pH 7 therefore to account for all pre-performing errors, and furthermore the sample volume was increased to 50 mL to have a very low air space in the sample tube to lower the potential outgassing of ozone.

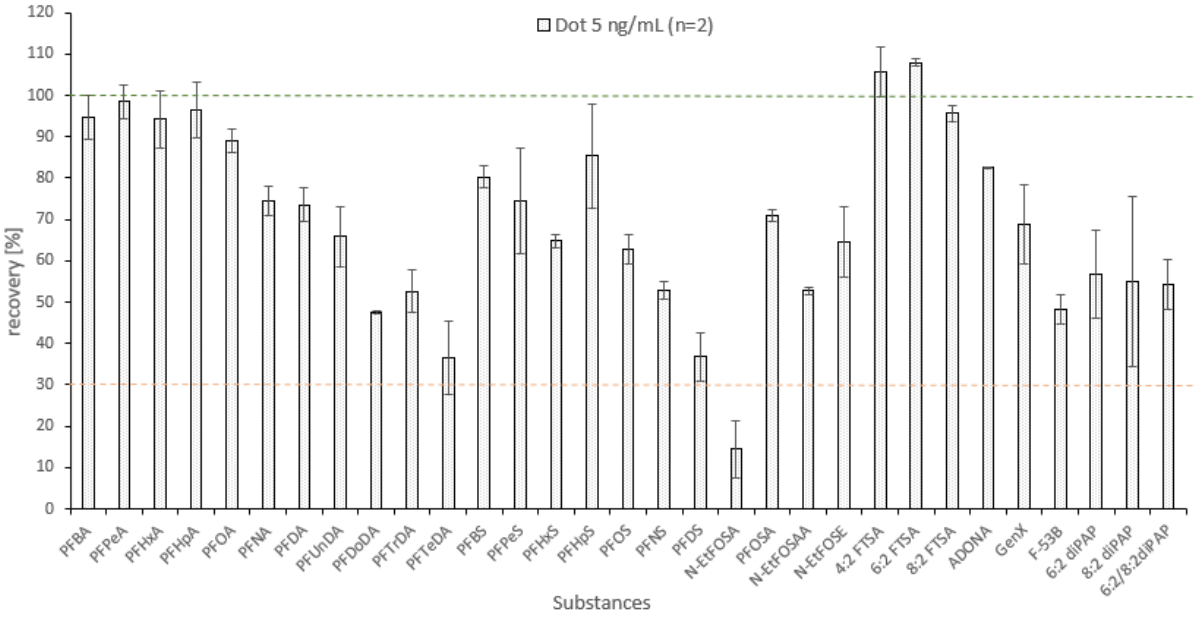


Figure 9: Achieved recoveries by applying the original ISO 21675 (2019)

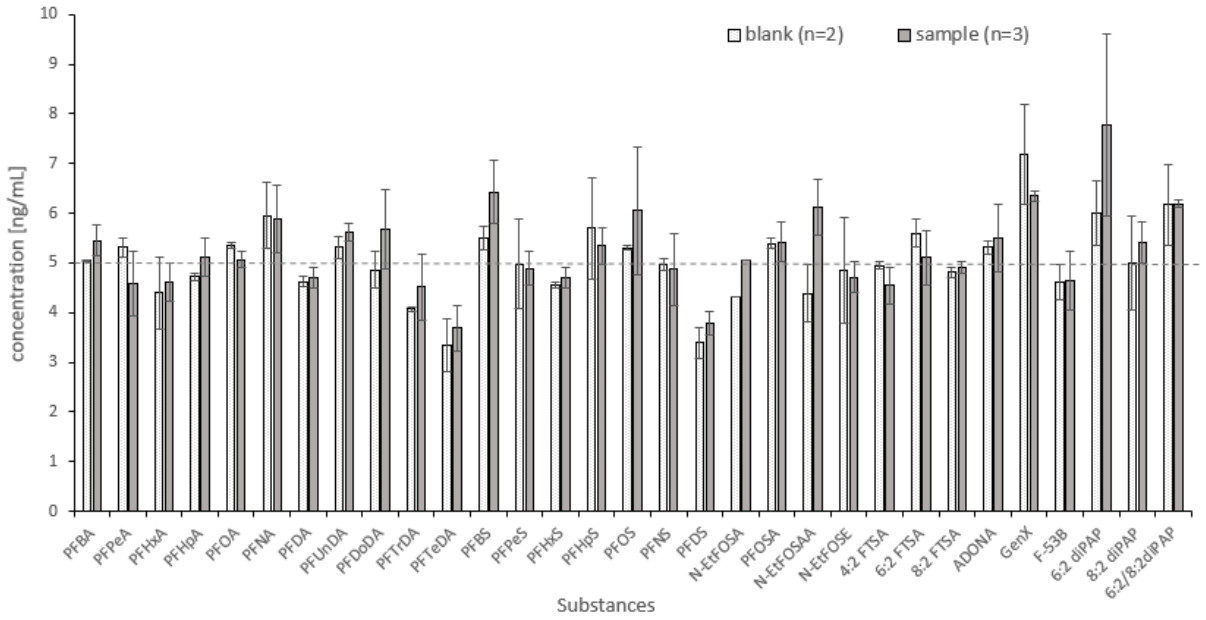


Figure 10: Sample concentrations after ozonation in comparison to their related blanks for the second experiment

3.5 Experiment III

3.5.1 Recoveries of native and internal isotope labeled standards

The mean recoveries of all 14 internal isotope labeled standards in 6 samples was 90.4% (± 18) and for all native standards 78.2 (± 14%). The results were much better compared to exp-I and exp-II. Figure 14 shows the recoveries for each of the 31 observed PFAS. The blue bars represent the recoveries for all 31 native PFAS of one spiked blank that was not ozonated. The bars with the bright patterns in Figure 11 represent the recoveries of the 14 internal isotope labeled standards including the standard deviation (n=6). Although the standard deviations for some substances (e.g. 6:2 FTS) are a bit wider, except for N-EtFOSA all recoveries can be considered as very good, and N-EtFOSA as quite acceptable. As shown in exp-II the recoveries of the carboxylic acids declined with an increasing chain length, and in exp-I the recoveries for PFTrDA and PFTeDA were even below 30%. The recoveries though in exp-III for PFTrDA and PFTeDA were > 75%. Montes et al. (2020) reported recoveries for these compounds as well of 80-100% using WAX-cartridges. Considering the reported recoveries by Montes et al. (2020) as well as the presented recoveries in the present master thesis I would strongly recommend to perform slight adjustments when using the ISO 21675 (2019).

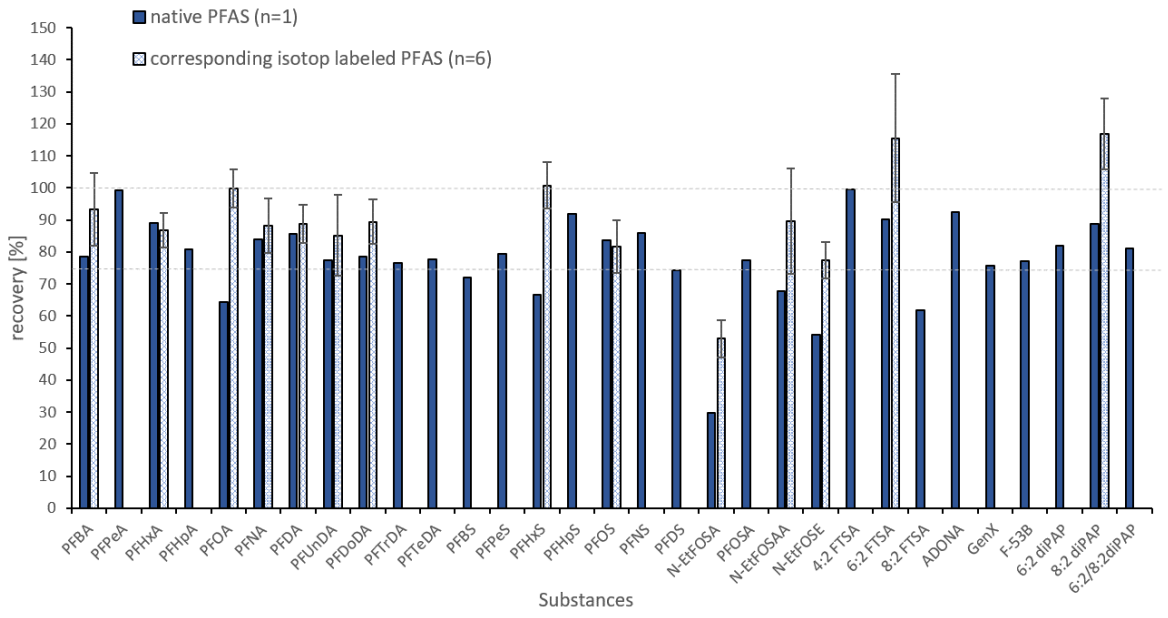


Figure 11: PFAS recoveries after the extraction for experiment III.

Looking at the sample concentrations, the mean of three blanks (including two from exp-II and one form exp-III) were 4.85 ng/mL (±0.84 ng/mL, or ± 17%). Figure 12 shows that especially the PFOS concentrations increased after the ozone treatment (only one blank from exp-III is shown in Figure 12). The PFOS concentrations were much higher after the treatment with a higher ozone dose (sample H-J) compared to a lower ozone dose (sample a). The substances that showed an observable decrease were N-EtFOSA and N-EtFOSE.

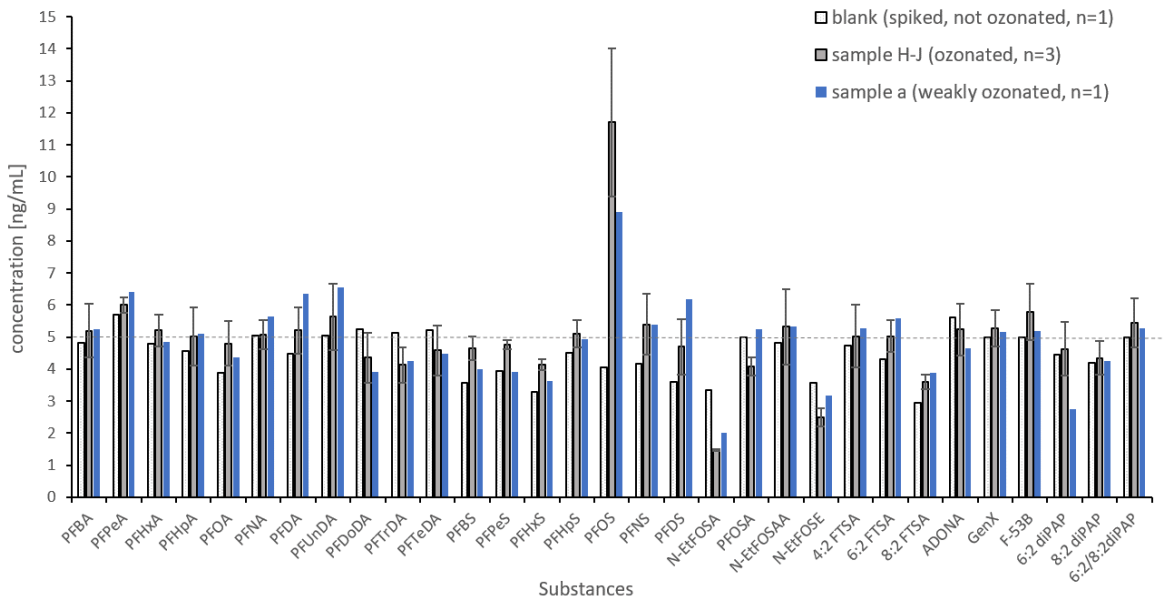


Figure 12: Concentrations of non-ozonated and ozonated samples from experiment III

3.5.2 Evaluation of the applied TOP assay

Comparison of the molarity of blank samples versus ozonated samples:

The molarity of the mean blanks from all substances (0.3380 µM) was compared to the molarity of the ozonated samples H, I and J (0.3469 µM). The molarities from the mean blanks and the ozonated samples showed an accordance of 97.4%. When the branched-PFOS (see chromatograms in Appendix F) were included the accordance sligthly decreased to 95.2%.

In contrast, the molarity of the mean blank compared to the molarity of sample a (0.3367 µM) was 100.4% and after the inclusion of the branched-PFOS 99.1%.

This results demonstrate a very good accuracy considering, that the added PFAS concentration was equal in the blanks and in the ozonated samples. The idea of the TOP

assay is, that only the substances that are present in one sample can be transformed from a less persistent PFAS to a more persistent one.
For the ozonated samples of experiment III (samples H, I and J, containing the PFAS-standard mix and tap water) the molarity of the increased substances (PFOS, PFDS and F-53B) was 0.0177 µM and for the decreased substances (N-EtFOSA, PFOSA, NEtFOSE and 8:2 diPAP) was 0.0114 µM. This is an accordance of 154.8%, which demonstrated the balance in molarity could not be precisely explained.
For sample a, for which a lower ozone dose was used, around 120.3% of the molarity balance could be explaind, which is slightly better, indicating that lower ozone doses might be better for the usage of the TOP assay. The increases and decreases of different PFAS after the ozone treatment are shown in Table 15.

