Radiosynthesis of Perfluoroalkyl Substances

- Chemical analysis, uptake, distribution,

and partitioning studies

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Abstract

Perfluoroalkyl substances (PFASs) are widely utilized manmade chemicals. Their properties have made them highly appreciated in a variety of industrial and consumer product applications, including fire-fighting foams, hydraulic fluids, as well as in cookware and food contact papers.

However, some of the PFASs are highly persistent in the environment and their toxicological profiles are of concern. Voluntary and regulatory efforts have been taken to reduce the environmental levels of PFASs. These actions have resulted in a reduction of PFASs in human milk from Stockholm as presented in this thesis.

The radiosyntheses of ^{35}S-PFOS, ^{35}S-PFBS, and ^{14}C-PFOA presented herein were applied for distribution studies in mice but also for solubility and adhesion experiments of common laboratory solvents and buffers. The radiosynthesis employed reactive Grignard reagents, perfluoroalkyliodides, and ^{35}S-sulfur dioxide or ^{14}C-carbon dioxide. The distribution studies were performed with ^{35}S-PFOS on both pregnant mice and their offspring as well as on male mice. The mice were subjected to whole-body autoradiography and the tissues were analyzed by liquid scintillation counting. Liver and lungs were the target organs for ^{35}S-PFOS in the dams. The fetuses and pups had remarkable high levels of ^{35}S-PFOS in their lungs as well as in the brain. The male mice were given a high dose and a more environmental relevant dose of ^{35}S-PFOS. PFOS was transferred from the blood to the tissues as the dose increased.

In another study the distribution pattern of the shorter homologue PFBS was compared to PFOS. ^{35}S-PFBS was utilized and demonstrated a 5-40 fold lower tissue levels in comparison to PFOS.

The pharmacokinetic parameters determined for PFHxS in mice, rats, and monkeys will provide valuable insight in establishing a proper risk assessment for this compound. The study confirms the common species differences in serum elimination half-life that are associated with PFASs.

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”För att ingenting är omöjligt”

List of papers

This thesis is based on following papers. Permission to print the papers was kindly obtained by the publishers.

I. **Sundström, M**., Bogdanska, J., Pham, H.V., Athanasios, V., Nobel, S., McAlees, A., Eriksson, J., DePierre, J.W., Bergman, Å., 2012. Radiosynthesis of perfluorooctanesulfonate (PFOS) and perfluorobutanesulfonate (PFBS), including solubility, partition and adhesion studies. Chemosphere 87, 865-871.

II. **Sundström, M**., Ehresman, D.J., Bignert, A., Butenhoff, J.L., Olsen, G.W., Chang, S.C., Bergman, Å., 2011. A temporal trend study (1972-2008) of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in pooled human milk samples from Stockholm, Sweden. Environment International 37, 178-183.

III. Borg, D., Bogdanska, J., **Sundström, M.**, Nobel, S., Håkansson, H., Bergman, Å., DePierre, J.W., Halldin, K., Bergström, U., 2010. Tissue distribution of S-35-labeled perfluorooctane sulfonate (PFOS) in C57Bl/6 mice following late gestational exposure. Reproductive Toxicology 30, 558-565

IV. Bogdanska, J., Borg, D., **Sundström, M.**, Bergström, U., Halldin, K., Valugerdi, M., Bergman, Å., Nelson, B., DePierre, J., Nobel, S., 2011. Tissue distribution of ^{35}S-labeled perfluorooctane sulfonate in adult mice after oral exposure to a low environmentally relevant dose or a high experimental dose. Toxicology 284, 54-62.

V. **Sundström, M.**, Chang, S.C., Noker, P.E., Gorman, G.S., Hart, J.A., Ehresman, D.J., Bergman, Å., Butenhoff, J.L., 2012. Comparative pharmacokinetics of perfluorohexanesulfonate (PFHxS) in rats, mice, and monkeys. Reproductive Toxicology 33, 441-451.

VI. Bogdanska, J., **Sundström, M**., Bergström, U., Borg, D., Abedi-Valugerdi, M., Bergman, Å., Nelson, B., DePierre, J., Nobel, S. Tissue distribution of ^{35}S-labeled perfluorobutane sulfonic acid in adult mice following dietary exposure for 1-5 days. *Manuscript.*

Papers not included in this thesis:

- Cantillana, T., **Sundström, M.**, Bergman, Å., 2009. Synthesis of 2-(4-chlorophenyl)-2-(4-chloro-3-thiophenol)-1,1-dichloroethene (3-SH-DDE) via Newman-Kwart rearrangement - A precursor for synthesis of radiolabeled and unlabeled alkylsulfonyl-DDEs. Chemosphere 76, 805-810.

- Teclechiel, D., **Sundström, M**., Marsh, G., 2009. Synthesis of polybrominated diphenyl ethers via symmetrical tetra- and hexabrominated diphenyliodonium salts. Chemosphere 74, 421-427.