Comparison of the molarity of effluent samples versus ozonated effluent samples:
In the effluent the molarity of all detected PFAS was 0.0054 µM and in the ozonated effluent 0.0613 µM. These results indicate that after the ozonation the detected PFAS increased by 91.1%. These results would be inline with previous studies that stated that approximately 65% to 75% of PFCAs are generated by the oxidation of precursors (Houtz et al., 2018). Althought in the present study 6:2 FTS accounted for the highest increase short chain PFCAs contributed a lot to the increase as well (see Table 15). Before the ozonation it was estimated that the effluent contained 74.7 ng PFAS/L. After the applied TOP assay it can be estimated that the effluent contains 840 ng PFAS/L. The reported range of PFOS concentrations alone in 8 observed wastewater effluents in Austria was 1-100 ng/L (BMLFUW, 2017). Houtz et al. (2018) estimated that sometimes the TOP assay tends even to underreport the total PFAS concentrations – organic fluorine can convert to inorganic fluorine, which than is not captured with the HPLC-MS/MS - and therefore it can´t be excluded that the PFAS concentrations in the effluent might even be higher than estimated with the applied TOP assay.

Comparison of the results with reports in literature:
In the present study the perfluoroakly sulfonamides degraded at around 22-62% and mostly the PFOS concentrations increased after the ozonation (see Table 15).
Neither the PFOA concentration nor the concentration of short chain carboxylic acids (< C7) increased in the spiked samples after the ozonation.
This results are quite in contrast to the observation of a biodegradation study that reported that N-EtFOSE transformed to perfluoroocantoate (Lange, 2000).
Martin et al. (2006) suggested for example that perfluoroalkyl sulfonamides degrade under atmospheric oxidative conditions to PFCAs, while the present study would indicate that perfluoroalkyl sulfonamides degrade mostly to PFOS in water samples under oxidative conditions.
These differences might be explained considering that transformation products depend on the matrix where the degradation or metabolism takes place.

A previous study in which surface water samples were ozonated reported the transformation of polyfluoroalkyl amides (FA) and polyfluoroalkyl sulfonamides (FS) to both PFOA and PFOS (Xiao et al., 2018). In contrast to the present master thesis Xiao et al. (2018) used other FA and FS for their experiments, but those compounds were at least structurally similar to e.g. N-EtFOSE or N-EtFOSA. Xiao et al. (2018) stated that FS are less susceptible than FA to mild oxidation treatments (e.g. chlorination), but FS are quickly degraded to both PFOS and PFOA during conventional ozonation (Xiao et al., 2018). In this study, only the degradation from FS to PFOS was observed in the spiked samples.

It is unsure if any FA or FS in general have been present in the effluent, however, the effluent showed no increase in PFOA or PFOS after the ozonation. But the concentrations of short chain PFCAs increased with a decreasing chain length in the effluent after the ozonation. Furthermore, the PFBS and the 6:2 FTS concentrations increased in the effluent after the ozonation. The substance in the effluent that showed the highest increase after ozonation was 6:2 FTS. Althought it was recently reported that 6:2 FTS could not be removed by ozonation in wastewater (Glover et al., 2018) it was quite surprising that the concentration increased to such an extent.
In contrast to Glover et al. (2018) and the present master thesis Dombrowski et al. (2018) reported that 6:2 FTS can degrade under oxidative conditions (using persulfate $S_2O_8^{2-}$) to PFHxA and to a lesser extent to PFPeA and PFBA. Martin et al. (2019) as well reported that 6:2 FTS converted to PFCAs in the TOP assay by 10-40mol%. However, as mentioned before the applied TOP assay in the previous studies were conducted by the method presented by Houtz and Sedlak (2012) using persulfate $S_2O_8^{2-}$ instead of ozone.
Glover et al. (2018) reported that ozonation resulted in an increase of PFAA levels, especially PFPeA (+53%), PFHxA (+30%), PFHpA (53%) and PFOA (+24%). Wu et al. (2018) reported that PFOA could be degraded with persulfate-assisted photocatalytic ozonation with occurring main intermediates such as PFHpA, PFHxA, PFPeA and PFBA and a similar study experiment reported analogical results (Huang et al., 2016).
Similar increases or even higher (probably since higher ozone doses were used) were observed in the ozonated effluent but not in the spiked samples.

This would initiate two hypotheses: 1) that other PFAS as well which were not included in the present study were present in the effluent, which were transformed to short PFAAs by ozone, and 2) the matrix of the effluent enabled the creation of different radicals during the ozone exposure and these radicals were able to transform different PFAS to different short chain PFAAs.

Furthermore, Pisarenkto et al. (2015) stated that PFHxA and PFBS are the most commonly detected PFAS after ozonation of wastewater which would be in line with the present results. Houtz and Sedlak (2012) reported for their TOP assay where they used generated hydroxyl radicals by thermolysis of persulfate ($S_2O_8^{2-}$) under basic pH (≥12) conditions that 6:2 FTS,

and 6:2 diPAP transforms primarily to PFBA, PFPeA and PFHxA; the 8:2 analogs transform to PFOA and PFNA and that N-EtFOSAA oxidizes primarily to PFOA. Glover et al. (2018) reported as well that mostly PFCAs (primarily PFBA, PFPeA, PFHxA, PFHpA, PFOA and PFNA) increased in the TOP assay and that the increase rate decreased with the chain-length. The same observation was made in the present master thesis for the effluent but not for the spiked samples.

In a study conducted by Yi et al. (2018) it was observed that thioether groups in polyfluorinated substances were quite stable under sulfate reducing conditions. Zhang et al. (2019) investigated the fate of 15 PFEAs (including ADONA, F53B and GenX) in the TOP assay using persulfate as oxidant as suggested by Houtz and Sedlak (2012). The results of the latest two mentioned studies showed that most monoether and multiether PFEAs were quite stable in the TOP assay (Yi et al., 2018; Zhang et al., 2019). Only ADONA was converted into another PFEAs probably due to it´s accessibility via the hydrogen atom in the O-CFH-bond (Zhang et al., 2019). Another study indicated that GenX is neither oxidized in systems containing hydroxyl radicals nor sulfate radicals (Hatton et al., 2018). In contrast to these previous studies the present study observed increases of F-53B and decreases of GenX and no significant changes of ADONA in exp-III. In exp-I the opposite for GenX was observed, GenX increased by ozonation in the samples. Although it doesn´t seem very plausible on first sight that fluorotelomers, fluorosulfonamides or PFAAs are transformed into PFEAs, the potential occurring of such a process can´t be completely excluded. However, it is very difficult to explain results of the present study concerning GenX, F-53B and ADONA and probably the best would be to look at trends for GenX, F-53B and ADONA in further experiments. In any case as recommended by Zhang et al. (2019) PFEAs should also be included in the TOP assay instead of only focusing on PFAAs.

Comparing the two TOP assays, the one from Houtz and Sedlak (2012) and the one with ozone applied in the present master thesis, shows that the different mechanisms lead to different outcomes (especially for the spiked samples). This does not mean that one or the other method is better, but rather that this should be taken into consideration when one of these both TOP assays is applied. Moreover, the TOP assay with ozone in this form was up to my best knowledge used for the first time and certainly some improvements are necessary. For example, one study demonstrated that PFOA and PFOS are better degraded by ozone at alkaline conditions (pH ≥ 11) and that those substances hardly degrade at lower pH values (pH ≤ 5) (Lin et al., 2012). It is hypothesized that alkaline conditions are more favorable for the formation of superoxide radicals ($\cdot O_2^-$), which achieves the degradation of PFOS and PFOA (Lin et al., 2012). In a wastewater treatment plant it would be impossible to adjust the pH at alkaline conditions even though Lin et al. (2012) suggested that alkaline ozonation can be readily implemented in large scales. Additionally, they absolutely blinded out the short chain intermediates that would remain in the effluent. However, in the case of the TOP assay with ozone a pH adjustment to gain alkaline conditions (pH ≥ 11) can very

easily be applied and this might improve the performance of the assay and strengthen its applicability.

Why the PFOS concentation increased to such a hight contant in the spiked tap water samples can not be well explained. The molarity of the spiked untreated and spiked treated samples with ozone was quite similar. Since the same results could not be observed for the exp-I (pH 7.5; filtered tap water) compared to exp-III (pH 7; unfiltered tap water), it probably had something to do with the presence of different ions in the unfiltered tap water which might trigger radical production. Franke et al. (2019) noted in laboratory-scale trail using ozone that the removal of PFAS increased with the perfluorocarbon chain length and that the removal did not show a general trend regarding the PFAS functional group. For the present study a slightly similar trend was observed, PFDoDA decreased in the spiked samples compared to the shorter carboxylic acids. Even PFTrDA and PFTeDA slightly decreased after ozonation (shown in the appendix). But in contrast to Franke et al. (2019) the same could not be observed for the PFSAs but it needs to be mentioned that Franke et al. (2019) used an AOP with catalyst material and persulfate instead of ozone alone.

Additionally, another study did demonstrate that fluorotelomer unsaturated carboxylic acids (FTUCAs) were transformed into PFHxA (and PFPeA in much smaller quantities) from 6:2 FTUCA and PFOA (and PFHpA in much smaller quantities) from 8:2 FTUCA in ozone processes (Anumol et al., 2016). FTUCAs were not observed in the present study, but the latest observation might initiate that hypothesis that FTUCAs might have been present in the effluent.

Table 15: Increases and decreases of different PFAS after ozonation – „↑“ = in the effluent the respective compound was not detected but in the effluent after the ozonation

compound	high ozone dose	low ozone dose	effluent
PFBA			↑ 321%
PFPeA			↑ 130%
PFHxA			↑ 59%
PFHpA			↑ 53%
PFOA			↑
PFNA			↑
PFDA		↑ 39%	↑
PFUnDA		↑ 25%	
PFDoDA		↓ 21%	↑
PFBS			↑ 189%
PFHxS			↑
PFOS	↑ 140%	↑ 82%	
PFDS	↑ 36%	↑ 78%	
N-EtFOSA	↓ 62%	↓ 48%	
PFOSA	↓ 22%		
N-EtFOSE	↓ 44%	↓ 28%	
4:2 FTS			↑
6:2 FTS			↑ 3528%
GenX		↓ 20%	
F-53B	↑ 22%		
6:2 diPAP		↓ 43%	
8:2 diPAP	↓ 21%	↓ 23%	

3.5.3 Evaluation of ozone for wastewater treatment regarding PFAS

The present results showed that ozone alone is not capable to remove PFAS in water samples, which is in line with previous results (Schröder and Meesters, 2005; Eschauzier et al., 2012; Yang et al., 2014; Sun et al., 2018). AOPs though might enhance the removal efficiencies for some PFAS. It was argued for example that hydroxyl radicals are not responsible for PFAAs degradation (Dombrowski et al., 2018; Houtz and Sedlak, 2012)

which therefore would exclude the AOP/H_2O_2 process, and make other AOPs more promising.

According to Franke et al. (2019) the average removal efficiencies for PFAAs can be improved up to 77% with an additional catalyst material and/or persulfate compared to ozone alone. But in the review from Trojanowicz et al. (2018) on different AOPs it was reported that almost all tested methods leaded to the formation of shorter chain PFAS as the product of decomposition (Trojanowicz et al., 2018). In a UV/ozone combined treatment of 28 PFAS an increase of PFHxA and PFHpA was observed by an achieved removal of PFOA (Dai et al., 2019). In another study it was reported that the photodegradation (254 nm, 32 W) of PFOS resulted in C_7HF_{15} and $C_7F_{15}OH$, and furthermore in short-chain fluorocarbons such as CF_4, C_2F_6 and C_3F_8 (Yamamoto et al., 2007). In comparison previous studies demonstrated that GAC filters alone were able to remove PFNA, PFOS and PFHxS effectively and partially removed PFOA (around 50%) but failed to remove shorter-chain compounds (Eschauzier et al., 2012). The removal efficiencies, however, can differ by the type and the freshness of GAC (Merino et al., 2016). A combination of GAC with a pre-ozonation or better a pre-AOP step might improve the removal efficiencies. Ozone alone or an AOP could eliminate substances which are highly reactive with ozone and therefore disburden the GAC which than might remove more efficiently short-chain PFAAs as well. Mitchell et al. (2014) reported that hydroperoxide anions (HO_2^-) and superoxide radicals ($\cdot O_2^-$) are both reactive with PFOA and that both together can rapidly degrade PFOA. Furthermore, Mitchell et al. (2014) suggested that both radicals together have the potential to mineralize PFOA (i.e. release all fluorine atoms of the degraded PFOA). An AOP with high HO_2^- and $\cdot O_2^-$ yields therefore might be good choice as a pretreatment for GAC. More research on combined treatments with ozone and AOP with GAC to investigate the most efficient removal techniques for PFAS is certainly necessary. Focus should also be given on short- and ultra-short chain PFAS. High-pressure membrane applications (i.e. NF and RO) seem the most promising techniques to remove PFAS in water. But although the cost-effectivity of NF and RO might improve with advances in energy efficiency (Rahman et al., 2014) a proper disposal for the remained PFAS-enriched concentrate still needs to be considered. In general, reducing emissions from certain point sources would be much more efficient than spending money on high level technologies (e.g. RO, NF and AOP).

4 Summary and Conclusion

For the present master thesis, a review on the current knowledge on PFAS with a special focus on wastewater treatment was performed. Three laboratory experiments were carried out (i) to investigate the removal efficiencies of PFAS with ozone, (ii) to investigate which precursors and PFEAs are transformed by ozonation to PFAAs, and (iii) to evaluate the usefulness and applicability of the TOP assay with ozone as oxidizing agent. The knowledge on PFAS is steadily rising but in parallel growing complexity (e.g. development of new PFAS) might inhibit the compensation of the gained knowledge. Techniques which can efficiently remove PFAS in large-scale wastewater treatment plants are still under investigation but a combined treatment technique with AOP and GAC seems beside RO and NF the most promising one. Ozone alone is not able to remove PFAAs in water samples, but it can decrease the precursor content between approximately 20-60%. The decrease of precursors, however, leads to an increase of PFAAs. In tap water it was observed that a decrease of N-EtFOSA, PFOSA, N-EtFOSE, 6:2 diPAP and 8:2 diPAP increased mostly the linear and branched PFOS concentration. In the effluent the ozonation increased the PFCAs (C4-C10) and PFSAs (C4-C6) concentrations. In contrast to previous studies the 6:2 FTS concentration in the effluent highly increased after the ozonation. The applied TOP assay with ozone showed an accordance in the molarity between spiked untreated and spiked treated samples with ozone of 97,4% (treated with 0.75 mg O_3) and 100.4% (treated with 0.3 mg O_3). These results would indicate that the TOP assay with ozone is quite accurate. Unfortunately, the increases of PFOS could not precisely explain the decreases of precursors - 155% when treated with 0.75 mg O_3 and 120% when treated with 0.3 mg O_3. This precision might be improved using alkaline conditions. However, regarding the very good molarity accordance of the applied TOP assay it was estimated that the PFAS concentration (or molarity) in the effluent is probably 91% higher than determined with the target analysis alone. For a detailed comparison of the TOP assay presented by Houtz and Sedlak (2012) with the TOP assay applied for this master thesis more experiments would be recommended. One disadvantage that comes along with the presented TOP assay with ozone is that the respective set-up in a laboratory is required and not all laboratories are equipped with an ozone generator. The advantage is that if laboratory experiments are performed using an ozone generator, then the TOP assay can easily be performed in parallel to estimate the total PFAS content in a sample. Since the presented TOP assay was - to my best knowledge - performed for the first time, I would recommend more quality control measurements (find suggestions in Appendix H) in related follow up studies to confirm and strengthen the accuracy of the presented method for the estimation of the total PFAS content in (waste)water samples.

Bibliography

3M, 2020. 3M's Commitment to PFAS Stewardship - PFAS History. Available: https://www.3m.com/3M/en_US/pfas-stewardship-us/pfas-history/ [accessed: 10.02.2020].

Ahrens, L., Felizeter, S., Sturm, R., Xie, Z., Ebinghaus, R., 2009. Polyfluorinated compounds in waste water treatment plant effluents and surface waters along the River Elbe, Germany. Marine Pollution Bulletin 58, 1326–1333. https://doi.org/10.1016/j.marpolbul.2009.04.028

Anumol, T., Dagnino, S., Vandervort, D.R., Snyder, S.A., 2016. Transformation of Polyfluorinated compounds in natural waters by advanced oxidation processes. Chemosphere 144, 1788–1787. https://doi.org/10.1016/j.chemosphere.2015.10.070

ATSDR, 2018. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Perfluoroalkyls - Draft for Public Comment. 1–852.

Banks, R.E., Smart, B.E., Tatlow, J.C., 1994. Organofluorine Chemistry - Principles and Commercial Applications. Springer US, New York.

Barry, V., Winquist, A., Steenland, K., 2013. Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. Environmental Health Perspectives 121 (11-12), 1313–1318. https://doi.org/10.1289/ehp.1306615

Benskin, J.P., Yeung, L.W.Y., Yamashita, N., Taniyasu, S., Lam, P.K.S., Martin, J.W., 2010. Perfluorinated Acid Isomer Profiling in Water and Quantitative Assessment of Manufacturing Source. Environmental Science and Technology 44, 9049–9054. https://doi.org/10.1021/es102582x

BMLFUW, 2017. Bundesministerium für Land- und Forstwirtschaft, Umwelt und Wasserwirtschaft. Emissionen ausgewählter prioritärer und sonstiger Stoffe aus kommunalen Kläranlagen. 1–112.

Buck, R.C., Frankline, J., Berger, U., Conder, J.M., Cousins, I.T., de Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P., 2011. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. Integrated Environmental Assessment and Management 7 (4), 513–541. https://doi.org/10.1002/ieam.258

Dagnino, S., 2015. Analysis of PFASs in Biological Tissues and Fluids. In: DeWitt, J.C. (Ed.), Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Springer, Humana Press, 23–49. https://doi.org/10.1007/978-3-319-15518-0_2

D'Agostino, L.A., Mabury, S.A., 2014. Identification of novel fluorinated surfactants in aqueous film forming foams and commercial surfactant concentrates. Environmental Science and Technology 48, 121–129.

Dai, X., Xie, Z., Dorian, B., Gray, S., Zhang, J., 2019. Comparative study of PFAS treatment by UV, UV/ozone, and fractionations with air and ozonated air. Environmental Science: Water Research and Technology 5, 1897–1907. https://doi.org/10.1039/c9ew00701f

D'eon, J., Crozier, P.W., Furdui, V.I., Reiner, E.J., Libelo, E.L., Mabury, S.A., 2009. Observation of a Commercial Fluorinated Material, the Polyfluoroalkyl Phosphoric Acid Diesters, in Human Sera, Wastewater Treatment Plant Sludge, and Paper Fibers. Environmental Science and Technology 43, 4589–4594. https://doi.org/10.1021/es900100d

DIN 38408-3, 2011. Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung – Gasförmige Bestandteile (Gruppe G) – Teil 3: Bestimmung von Ozon (G 3).

Dombrowski, P.M., Kakarla, P., Caldicott, W., Chin, Y., Sadeghi, V., Bogdan, D., Barajas-Rodriguez, F., Chiang, S.-Y., 2018. Technology review and evaluation of different chemical oxidation conditions on treatability of PFAS. Remediation 28, 135–150. https://doi.org/10.1002/rem.21555

Du, G., Huang, H., Hu, J., Qin, Y., Wu, D., Song, L., Xia, Y., Wang, X., 2013. Endocrine-related effects of perfluorooctanoic acid (PFOA) in zebrafish H295R steroidogenesis and receptor reporter gene assays. Chemosphere 91, 1099–1106. https://doi.org/10.1016/j.chemosphere.2013.01.012

EFSA, 2018. European Food Safety Authority. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Journal 16(12):5194, 1–284. https://doi.org/10.2903/j.efsa.2018.5194

Eschauzier, C., Beerendonk, E., Scholte-Veenendaal, P., De Voogt, P., 2012. Impact of treatment processes on the removal of perfluoroalkyl acids from the drinking water production chain. Environmental Science and Technology 46, 1708–1715. https://doi.org/10.1021/es201662b

Eschauzier, C., Hoppe, M., Schlummer, M., De Voogt, P., 2013. Presence and sources of anthropogenic perfluoroalkyl acids in high-consumption tap-water based beverages. Chemosphere 90, 36–41. https://doi.org/0.1016/j.chemosphere.2012.06.070

Fei, C., McLaughlin, J.K., Tarone, R.E., Olsen, J., 2007. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environmental Health Perspectives 115 (11), 1677–1682. https://doi.org/10.1289/ehp.10506

Franke, V., Schäfers, M.D., Lindberg, J.J., Ahrens, L., 2019. Removal of per- and polyfluoroalkyl substances (PFASs) from tap water using heterogeneously catalyzed ozonation. Environmental Science Water Research & Technology 5, 1887–1896. https://doi.org/10.1039/C9EW00339H

Freberg, B.I., Haug, L.S., Olsen, R., Daae, H.L., Hersson, M., Thomsen, C., Thorud, S., Becher, G., Molander, P., Ellingsen, D.G., 2010. Occupational Exposure to Airborne Perfluorinated Compounds during Professional Ski Waxing. Environmental Science and Technology 44, 7723–7728. https://doi.org/10.1021/es102033k

Fromme, H., Wöckner, M., Roscher, E., Völkel, W., 2017. ADONA and perfluoroalkylated substances in plasma samples of German blood donors living in South Germany. International Journal of Hygiene and Environmental Health 220, 455–460. https://doi.org/10.1016/j.ijheh.2016.12.014

Giesy, J.P., Kannan, K., 2001. Global Distribution of Perfluorooctane Sulfonate in Wildlife. Environmental Science and Technology 35, 1339–1342. https://doi.org/10.1021/es001834k

Glover, C.M., Quinones, O., Dickenson, E.R.V., 2018. Removel of perfluoroalkyl and polyfluoroalkyl substances in potable reuse systems. Water Research 144, 454–461. https://doi.org/10.1016/j.watres.2018.07.018

Gordon, S.C., 2011. Toxicological evaluation of ammonium 4,8-dioxa-3H-perfluorononanoate, a new emulsifier to replace ammonium perfluorooctanoate in fluoropolymer manufacturing. Regulatory Toxicology and Pharmacology 59, 64–80. https://doi.org/10.1016/j.yrtph.2010.09.008

Gottschalk, C., Libra, J., Saupe, A., 2010. Ozonation of Water and Waste Water - A Practical Guide to Understanding Ozone and its Applications. WILEY-VCH, Weinheim.

Gump, B.B., Wu, Q., Dumas, A.K., Kannan, K., 2011. Perfluorochemical (PFC) exposure in children: associations with impaired response inhibition. Environmental Science and Technology 45, 8151–8159. https://doi.org/10.1021/es103712g

Gutmann, V., Hengge, E., 1988. Anorganisch Chemie - Eine Einführung, VCH.

Hakizimana, J.N., Gourich, B., Chafi, M., Stiriba, Y., Vial, C., Drogui, P., Naja, J., 2017. Electrocoagulation process in water treatment: A review of electrocoagulation modeling approaches. Desalination 404, 1–21. https://doi.org/10.1016/j.desal.2016.10.011

Hansen, K.J., Clemen, L.A., Ellefson, M., Johnson, H.O., 2001. Compound-Specific, Quantitative Characterization of Organic Fluorochemicals in Biological Matrices. Environmental Science and Technology 35 (4), 766–770.

Hatton, J., Holton, C., DiGuiseppi, B., 2018. Occurrence and behavior of per- and polyfluoroalkyl substances from aqueous film-forming foam in groundwater systems. Remediation 28, 89–99. https://doi.org/10.1002/rem.21552

Haug, L.S., Thomsen, C., Brantsaeter, A.L., Kvalem, H.E., Haugen, M., Becher, G., Alexander, J., Meltzer, H.M., Knutsen, H.K., 2010. Diet and particularly seafood are major sources of perfluorinated compounds in humans. Environment International 36, 772–778. https://doi.org/10.1016/j.envint.2010.05.016

Hoigné, J., Bader, H., 1983. Rate constants of reactions of ozone with organic and inorganic compounds in water - I. Water Research 17, 173–183.

Hoigne, J., Bader, H., 1977. Beeinflussung der Oxidationswirkung von Ozon und OH-Radikalen durch Carbonat. Vom Wasser 48., 283–304.

Hölzer, J., Midasch, O., Rauchfuss, K., Kraft, M., Reupert, R., Angerer, J., Kleeschulte, P., Marschall, N., Wilhelm, M., 2008. Biomonitoring of Perfluorinated Compounds in Children and Adults Exposed to Perfluorooctanoate-Contaminated Drinking Water. Environmental Health Perspectives 116, 651–657. https://doi.org/10.1289/ehp.11064

Hori, H., Yamamoto, A., Koike, K., Kutsuna, S., Osaka, I., Arakawa, R., 2007. Photochemical decomposition of environmentally persistent short-chain perfluorocarboxylic acids in water mediated by iron(II)/(III) redox reactions. Chemosphere 68, 572–578. https://doi.org/10.1016/j.chemosphere.2006.12.038

Houtz, E., Wang, M., Park, J.-S., 2018. Identification and Fate of Aqueous Film Forming Foam Derived Perand Polyfluoroalkyl Substances in a Wastewater Treatment Plant. Environmental Science and Technology 52, 13212–13221. https://doi.org/10.1021/acs.est.8b04028

Houtz, E.F., Sedlak, D.L., 2012. Oxidative Conversion as a Means of Detecting Precursors to Perfluoroalkyl Acids in Urban Runoff. Environmental Science and Technology 46, 9342–9349.

Huang, J., Wang, X., Pan, Z., Li, X., Ling, Y., Li, L., 2016. Efficient degradation of perfluorooctanoic acid (PFOA) by photocatalytic ozonation. Chemical Engineering Journal 296, 329–334. https://doi.org/10.1016/j.cej.2016.03.116

Hübner, U., Zucker, I., Jekel, M., 2015. Options and limitations of hydrogen peroxide addition to enhance radical formation during ozonation of secondary effluents. Journal of Water Reuse and Desalination 05.1, 8–16.

Huling, S.G., Pivetz, B., 2007. In-situ chemical oxidation: Engineering issue. EPA/600/R-06/072. Washington, DC: USEPA.

IPAN, 2018. International Pollutants Elimination Network. Fluorine-Free Firefighting Foams (3F) - Viable Alternatives to Fluorinated Aqueous Film-Forming Foams (AFFF).

IPEN, 2019. International Pollutants Elimination Network. Say no to sulfuramid: reasons for a worldwide ban on this agrotoxic chemical. Available: https://ipen.org/sites/default/files/documents/en_ipen-sulfuramide-factsheet-v1_10a-en.pdf [accessed: 21.02.2020].

ISO 21675, 2019. Water quality — Determination of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in water — Method using solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Janda, J., Nödler, K., Scheurer, M., Happel, O., Nürenberg, G., Zwiener, C., Lange, F.T., 2019. Closing the gap – inclusion of ultrashort-chain perfluoroalkyl carboxylic acids in the total oxidizable precursor (TOP) assay protocol. Environmental Science Process and Impacts 21, 1926–1935. https://doi.org/10.1039/c9em00169g

Joensen, U.N., Bossi, R., Leffers, H., Jensen, A.A., Skakkebaek, N.E., Jorgensen, N., 2009. Do perfluoroalkyl compounds impair human semen quality? Environmental Health Perspectives 117 (6), 923–927.

Kannan, K., Corsolini, S., Falandysz, J., Fillmann, G., Kumar, K.S., Loganathan, B.G., Mohd, M.A., Olivero, J., Van Wouwe, N., Yang, J.H., Aldous, K. m., 2004. Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries. Environmental Science and Technology 38, 4489–4495. https://doi.org/10.1021/es0493446

Kato, K., Ye, X., Calafat, A.M., 2015. PFASs in the General Population. In: DeWitt, J.C. (Ed.), Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Springer, Humana Press, 51–76. https://doi.org/10.1007/978-3-319-15518-0_3

Kennedy, G., Butenhoff, J., Olsen, G., O´Connor, J., Seacat, A.M., Perkins R., 2004. The toxicology of perfluorooctanoate. Critical Reviews in Toxicology 34 (4), 351–384.

Kirchhof, B., Wong, D., Van Meurs, J., Hilgers, R.D., Macek, M., Lois, N., Schrage, N.F., 2002. Use of perfluorohexyloctane as a long-term internal tamponade agent in complicated retinal detachment surgery. American Journal of Ophthalmology 133, 95–101. https://doi.org/10.1016/s0002-9394(01)01295-8

Kissa, E., 2001. Fluorinated Surfactants and Repellents: 2nd ed. Volume 97. Marcel Dekker, New York.

Kissa, E., 1994. Fluorinated surfactants: synthesis, properties, applications. Marcel Dekker, New York.

Knox, S.S., Jackson, T., Javins, B., Frisbee, S.J., Shankar, A., Ducatman, A.M., 2011. Implications of early menopause in women exposed to perfluorocarbons. The Journal of Clinical Endocrinology and Metabolism 96, 1747–1753. https://doi.org/10.1210/jc.2010-2401

Kudo, N., 2015. Metabolism and Pharmacokinetics. In: DeWitt, J.C. (Ed.), Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Springer, Humana Press, 151–175. https://doi.org/10.1007/978-3-319-15518-0_6

Lange, C.C., 2000. The Aerobic Biodegradation of N-EtFOSE Alcohol by the Microbial Activity Present in Municipal Wastewater Treatment Sludge. 3M Project ID: LIMS E00-2252, 1–55.

Lau, C., 2015. Perfluorinated Compounds: An Overview. In: DeWitt, J.C. (Ed.), Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances. Springer, Humana Press, 1–21. https://doi.org/10.1007/978-3-319-15518-0_1

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl Acids: A Review of Monitoring and Toxicological Findings. Toxicological Sciences 99 (2), 366–394.

Lee, H., D'eon, J., Mabury, S.A., 2010. Biodegradation of Polyfluoroalkyl Phosphates as a Source of Perfluorinated Acids to the Environment. Environmental Science and Technology 44, 3305–3310.

Lee, H., Mabury, S.A., 2011. A Pilot Survey of Legacy and Current Commercial Fluorinated Chemicals in Human Sera from United States Donors in 2009. Environmental Science and Technology 45, 8067–8074. https://doi.org/10.1021/es200167q

Lee, J.W., Choi, K., Park, K., Seong, C., Yu, S.D., Kim, P., 2020. Adverse effects of perfluoroalkyl acids on fish and other aquatic organisms: A review. Science of the Total Environment 707, 1–31. https://doi.org/10.1016/j.envpol.2019.113721

Lehmler, H.-J., 2005. Synthesis of environmentally relevant fluorinated surfactants - a review. Chemosphere 58, 1471–1496. https://doi.org/10.1016/j.chemosphere.2004.11.078

Li, Y., Fletcher, T., Mucs, D., Scott, K., Lindh, C.H., Tallving, P., Jakobsson, K., 2018. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occupational and Environmental Medicine 75, 46–51. https://doi.org/10.1136/oemed-2017-10465

Lim, X., 2019. The fluorine detectives - researchers are battling to identify and assess a worring class of persistent chemicals. Nature 566, 27–29.

Lin, A.Y.-C., Panchangam, S.C., Chang, C.-Y., Hong, P.K.A., Hsueh, H.-F., 2012. Removal of perfluorooctanoic acid and perfluorooctane sulfonate via ozonation under alkaline condition. Journal of Hazardous Materials 243, 272–277. https://doi.org/10.1016/ j.jhazmat.2012.10.029

Lin, H., Wang, Y., Niu, J., Yue, Z., Huang, Q., 2015. Efficient sorption and removal of perfluoroalkyl acids (PFAAs) from aqueous solution by metal hydroxides generated in situ by electrocoagulation. Environmental Science and Technology 49, 10562–10569. https://doi.org/10.1021/acs.est.5b02092

Lopez-Espinosa, M., Fletcher, T., Armstrong, B., Genser, B., Dhatariya, K., Mondal, D., Ducatman, A., Leonardi, G., 2011. Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with age of puberty among children living near a chemical plant. Environmental Science and Technology 45, 8160–8166. https://doi.org/10.1021/es1038694

Martin, D., Munoz, G., Mejia-Avendano, S., Vo Duy, S., Yao, Y., Volchek, K., Brown, C.E., Liu, J., Sauve, S., 2019. Zwitterionic, cationic, and anionic perfluoroalkyl and polyfluoroalkyl substances integrated into total oxidizable precursor assay of contaminated groundwater. Talanta 195, 533–542. https://doi.org/10.1016/j.talanta.2018.11.093

Martin, J.W., Ellis, D.A., Mabury, S.A., Hurley, M.D., Wallington, T.J., 2006. Atmospheric Chemistry of Perfluoroalkanesulfonamides: Kinetic and Product Studies of the OH Radical and Cl Atom Initiated Oxidation of N-Ethyl Perfluorobutanesulfonamide. Environmental Science and Technology 40, 864–872. https://doi.org/10.1021/es051362f

Melzer, D., Rice, N., Depledge, M.H., Henley, W.E., Galloway, T.S., 2010. Association between serum perfluorooctanoic acid (PFOA) and thyroid disease in the US national health and nutrition examination survey. Environmental Health Perspectives 118 (5), 686–692. https://doi.org/10.1289/ehp.0901584

Merino, N., Qu, Y., Deeb, R.A., Hawley, E., Hoffmann, M.R., Mahendra, S., 2016. Degradation and Removal Methods for Perfluoroalkyl and Polyfluoroalkyl Substances in Water. Environmental Engineering Science 33, 615–649. https://doi.org/10.1089/ees.2016.0233

Mitchell, S.M., Ahmad, M., Teel, A.L., Watts, R.J., 2014. Degradation of perfluorooctanoic acid by reactive species generated through catalyzed H2O2 propagation reactions. Environmental Science and Technology Letters 1, 117–121. https://doi.org/10.1021/ez4000862

Mollah, M.Y.A., Morkovsky, P., Gomes, J.A.G., Kesmez, M., Parga, J., Cocke, D.L., 2004. Fundamentals, present and future perspectives of electrocoagulation. Journal of Hazardous Materials 114, 199–210. https://doi.org/10.1016/j.jhazmat.2004.08.009

Montes, R., Rodil, R., Placer, L., Wilms, J.M., Cela, R., Quintana, J.B., 2020. Applicability of mixed-mode chromatography for the simultaneous analysis of C1-C18 perfluoroalkylated substances. Analytical and Bioanalytical Chemistry. https://doi.org/10.1007/s00216-020-02434-w

Munter, R., 2001. Advanced oxidation processes – current status and prospects. Proceedings of the Esonian Academy of Sciences 50, 59–80.

NCM, 2019. Nordic Council of Ministers. PFASs in the Nordic environment - Screening of Poly- and Perfluoroalkyl Substances (PFASs) and Extractable Organic Fluorine (EOF) in the Nordic Environment. 1–156.

Nilsson, H., Kärrman, A., Rotander, A., Bavel, B. van, Lindström, G., Westberg, H., 2013. Professional ski waxers' exposure to PFAS and aerosol concentrations in gas phase and different particle size fractions. Environmental Science Processes and Impacts 15, 814–822. https://doi.org/10.1039/c3em30739e

Nöthe, T., 2009. Zur Ozonung von Spurenstoffen in mechanisch-biologische gereinigten Abwässern. SHAKER VERLAG.

OECD, 2018. Organisation for Economic Co-operation and Development. Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFASs): Summary report on updating the OECD 2007 list of per- and polyfluoroalkyl substances (PFASs). Available: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV-JM-MONO(2018)7&doclanguage=en [accessed: 28.05.2019] OECD Environment, Health and Safety Publications Series on Risk Management No. 39.

OECD, 2015. Organisation for Economic Co-operation and Development. Working towards a global emission inventory of PFASs: Focus on PFCAS - status quo and teh way forward. Available: http://www.oecd.org/chemicalsafety/Working%20Towards%20a%20Global%20Emi

ssion%20Inventory%20of%20PFASS.pdf [accessed: 11.01.2020] OECD Environment, Health and Safty Publications Series on Risk Management No. 30.

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-Life of Serum Elimination of Perfluorooctanesulfonate, Perfluorohexanesulfonate, and Perfluorooctanoate in Retired Fluorochemical Production Workers. Environmental Health Perspectives 115, 1298–1305.

Olsen, G.W., Chang, S.-C., Noker, P.E., Gorman, G.S., Ehresman, D.J., Lieder, P.H., Butenhoff, J.L., 2009. A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans. Toxicology 256, 65–74.

Pan, Y., Zhang, H., Cui, Q., Sheng, N., Yeung, L.W.Y., Sun, Y., Guo, Y., Dai, J., 2018. Worldwide Distribution of Novel Perfluoroether Carboxylic and Sulfonic Acids in Surface Water. Environmental Science and Technology 52, 7621–7629. https://doi.org/10.1021/acs.est.8b00829

Pisarenkto, A.N., Marti, E.J., Gerrity, D., Peller, J.R., Dickenson, E.R.V., 2015. Effects of molecular ozone and hydroxyl radical on formation of N-nitrosamines and perfluoroalkyl acids during ozonation of treated wastewaters. Environmental Science: Water Research and Technology 5, 668–678. https://doi.org/10.1039/C5EW00046G

Pitter, G., Da Re, F., Canova, C., Barbieri, G., Jeddi, M.Z., Dapra, F., Manea, F., Zolin, R., Bettega, A.M., Stopazzolo, G., Vittorii, S., Zambelli, L., Martuzzi, M., Mantoan, D., Russo, F., 2020. Serum Levels of Perfluoroalkyl Substances (PFAS) in Adolescents and Young Adults Exposed to Contaminated Drinking Water in the Veneto Region, Italy: A Cross Sectional Study Based on a Health Surveillance Program. Environmental Health Perspectives 128, 1–12. https://doi.org/10.1289/EHP5337

Poothong, S., Papadopoulou, E., Padilla-Sanchez, J.A., Thomsen, C., Haug, L.S., 2020. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): From external exposure to human blood. Environment International 134, 1–9. https://doi.org/10.1016/j.envint.2019.105244

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, Fate and Transport of Perfluorocarboxylates. Environmental Science and Technology 40, 32–44.

Rahman, M.F., Peldszus, S., Anderson, W.B., 2014. Behaviour and fate of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in drinking water treatment: A review. Water Research 50, 318–340. https://doi.org/10.1016/j.watres.2013.10.045

Rappazzo, KristenM., Coffman, E., Hines, E.P., 2017. Exposure to Perfluorinated Alkyl Substances and Health Outcomes in Children: A Systematic Review of the Epidemiologic Literature. International Journal of Environmental Research and Public Health 14 (7), 691. https://doi.org/10.3390/ijerph14070691

Rayne, S., Forest, K., Friesen, K.J., 2008. Congener-specific numbering systems for the environmentally relevant C4 through C8 perfluorinated homologue groups of alkyl sulfonates, carboxylates, telomer alcohols, olefins, and acids, and their derivatives. Journal of Environmental Science and Health A 43, 1391–1401.

Renner, R., 2001. Growing concern over perfluorinated chemicals. Environmental Science and Technology 35, 154–160. https://doi.org/10.1021/es012317k

Riddell, N., Arsenault, G., Benskin, J.P., Chittim, B., Martin, J.W., McAlees, A., McCrindle, R., 2009. Branched perfluorooctane sulfonate isomer quantification and

characterization in blood serum samples by HPLC/ESI-MS(/MS). Environmental Science and Technology 43, 7902–7908.

Roeske, W., 2007. Trinkwasserdesinfektion: Grundlagen - Verfahren - Anlagen - Geräte - Mikrobiologie - Chlorung - Ozonung - UV-Bestrahlung - Membranfiltration - Qualitätssicherung, 2nd ed. Oldenbourg Industrieverlag, München.

Rogowski, I., Leonard, D., Gauvrit, J.-Y., Lanteri, P., 2007. Influence of fluorine-based additive content on the physical and physicochemical properties of ski gliding wax. Cold Regions Science and Technology 49, 145–150. https://doi.org/10.1016/j.coldregions.2007.03.010

Sadia, M., Yeung, L.W.Y., Fiedler, H., 2020. Trace level analyses of selected perfluoroalkyl acids in food: Method development and data generation. Environmental Pollution 263, 1–9. https://doi.org/10.1016/j.envpol.2019.113721

Sandford, G., 2000. Organofluorine chemistry. Philosophical Transactions of the Royal Society 358, 455–471.

Schaar, H., 2016. Ozonung von Kläranlagenablauf zur weitergehendenden Abwasserreinigung. Dissertation. Technische Universität Wien.

Scheirs, J. (Ed.), 1997. Modern Fluoropolymers. New York.

Schröder, H., Meesters, R., 2005. Stability of fluorinated surfactants in advanced oxidation processes--A follow up of degradation products using flow injection-mass spectrometry, liquid chromatography-mass spectrometry and liquid chromatography-multiple stage mass spectrometry. Journal of Chromatography A, 1082, 110–119. https://doi.org/10.1016/j.chroma.2005.02.070

Schultz, M.M., Barofsky, D.F., Field, J.A., 2004. Quantitative determination of fluorotelomer sulfonates in groundwater by LC MS/MS. Environmental Science and Technology 38, 1828–1835. https://doi.org/10.1021/es035031j

Shoeib, M., Harner, T., Ikonomou, M., Kannan, K., 2004. Indoor and outdoor air concentrations and phase partitioning of perfluoroalkyl sulfonamides and polybrominated diphenyl ethers. Environmental Science and Technology 38, 1313–1320. https://doi.org/10.1021/es0305555

Sun, Y., Angelotti, B., Brooks, M., Dowbiggin, B., Evans, P.J., Devins, B., Wang, Z.-W., 2018. A pilot-scale investigation of disinfection by-product precursors and trace organic removal mechanisms in ozone-biologically activated carbon treatment for potable reuse. Chemosphere 210, 539–549. https://doi.org/10.1016/j.chemosphere.2018.06.162

Taniyasu, S., Kannan, K., Yeung, L.W.Y., Kwok, K.Y., Lam, P.K.S., Yamashita, N., 2008. Analysis of trifluoroacetic acid and other short-chain perfluorinated acids (C2–C4) in precipitation by liquid chromatography–tandem mass spectrometry: Comparison to patterns of long-chain perfluorinated acids (C5–C18). Analytica Chimica Acta 619, 221–230. https://doi.org/10.1016/j.aca.2008.04.064

Trojanowicz, M., Bojanowska-Czajka, A., Bartosievicz, I., Kulisa, K., 2018. Advanced Oxidation/Reduction Processes treatment for aqueous perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) – A review of recent advances. Chemical Engineering Journal 336, 170–199. https://doi.org/10.1016/j.cej.2017.10.153

Tsitonaki, A., Petri, B., Crimi, M., Mosbaek, H., Siegrist, R., Bjerg, P.L., 2010. In Situ Chemical Oxidation of Contaminated Soil and Groundwater Using Persulfate: A

Review. Critical Reviews in Environmental Science and Technology 40, 55–91. https://doi.org/10.1080/10643380802039303

Vecitis, C.D., Park, H., Cheng, J., Mader, B.T., Hoffmann, M.R., 2009. Treatment technologies for aqueous perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA). Frontiers of Environmental Science and Engineering 3, 129–151. https://doi.org/10.1007/s11783-009-0022-7

Vestergren, R., Cousins, I.T., 2009. Tracking the pathways of human exposure to perfluorocarboxylates. Environmental Science and Technology 43, 5565–5575. https://doi.org/10.1021/es900228k

Wang, B., Yao, Y., Chen, H., Chang, S., Tian, Y., Sun, H., 2020. Per- and polyfluoroalkyl substances and the contribution of unknown precursors and short-chain (C2–C3) perfluoroalkyl carboxylic acids at solid waste disposal facilities. Science of the Total Environment 705, 135832. https://doi.org/10.1016/j.scitotenv.2019.135832

Wang, Y., Zhang, P., Pan, G., Chen, H., 2008. Ferric ion mediated photochemical decomposition of perfluorooctanoic acid (PFOA) by 254 nm UV light. Journal of Hazardous Materials 160, 181–186. https://doi.org/10.1016/j.jhazmat.2008.02.105

Wang, Z., Cousins, I.T., Scheringer, M., Buck, R.C., Hungerbühler, K., 2014. Global emission inventories for C4-C14 perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources. Environment International 70, 62–75. https://doi.org/10.1016/j.envint.2014.04.013

Wardman, P., 1989. Reduction Potentials of One-Electron Couples Involving Free Radicals in Aqueous Solution. Joruncal of Physical and Chemical Reference Data 18, 1637. https://doi.org/10.1063/1.555843

Wolf, C.J., Takacs, M.L., Schmid, J.E., Lau, C., Abbott, B.D., 2008. Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicological Sciences 106, 162–171. https://doi.org/10.1093/toxsci/kfn166

Wu, D., Li, X., Zhang, J., Chen, W., Lu, P., Tang, Y., Li, L., 2018. Efficient PFOA degradation by persulfate-assisted photocatalytic ozonation. Separation and Purification Technology 207, 255–261. https://doi.org/10.1016/j.seppur.2018.06.059

Xiao, F., Hanson, R.A., Golovko, S.A., Golovko, M.Y., Arnold, W.A., 2018. PFOA and PFOS Are Generated from Zwitterionic and Cationic Precursor Compounds During Water Disinfection with Chlorine or Ozone. Environmenal Science and Technology 5, 382–388. https://doi.org/10.1021/acs.estlett.8b00266

Yamamoto, T., Noma, Y., Sakai, S., Shibata, Y., 2007. Photodegradation of Perfluorooctane Sulfonate by UV Irradiation in Water and Alkaline 2-Propanol. Environmental Science and Technology 41, 5660–5665. https://doi.org/10.1021/es0706504

Yang, X., Huang, J., Yu, G., Deng, S., Wang, B., 2014. Stability of 6:2 fluorotelomer sulfonate in advanced oxidation processes: degradation kinetics and pathway. Environmental Science and Pollution Research 21, 4634–4642. https://doi.org/10.1007/s11356-013-2389-z

Yi, L.B., Chai, L.Y., Xie, Y., Peng, Q.J., Peng, Q.Z., 2016. Isolation, identification, and degradation performance of a PFOA-degrading strain. Genetics of Molecular Research 15, 1–12. https://doi.org/10.4238/gmr.15028043

Yi, S., Harding-Marjanovic, K.C., Houtz, E., Gao, Y., Lawrence, J.E., Nichiporuk, R.V., Iavarone, A.T., Zhuang, W.Q., Hansen, M., Field, J.A., Sedlak, D., Alvarez-Cohen, L., 2018. Biotransformation of AFFF Component 6:2 Fluorotelomer Thioether Amido Sulfonate Generates 6:2 Fluorotelomer Thioether Carboxylate under Sulfate-Reducing Conditions. Environmental Science and Technology 8, 283–288. https://doi.org/10.1021/acs.estlett.8b00148

Young, C.J., Mabury, S.A., 2010. Atmospheric perfluorinated acid precursors: chemistry, occurrence, and impacts. Reviews of Environmental Contamination and Toxicology 208, 1–102. https://doi.org/10.1007/978-1-4419-6880-7_1

Zappatini, A., Götz, C., 2015. Testverfahren zur Beurteilung der Behandelbarkeit von Abwasser mit Ozon - Anleitung und Durchführung von Laborversuche. ENVILAB AG, 1–36.

Zhang, C., Hopkins, Z.R., McCord, J., Strynar, M.J., Knappe, D., 2019. Fate of Per- and Polyfluoroalkyl Ether Acids in the Total Oxidizable Precursor Assay and Implications for the Analysis of Impacted Water. Environmental Science and Technology 6, 662–668. https://doi.org/10.1021/acs.estlett.9b00525

Zheng, G., Boor, B.E., Schreder, E., Salamova, A., 2020. Indoor exposure to per- and polyfluoroalkyl substances (PFAS) in the childcare environment. Environmental Pollution 258, 1–8. https://doi.org/10.1016/j.envpol.2019.113714

List of Figures

List of Tables

List of Abbreviations

9Cl-PF3ONS	6:2 chlorinated polyfluorinated ether sulfonate (major F-53B)
AC	activated carbon
ADONA	polyfluoroether carboxylic acid
AFFFs	aqueous film-forming foams
ASS	acid stock solution
diPAP	polyfluoroalkyl phosphate diester
DOC	dissolved organic carbon
ESI	electrospray ionization
FA	polyfluoroalkyl amides
FS	polyfluoroalkyl sulfonamides
FTSA	fluorotelomer sulfonate
FTUCAs	fluorotelomer unsaturated carboxylic acids
GAC	granulated activated carbon
HFPO-DA	perfluoroether carboxylic acid (GenX)
HPLC-MS/MS	high-performance liquid chromatography tandem-mass spectrometry
IOD	instantaneous ozone demands
IRS	indigo reagent solution
IS	internal standards
ISS	indigo stock solution
MS	mass spectrometry
N-EtFOSA	N-ethyl-perfluoro-1-octane sulfonamide
N-EtFOSAA	N-ethyl-perfluoro-1-octane sulfonamido acetic acid
N-EtFOSE	N-ethyl-perfluoro-1-octane sulfonamido-ethanol

NF	nanofiltration
PAPs	polyfluoroalkyl phosphate esters
PFAAs	perfluoroalkyl acids
PFAS	perfluoro- and polyfluoroalkyl substances
PFBA	perfluoro-n-butanoate (perfluoro-n-butanoic acid)
PFBS	perfluoro-1-butane sulfonate
PFDA	perfluoro-n-decanoate (perfluoro-n-decanoic acid)
PFDS	perfluoro-1-decane sulfonate
PFEAs	per – and polyfluoroalkyl ether acids
PFHpA	perfluoro-n-heptane carboxylate (perfluoro-n-heptanoic acid)
PFHpS	perfluoro-1-heptane sulfonate
PFHxA	perfluoro-n-hexane carboxylate (perfluoro-n-hexanoic acid)
PFHxS	perfluoro-1-hexane sulfonate
PFNA	perfluoro-n-nonane carboxylate (perfluoro-n-nonanoic acid)
PFNS	perfluoro-1-nonane sulfonate
PFOA	perfluorooctanoic acid (or perfluorooctanoic carboxylate)
PFOS	perfluoro-1-octane sulfonate
PFOSA	perfluoro-1-octane sulfonamide
PFPeA	perfluoro-n-pentane carboxylate (perfluoro-n-pentanoic acid)
PFPeS	perfluoro-1-pentane sulfonate
PFTeDA	perfluoro-n-tetradecane carboxylate (perfluoro-n-tetradecanoic acid)
PFTrDA	perfluoro-n-tridecane carboxylate (perfluoro-n-tridecanoic acid)
PFUnDA	perfluoro-n-undecane carboxylate (perfluoro-n-undecanoic acid)
POPs	persistent organic pollutants

PP	polypropylene
QC	quality control
RO	reverse osmosis
TOP	total oxidizable precursor

Appendix A - LODs and LOQs

Table 16 shows the calculated limits of detections (LODs) and limits of quantifications (LOQs) considering the related recovery for each compound, from the first experiment (I), the second experiment (II) and the third experiment (III) – concentrations reported for concentrated samples to 1 mL. Here it shall be mentioned that the unit of the LODs and LOQs could be reported as well as ng/44 mL or ng/50 mL (the respective water volume that was used), but since the samples were spiked the concentrations are reported in ng/mL.

Table 16: Calculated limit of detections (LODs) and limit of quantifications (LOQs) in the concentrated samples

compound	concentration in ng/mL					
	LOD I	LOQ I	LOD II	LOQ II	LOD III	LOQ III
PFBA	0.075	0.15	0.10	0.20	0.10	0.20
PFPeA	0.17	0.34	0.18	0.36	0.19	0.38
PFHxA	0.035	0.070	0.030	0.060	0.035	0.070
PFHpA	0.050	0.10	0.040	0.080	0.040	0.080
PFOA	0.14	0.28	0.090	0.18	0.085	0.17
PFNA	0.10	0.20	0.055	0.11	0.050	0.10
PFDA	0.50	0.10	0.027	0.054	0.025	0.050
PFUnDA	0.075	0.15	0.050	0.10	0.040	0.080
PFDoDA	0.050	0.10	0.026	0.052	0.020	0.040
PFTrDA	0.15	0.30	0.075	0.15	0.050	0.10
PFTeDA	0.20	0.40	0.14	0.28	0.070	0.14
PFBS	0.175	0.35	0.080	0.16	0.060	0.12
PFPeS	0.90	1.8	0.40	0.80	0.30	0.60
PFHxS	0.14	0.28	0.065	0.13	0.050	0.10
PFHpS	0.22	0.44	0.20	0.40	0.15	0.30
PFOS	0.21	0.42	0.20	0.40	0.15	0.30
PFNS	0.48	0.96	0.42	0.84	0.30	0.60

PFDS	0.17	0.34	0.20	0.40	0.10	0.20
N-EtFOSA	5.1	10.2	0.80	1.6	0.20	0.40
PFOSA	0.14	0.28	0.13	0.26	0.125	0.25
N-EtFOSAA	0.85	1.7	0.55	1.1	0.45	0.90
N-EtFOSE	0.55	1.1	0.35	0.70	0.25	0.50
4:2 FTSA	0.18	0.36	0.14	0.28	0.12	0.24
6:2 FTSA	0.40	0.80	0.28	0.56	0.25	0.50
8:2 FTSA	0.40	0.80	0.30	0.60	0.25	0.50
ADONA	0.40	0.80	0.26	0.52	0.25	0.50
GenX	0.34	0.68	0.36	0.72	0.33	0.66
F-53B	0.85	1.7	0.80	1.6	0.50	1.0
6:2 diPAP	0.60	1.2	0.32	0.64	0.25	0.50
8:2 diPAP	4.2	8.4	2.0	4.0	1.7	3.4
6:2/8:2diPAP	1.5	3.0	1.3	2.6	0.9	1.8

Appendix B - additional data: O_3 concentrations

Table 17 provides a summary of all the single measured O_3-concentrations from each experimental day inlcuding the respective extincation values. On each day two references were used where the mean of both references was further used for the calculations. For the determination of the O_3-concentration in genereal dublicats were used as shown in Table 17 named sample I and sample II. Furthermore, the calculated O_3-concentrations from the "UV-detection direct method" and the effect of (20 µL) methanol on O_3-concentrations are shown in Table 17 as well. On "Day I" the set up was tested and the O_3-concentration was measured a few times to evaluate the O_3-concentration at the equilibrium. On "Day II" the first experiment was performed; during the experiment some errors occurred (explained in detail in Appendix C - additional data from the first experiment) and the experiment was repeated in an improved and modified way on "Day III". Further explanation: "time" means the moment at which the extinction was noted, the extinction was continuously and directly measured with the UV-spectrophotometer during the experiment, but only four times the values of the extinction were noted.

Table 17: Summary of all measurements of the O_3-stock solution

sample (method)	**extinction [1/cm]**	**O_3-concentration [mg O_3/L]**
Day I: reference I (indigo-method)	0.855	-
Day I: reference II (indigo-method)	0.850	-
sample I (indigo-method)	0.398	55
Sample II (indigo-method)	0.439	50
Sample I (indigo-method)	0.392	55
Sample II (with 20 mL MeOH)	0.346	61
Day II: reference I (indigo-method)	0.839	-
Day II: reference II (indigo-method)	0.845	-
sample I (indigo-method)	0.536	37
sample II (indigo-method)	0.532	37
sample I (indigo-method)	0.546	36
sample II (indigo-method)	0.530	37
sample I (20 µL MeOH, indigo-m.)	0.578	32

sample II (20 µL MeOH, indigo-m.)	0.537	37
sample I (indigo-method)	0.468	45
sample II (indigo-method)	0.450	46
time I (UV-detection direct method)	1.8320	41
time II (UV-detection direct method)	1.8758	42
time III (UV-detection direct method)	2.0100	45
time IV (UV-detection direct method)	2.1444	48
Day III: reference I (indigo-method)	0.872	-
Day III: reference II (indigo-method)	0.862	-
sample I (indigo-method)	0.559	37
sample II (indigo-method)	0.587	34
sample I (indigo-method)	0.530	40
sample II (indigo-method)	0.528	41
sample I (indigo-method)	0.545	39
sample II (indigo-method)	0.495	45
time I (UV-detection direct method)	around 1.640 to 2.000	around 36 to 44
Day IV: reference I (indigo-method)	0.845	-
Day IV: reference II (indigo-method)	0.832	-
sample I (indigo-method)	0.579	31
sample II (indigo-method)	0.559	34
sample I (indigo-method)	0.565	33
sample II (indigo-method)	0.569	32
time I (UV-detection direct method)	around 0.8520 to 1.499	around 18.9 to 33.3

Appendix C - additional data from the first experiment

Introduction. Some working steps for the first experiment were not well thought out and some minor mishaps influenced the results. Some of the experimental procedures and results of the first experiment are therefore only reported and discussed in Appendix C. The mistakes from the first experiment though helped to modify the working steps and therefore the detailed description was provided.

Method. For the first experiment 7 samples were prepared. First, six 50 mL polypropylene (PP) tubes were filled with 40 mL of tap water that was pre-filtered with Oasis® HLB-column (6 mL, 500 mg) cartridges. All six samples were spiked with 10 µL of a PFAS-standard mix containing 31 PFAS with a concentration of 0.5 µg/mL – the PFAS-standard mix contained all 31 PFAS which are presented in Table 1-5. The samples were shortly shaken and vortexed. After one hour the temperature and the pH was measured for three of the samples with a standard-pH-meter PHM 210 from Radiometer Copenhagen. Only three samples were checked for the pH to have the possibility to encounter potential recovery loses by an adsorption of PFAS on the pH glass electrode (one blank and two samples). After the O_3-stock solution reached its equilibrium of 40 mg O_3/L, 4 mL of the O_3-stock solution were extracted with a glass syringe and injected in the sample (approximately 1-2 cm below the surface). The sample than was closed and shaken for 15 seconds. This procedure was repeated three times to have four PFAS spiked and ozonated samples. Two blanks were filled up to 44 mL with ozone free deionized water. One blank, which was an empty 50 mL PP-tube was filled up to 44 mL with the pure O_3-stock solution; this blank was kept open under the fume hood. The last blank was used to check for possible contaminations from the ozone reactor, especially concerning the polytetrafluoroethylene tubes. After 30 min the samples were checked for ozone residues with a potassium iodide paper. Subsequently the pH was measured again for the samples for which the pH was determined before the ozonation as well. The samples were stored at room temperature for approximately 3-4 hours and then all seven samples were spiked with 20 µL of a standard containing 0.5 µg/mL of isotope labeled PFAS. Subsequently the samples were shaken and vortexed for 10 seconds. Although the potassium iodide paper did not detect the presents of ozone residues before, the samples might still have contained some ozone residues, since ozone was perceived by smell during the spiking with the isotope labeled standards. Table 18 shows the summery of used sample volumes, pH values and temperatures from the first pre-experiment.

Table 18: Additional data summary of the first experiment

sample name	mass [g]	pH and temp (°C) before ozonation	volume of O_3-stock solution (concentration of O_3-stock solution [mg O_3 /mL])	pH and temp (°C) after ozonation
BW I	-	-	44 mL	-
BW II	40.01	7.62 (19.7 °C)	-	-
BW III	40.01	-	-	-
sample A (A)	40.01	7.60 (19.7 °C)	4 mL (40)	7.29 (20.7 °C)
~~sample B~~	~~40.00~~	~~7.45 (19.7 °C)~~	~~4 mL (40)~~	~~7.10 (21.2 °C)~~
sample C (B)	40.01	7.38 (19.7 °C)	4 mL (40)	6.99 (21.2 °C)
sample D (C)	40.01	-	4 mL (40)	-
sample E (D)	40.01	-	4 mL (40)	-

Results and discussion. The results of the first experiment were not satisfying (see Figure 13). This unsatisfying results might be explained as follows: (i) the samples were ozonated and stored at room temperature, but to keep more ozone in solution the temperature should be lower (around 1-4 °C), so the samples should be stored before and after ozonation at 4 °C; (ii) after the O_3-stock solution has been added to the samples (approximately 1-2 cm below the surface), the samples were closed and shaken for 10 seconds and then stored at room temperature till the sample preparation, but it is better to add the O_3-stock solution and let the needle touch (almost) the ground[8], stir the samples carefully for a few seconds with the needle (avoid air bubbles), close the samples and store them at 4 °C again till the sample preparation; (iii) unfortunately during the sample preparation an error occurred in the "*washing-step*", instead of mixing 10 mL of the ammonium solution (96.67 mg ammonium acetate (97%) in 50 mL water) with 40 mL of the acetic acid solution (71.5 µL acetic acid (97-98%) in 50 mL water), the acetate buffer contained incorrectly 40 mL of the ammonium solution and 10 mL of the acetic acid solution – so this might have been one cause of the very bad recoveries for some substances; (iv) the samples were centrifuged at 1500 rpm for 2 min and a paper tissue was inserted in the chambers before, to collect the water residuals,

[8] The injected ozone shall interact with the diluted substances while moving upwards, if the ozone is injected only slightly below the surface it gasses out without any interaction with the target compounds.

but most of the water contained in the cartridges which was observed during the evaporation under the N_2 flow, since an evaporation under 0.5 mL was not achievable, so the sample in the end contained probably only water, which in my opinion is the second cause for the very bad recoveries for some substances. To avoid the last potential error during the sample preparation I suggest to use tubes or vials as a form of cups to put the cartridges into, so that is observable after the centrifugation of the samples if all the water residuals have been removed. Table 19 shows the results of the calculated O_3-concentrations of the first pre-experiment.

Table 19: Measured and calculated O_3-concentations; only means of the duplicates are presented

sample	**O_3-concentration [mg O_3/L]**	**quantity**
before the experiment	37	n = 2
during the experiment	36	n = 2
during the experiment (MeOH)	34	n = 2
after the experiment	46	n = 2
mean	40 (± 5)	n = 6

The measured PFAS concentrations from the first experiment are shown in Table 20 and Figure 13. The spiked samples had a concentration of 113.6 ng/L or 5 ng/mL if the 44 mL were concentrated to 1 mL. The respective LODs and LOQs are presented in Table 18. The O_3-stock solution was at least contaminated with PFBA (16.6 ng/L), PFPeA (29.5 ng/L), PFHxA (< LOQ) and PFHpA (< LOQ), but to ensure that it is recommended to repeat this measurement. Focusing on the removal or transformation of PFAS, no differences were observed for PFCAs with carbon chain length from C4 to C11 in comparison to the related blanks. Only N-EtFOSE was identifiably reduced after the ozonation, and probably PFOSA and N-EtFOSAA to same slight content as well. The slightly higher PFBS concentration in the ozonated samples compared to the blanks might be explained by the degradation of the three perfluoroalkyl sulfonamides. The higher GenX concentration detected in the ozonated samples was curious, since it was expected that the molecule would break down to smaller fractions. Furthermore, in my opinion it is very unlikely that any PFAS would be transformed to GenX under oxidative conditions. Much more plausible would be that ozone molecules (or maybe generated radicals) attack the ether linkage and take the GenX molecule apart.

Table 20: Concentrations from the first experiment; “n.a.” = result not available because the recovery was below 30%.

Compound	concentration in ng/L						
	0_3-reactor water	**blank I (no O_3)**	**blank II (no O_3)**	**sample A (O_3)**	**sample B (O_3)**	**sample C (O_3)**	**sample D (O_3)**
PFBA	17	85	93	80	87	90	n.a.
PFPeA	29	95	96	87	89	106	109
PFHxA	< LOQ	95	97	100	95	90	98
PFHpA	< LOQ	95	106	107	109	114	106
PFOA	n.n.	106	85	98	96	94	89
PFNA	n.n.	90	n.a.	103	98	104	98
PFDA	n.n.	102	n.a.	n.a.	114	n.a.	97
PFUnDA	n.a.	88	n.a.	n.a.	93	n.a.	n.a.
PFDoDA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PFTrDA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PFTeDA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PFBS	n.n.	140	n.a.	201	114	218	169
PFPeS	n.n.	150	n.a.	154	105	177	152
PFHxS	n.n.	110	n.a.	94	90	105	91
PFHpS	n.a.	n.a.	n.a.	n.a.	93	n.a.	n.a.
PFOS	n.a.	n.a.	n.a.	n.a.	95	n.a.	n.a.
PFNS	n.a.	n.a.	n.a.	n.a.	46	n.a.	n.a.
PFDS	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
N-EtFOSA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PFOSA	n.n.	108	75	65	77	69	87
N-EtFOSAA	n.n.	110	n.a.	n.a.	90	n.a.	n.a.

N-EtFOSE	n.n.	97	73	< LOQ	< LOQ	< LOQ	32
4:2 FTSA	n.n.	95	136	141	113	143	101
6:2 FTSA	n.n.	87	110	108	96	100	100
8:2 FTSA	n.n.	141	58	94	156	102	127
ADONA	n.n.	128	130	137	107	117	115
GenX	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.	n.n.
F-53B	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6:2 diPAP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8:2 diPAP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
6:2/8:2diPAP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

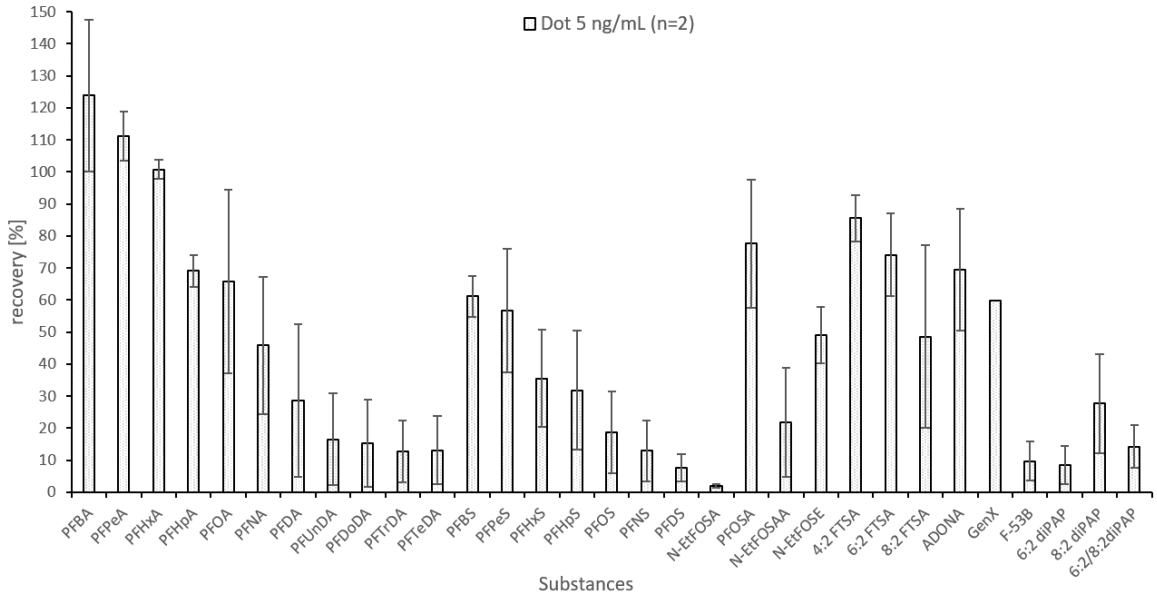


Figure 13: Recoveries in % from first experiment

Appendix D - additional data from the second experiment

Table 21 shows more detailed information on the second experiment considering the sample preparation and Table 22 shows the summary of the calculated concentrations. The temperature was not measured before the ozonation, but the pH values were determined with pH-strips. Since the samples were stored at 4 °C and only were taken out of the refrigerator at the moment of the experiment (approximately for 10-15 minutes at room temperature), it can be assumed that the temperature was around 4 °C. The pH value was 5 for all the samples, before and after the ozonation, and after the addition of the internal standards. Looking at Table 21 the blank (filtered tap water (ftw)) ran directly after 25 ng/mL standard, so PFHxA contamination might be caused by the 25 ng/mL standard that ran before that sample. Considering only the blanks (spiked samples which were not ozonated) then the mean concentration should be 113.6 ng/L (5 ng in 44 mL), the calculated mean is 114 ng/L (± 23.8).

Table 21: Additional data and sample size from the second experiment.

sample name	**mass [g]**	**pH and temp (°C) before ozonation; assumption after pre-test**	**volume of O_3-stock solution (concentration of O_3-stock solution [mg O_3 /mL])**	**pH and temp (°C) after ozonation**
BW I	40.09	5.0 (4 °C)	-	5
BW II	40.01	5.0 (4 °C)	-	5
deionized water		5.0 (4 °C)		
O_3-reactor water		5.0 (4 °C)		
sample A (E)	40.08	5.0 (4 °C)	4 mL (40)	5
sample B (F)	40.05	5.0 (4 °C)	4 mL (40)	5
sample C (G)	40.07	5.0 (4 °C)	4 mL (40)	5

Table 22: Concentrations of from the second experiment

Compound	concentration in ng/L							
	filtered tap water (ftw)	**deionized water**	**0_3-reactor water**	**blank I (no O_3)**	**blank II (no O_3)**	**sample A (O_3)**	**sample B (O_3)**	**sample C (O_3)**
PFBA	n.n.	n.n.	< LOQ	115	115	127	116	129
PFPeA	n.n.	n.n.	n.n.	124	118	103	90	119
PFHxA	< LOQ	< LOQ	< LOQ	112	88	98	102	114
PFHpA	n.n.	n.n.	n.n.	106	108	108	124	117
PFOA	n.n.	n.n.	n.n.	121	122	111	117	117
PFNA	n.n.	n.n.	n.n.	125	146	148	118	135
PFDA	n.n.	n.n.	n.n.	107	103	110	110	102
PFUnDA	n.n.	n.n.	n.n.	117	124	123	131	128
PFDoDA	n.n.	n.n.	n.n.	104	116	139	108	139
PFTrDA	n.n.	n.n.	n.n.	93	92	120	95	93
PFTeDA	n.n.	n.n.	n.n.	84	67	96	78	77
PFBS	n.n.	n.n.	n.n.	121	129	131	160	147
PFPeS	n.n.	n.n.	n.n.	98	127	102	117	114
PFHxS	n.n.	n.n.	n.n.	104	102	102	107	112
PFHpS	n.n.	n.n.	n.n.	113	146	112	126	126
PFOS	n.n.	n.n.	n.n.	120	121	122	171	119
PFNS	n.n.	n.n.	n.n.	111	115	92	117	123
PFDS	n.n.	n.n.	n.n.	82	72	92	85	81
N-EtFOSA	n.n.	n.a.	n.a.	98	n.a.	115	n.a.	n.a.
PFOSA	n.n.	n.n.	n.n.	121	124	116	120	134
N-EtFOSAA	n.n.	n.n.	n.n.	90	109	129	135	154

N-EtFOSE	n.n.	n.n.	n.n.	93	127	109	113	99
4:2 FTSA	n.n.	n.n.	n.n.	111	113	104	111	94
6:2 FTSA	n.n.	n.n.	n.n.	132	123	130	111	107
8:2 FTSA	n.n.	n.n.	n.n.	111	108	113	113	109
ADONA	n.n.	n.n.	n.n.	123	119	108	131	136
GenX	n.n.	n.n.	n.n.	179	147	144	147	142
F-53B	n.n.	n.n.	n.n.	110	99	102	121	94
6:2 diPAP	n.n.	n.n.	n.n.	129	99	133	123	114
8:2 diPAP	n.n.	n.n.	n.n.	153	127	142	141	139
6:2/8:2diPAP	n.n.	n.n.	n.n.	147	126	225	153	152

Appendix E - additional data from the third experiment

Table 23 shows some more detailed information on the third experiment and Table 24 shows a summary of the calculated concentrations. The temperature was not measured before the ozonation, the pH values were determined with pH-strips before and after the ozonation. The pH value was 7 for all the samples, before and after the ozonation, and after the addition of the internal standards.

Table 23: The additional data from the third experiment

sample name	**mass [g]**	**pH and temp (°C) before ozonation; assumption after pre-test**	**volume of O_3-stock solution (concentration of O_3-stock solution [mg O_3 /mL])**	**pH and temp (°C) after ozonation**
BW I	25.15	7.0 (~4 °C)	-	-
sample A (H)	25.14	7.0 (~4 °C)	25 mL (30)	
sample B (I)	25.07	7.0 (~4 °C)	25 mL (30)	
sample C (J)	25.09	7.0 (~4 °C)	25 mL (30)	
sample a	41.09	7.0 (~4 °C)	10 mL (30)	
effluent (no O_3)	25 (visual j.)	7.0 (~4 °C)		
effluent (O_3)	25 (visual j.)	7.0 (~4 °C)	25 mL (30)	
blank-blank	40.11	7.0 (~4 °C)		

Table 24: Calculated concentrations from the third experiment

Compound	concentration in ng/L							
	blank - blank	BW I	sample A (H)	sample B (I)	sample C (J)	sample a	effluent	effluent (O_3)
PFBA	n.n.	96	91	98	123	105	< LOQ	17
PFPeA	n.n.	114	119	116	125	128	< LOQ	17
PFHxA	n.n.	96	101	115	96	97	20	31
PFHpA	n.n.	91	81	116	104	102	9	13
PFOA	n.n.	78	92	111	85	88	8	7
PFNA	n.n.	101	108	91	105	113	n.n.	< LOQ
PFDA	n.n.	89	109	88	115	127	n.n.	2
PFUnDA	n.n.	101	100	101	136	131	n.n.	n.n.
PFDoDA	n.n.	105	94	98	69	78	n.n.	2
PFTrDA	n.n.	102	93	85	71	85	n.n.	n.n.
PFTeDA	n.n.	104	110	82	83	90	n.n.	n.n.
PFBS	n.n.	71	94	100	85	80	< LOQ	7
PFPeS	n.n.	78	95	98	93	78	n.n.	n.n.
PFHxS	n.n.	66	81	86	80	72	n.n.	< LOQ
PFHpS	n.n.	90	94	111	101	98	n.n.	n.n.
PFOS	n.n.	81	182	255	265	177	n.n.	n.n.
PFNS	n.n.	83	86	118	120	108	n.n.	n.n.
PFDS	n.n.	72	87	114	82	124	n.n.	n.n.
N-EtFOSA	n.n.	67	30	30	29	40	n.n.	n.n.
PFOSA	n.n.	100	88	80	77	105	n.n.	n.n.
N-EtFOSAA	n.n.	96	130	83	107	106	n.n.	n.n.
N-EtFOSE	n.n.	71	55	51	44	63	n.n.	n.n.

4:2 FTSA	n.n.	95	108	115	79	105	n.n.	< LOQ
6:2 FTSA	n.n.	86	93	112	96	112	24	683
8:2 FTSA	n.n.	59	68	77	72	77	n.n.	n.n.
ADONA	n.n.	112	89	104	121	93	n.n.	n.n.
GenX	n.n.	100	93	113	111	103	n.n.	n.n.
F-53B	n.n.	100	96	123	128	103	n.n.	n.n.
6:2 diPAP	< LOQ	89	108	96	74	55	n.n.	n.n.
8:2 diPAP	n.n.	84	83	99	79	85	n.n.	n.n.
6:2/8:2diPAP	n.n.	100	115	120	91	105	n.n.	n.n.

Appendix F - chromatograms

The Figures 14 and 15 show the chromatograms for PFOS in treated and untreated samples with ozone.

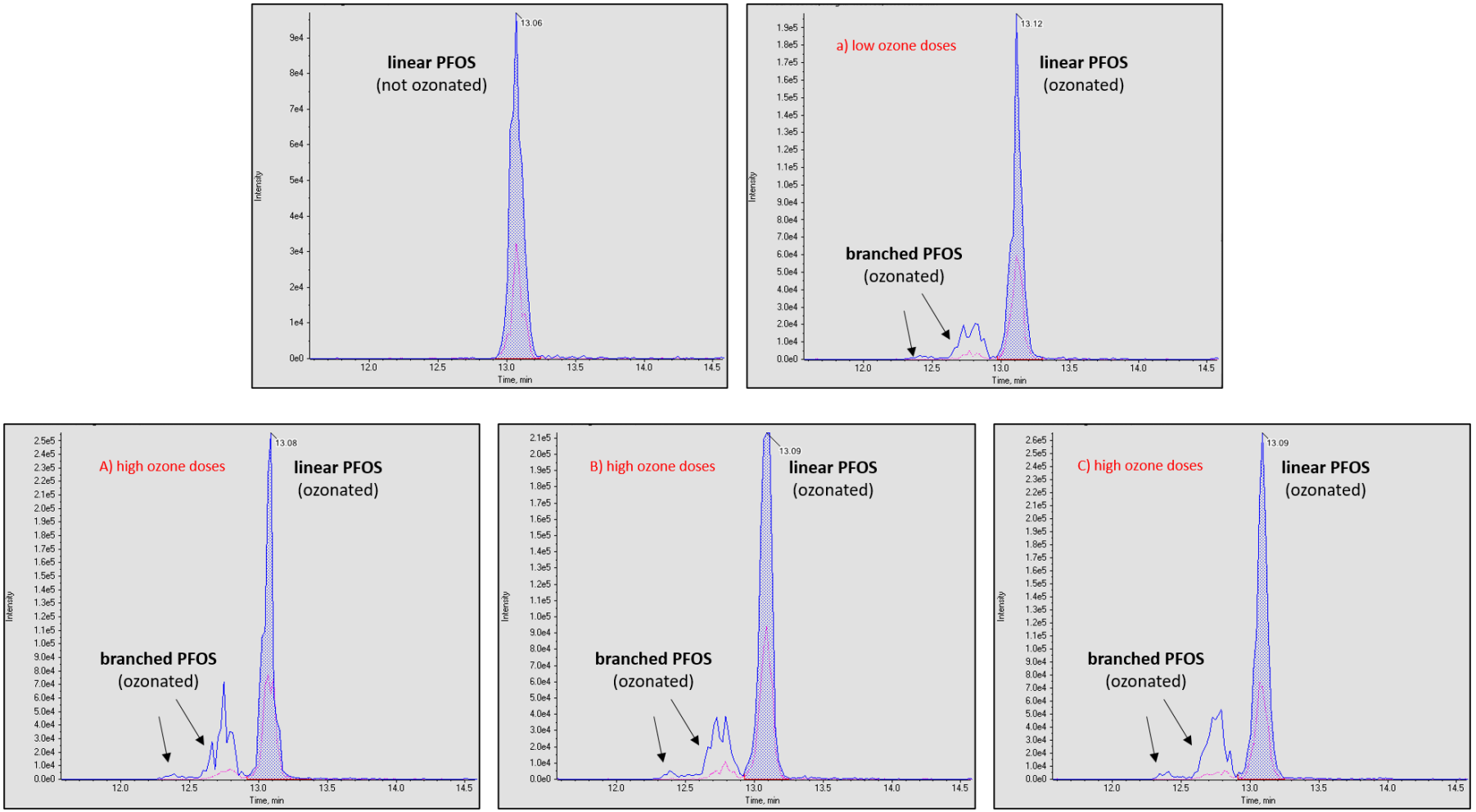
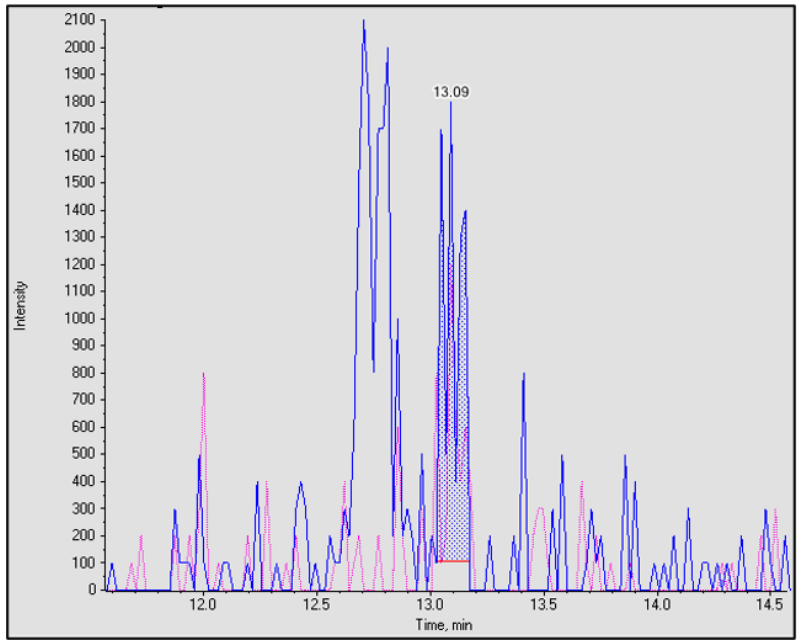


Figure 14: Chromatograms of PFOS in spiked tape water samples, not treated with ozone (upper left), treated with low ozone dose (upper right), and with high ozone doses (A, B, and C).

PFOS - effluent not ozonated



PFOS - effluent ozonated

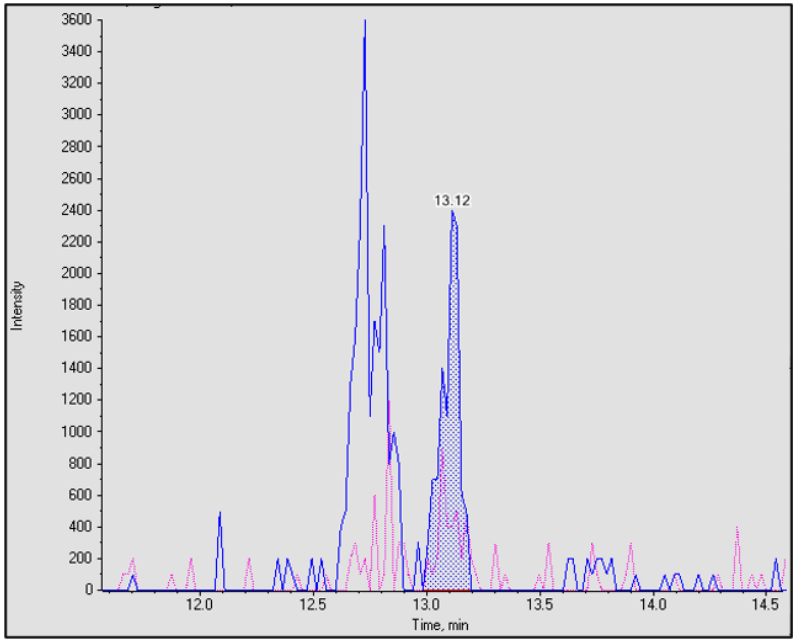


Figure 15: Chromatogram example from PFOS in the untreated effluent and in the treated effluent with ozone

Appendix G – performed statistical tests (R-codes)

Kruskal Wallis Test for the Blanks

```
> statistics_master_thesis.xlsx <- read_excel("C:/Users/Andeas/Statistik/Excelfiles/statistics_master_thesis.xlsx", sheet= 1,
                                  col_types = c("text", "text", "numeric",
                                                "text","numeric"))
> ozone <- statistics_master_thesis.xlsx
> View(ozone)

#check for normaldistribution
> qqnorm(ozone$µM_blank)
> qqline (ozone$µM_blank)
> shapiro.test(ozone$µM_blank)

                Shapiro-Wilk normality test

data:  ozone$µM_blank
W = 0.91288, p-value = 1.313e-05
```

Comment: Data are not normaldistributed (p-value is < 0.05)!

```
#check for the homogeneity of variance
> bartlett.test(ozone$µM_blank ~ ozone$group_blank)

                Bartlett test of homogeneity of variances

data:  ozone$µM_blank by ozone$group_blank
Bartlett's K-squared = 0.46595, df = 2, p-value = 0.7922
```

Comment: The variances are equal (p-value is > 0.05)!

```
> kruskal.test(ozone$µM_blank ~ ozone$group_blank)

                Kruskal-Wallis rank sum test

data:  ozone$µM_blank by ozone$group_blank
Kruskal-Wallis chi-squared = 1.755, df = 2, p-value = 0.4158
```

Comment: Their is no sig. difference in between the three blanks!
First two blanks are from experiment II and the third blank is from experiment III!

```
> dunnTest(ozone$µM_blank, ozone$group_blank, method = "bonferroni")

        Comparison          Z   P.unadj     P.adj
1   blank_I - blank_II -0.2018896 0.8400031 1.0000000 # "the p-values are > 0.05, so no significant differerence between blank I and blank II"
2  blank_I - blank_III  1.0368499 0.2998058 0.8994175 # "the p-values are > 0.05, so no significant differerence between blank I and blank III"
3 blank_II - blank_III  1.2302056 0.2186201 0.6558604 # "the p-values are > 0.05, so no significant differerence between blank II and blank III"
```

Kruskal Wallis Test for the ozonated samples!

```
#check for normaldistribution
> qqnorm(ozone$µM_ozone)
> qqline (ozone$µM_ozone)
> shapiro.test(ozone$µM_ozone)

                Shapiro-Wilk normality test

data:  ozone$µM_ozone
W = 0.93501, p-value = 0.0001726
```

Comment: Data are not normaldistributed (p-value is < 0.05)!

```
#check for the homogeneity of variance
> bartlett.test(ozone$µM_ozone ~ ozone$group_ozone)

                Bartlett test of homogeneity of variances

data:  ozone$µM_ozone by ozone$group_ozone
Bartlett's K-squared = 2.555, df = 2, p-value = 0.2787
```

Comment: The variances are equal (p-value is > 0.05)!

> kruskal.test(ozone$µM_ozone ~ ozone$group_ozone)

	Kruskal-Wallis rank sum test

data: ozone$µM_ozone by ozone$group_ozone
Kruskal-Wallis chi-squared = 0.28401, df = 2, p-value = 0.8676

Comment: Their is no sig. difference in between the three ozonated samples!

> dunnTest(ozone$µM_ozone ~ ozone$group_ozone, method = "bonferroni")

	Comparison	Z	P.unadj	P.adj
1	ozone_I - ozone_II	-0.5222908	0.6014679	1
2	ozone_I - ozone_III	-0.1693916	0.8654886	1
3	ozone_II - ozone_III	0.3528992	0.7241640	1

Comment: Their is no sig. difference in between the three ozonated samples!

Mann Whitney U test for the comparison between blanks and ozonated samples:

> wilcox.test(ozone_I$µM_mean ~ ozone_I$group_high_OD)

	Wilcoxon rank sum test

data: ozone_I$µM_mean by ozone_I$group_high_OD
W = 471, p-value = 0.8999
alternative hypothesis: true location shift is not equal to 0

Comment: Their is no sig. difference in between the blanks and the ozonated samples!

> wilcox.test(ozone_I$µM_low_OD ~ ozone_I$group_low_OD)

	Wilcoxon rank sum test

data: ozone_I$µM_low_OD by ozone_I$group_low_OD
W = 465, p-value = 0.8339
alternative hypothesis: true location shift is not equal to 0

Comment: Their is no sig. difference in between the blanks and the "weakly" ozonated samples!

---
bartlett.test(ozone_I$µM_effluent ~ ozone_I$group_effluent)

	Bartlett test of homogeneity of variances

data: ozone_I$µM_effluent by ozone_I$group_effluent
Bartlett's K-squared = 129.16, df = 1, p-value < 2.2e-16

Comment: Variances are not equal the Wilcoxon rank sum test would not make any sence!

Appendix H - timetable

Figure 16 shows the timetable for the present master thesis project.

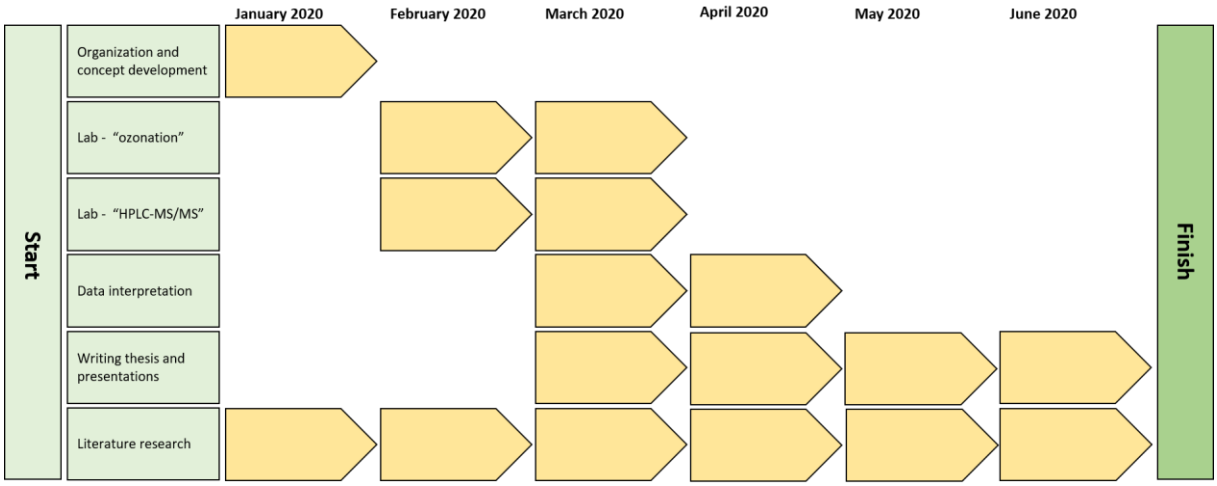


Figure 16: Timetable for the master thesis