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Abbreviations

^{14}C-PFOA	Carbon-14 labeled perfluorooctanoic acid
^{35}S-PFBS	Sulfur-35 labeled perfluorobutanesulfonic acid
^{35}S-PFOS	Sulfur-35 labeled perfluorooctanesulfonic acid
ADONA	Ammonium 4,8-dioxo-3H-perfluorononanoate
APFO	Ammonium perfluorooctanoate
DAST	N,N-diethyl aminosulfur trifluoride
DDT	1,1,1-Trichloro-2,2-di(4-chlorophenyl)ethane
DEOXO-FLOUR	Bis(2-methoxyetyl) aminosulfur trifluoride
DMSO	Dimethyl sulfoxide
ECF	Simons electrochemical fluorination
HF	Hydrogen fluoride
HFPO	Hexafluoropropylene oxide
iv	Intravenous
K_{ow}	Octanol-water partition coefficient
L-FABP	Liver fatty acid-binding protein
LLOQ	Lower limit of quantification
OATP	Organic anion transporting polypeptide
OECD	Organization for economic co-operation and development
PBDE	Polybrominated diphenyl ether
PBSF	Perfluorobutane sulfonyl fluoride
PBT	Persistent, bioaccumulative, toxic
PCB	Polychlorinated biphenyl
PDSF	Perfluorodecane sulfonyl fluoride
PFAAs	Perfluoroalkyl acids
PFASEs	Perfluoroalkyl sulfonamido ethanols
PFASs	Perfluoroalkyl and polyfluoroalkyl substances
PFBS	Perfluorobutanesulfonic acid

PFCAs	Perfluoroalkyl carboxylic acids
PFHxS	Perfluorohexanesulfonic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PFPAs	Perfluorinated phosphonic acids
PFSAs	Perfluoroalkane sulfonic acids
PHxSF	Perfluorohexane sulfonyl fluoride
pK_a	Logarithmic acid dissociation constant
po	*Per os* (by mouth)
POP	Persistent organic pollutant
POSF	Perfluorooctane sulfonyl fluoride
PPARα	Peroxisome proliferator-activated receptor-α
PTFE	Polytetrafluoroethylene
SPE	Solid phase extraction
$T_{1/2}$	Serum elimination half-life
UCB	Umbilical cord blood
US EPA	The United States environmental protection agency
V_d	Volume of distribution

These Unique Perfluoroalkylated Substances

1 Introduction

Development is a matter of finding solutions of existing problems, and it is one of the greatest driving forces in mankind. We have always been inspired to explore and develop new goods and materials that would make life a little bit easier. It does not matter if it was the development of a harvester in the field or a fabric resisting water and grease, just to mention two examples. All of the progresses made through different innovations, still have impacts on the environment, in one way or the other.

After the Second World War the American fluorine industry reached a paradigm shift with an enormous development of fluorochemicals. Prior to the war, Dr. Roy J. Plunkett discovered polytetrafluoroethylene (PTFE) when he was working to find a new improved refrigerant. This serendipity was the foundation that became Teflon® by DuPont [1]. Shortly after, chemists at the 3M Company accidently discovered the benefits of perfluorooctanesulfonic acid (PFOS) and the success story of Scotchgard® began. These discoveries of perfluoroalkylated substances and fluoropolymers revolutionized the plastic industry and are today highly precious and in some technical applications irreplaceable. The development of the perfluorochemistry has given us ordinary goods like the breathable and water resistant fabrics, the non-stick frying pan, and grease proof food-packing papers. The everyday-life is more or less dependent on the benefits these types of chemicals have given us. But all of this comes with a price. PFOS was discovered in wildlife [2] just prior the latest turn of the century and further analysis revealed perfluoroalkyl substances (PFASs) in non-exposed US citizens [3]. At this point the major fluorine industry decided, together with the US Environmental Protection, to phase-out their

perfluorooctanoyl production [4]. It was earlier known that PFAS may be present in occupational exposed workers blood [5]. However, the discovery of PFASs in the general populations and in the wildlife came as a surprise and it is one of the most recent global environmental scandals [6].

During the last ten years the knowledge of PFASs has grown tremendously and today the knowledge gaps surrounding this class of chemicals have decreased substantially. However, some questions remain and there is a constant drive in finding new fluorochemicals that could improve a technical application or consumer product. It is in these new substances future research needs to focus on. A close collaboration between the industry and research facilities such as universities is warranted, since working together is the best way to avoid release of future persistent organic pollutants (POPs) to the environment. The utopia should be a sustainable industry with a small environmental impact, since after all, this is what matters in the very end.

1.1 Aim of the thesis

The objective of my thesis was to develop a methodology for, and subsequently perform, syntheses of the radioisotope ^{35}S-perfluorooctane sulfonate (^{35}S-PFOS). At the point when ^{35}S–PFOS was synthesized (**Paper I**), we had unique possibilities to conduct detailed distribution studies in mice through autoradiographic imaging and liquid scintillation techniques (**Paper III, IV**). The methodology to synthesize ^{35}S-PFOS was exploited in the later radiosyntheses of ^{35}S-perfluorobutanesulfonic acid (^{35}S-PFBS) (**Paper I**) and also ^{14}C-perfluorooctanoic acid (^{14}C–PFOA). By using radiolabeled PFASs it was possible to conduct detailed distribution studies (**Paper III, IV, VI**) and solubility tests of the labeled compounds (**Paper I**), studies of importance for the pharmacokinetic understanding, the designing of biochemical studies, organic syntheses, as well as the development of analytical methodologies for

these compounds. Since PFOA and PFBS are still manufactured and used in a set of industrial processes, our studies may provide a complementary understanding of the toxicological and physicochemical aspects of these chemicals. Further was a time trend study of PFASs in Swedish mothers' milk conducted (**Paper II**).

1.2 Uniform acronyms

There is a great variety in classifying and naming perfluoroalkyl substances in the literature and by the chemical society. Buck *et al.* [7] have in a recent article tried to encourage the community to adopt a uniform terminology and classification regarding these substances. They suggest the term PFAS should be used for perfluoroalkyl and polyfluoroalkyl substances with the perfluoroalkyl moiety of C_nF_{2n+1}. Perfluoroalkyls are substances where all hydrogen atoms in the carbon chain are replaced with fluorine with the exception of the functional head group that may still contain hydrogen atoms, e.g. in the case for PFOA. Polyfluoroalkyls are a class of substances in which some of the hydrogen atoms are replaced with fluorine, but not all. However, in order to be included in the PFAS family, it is required that the substance contain at least one C_nF_{2n+1} moiety. Another commonly used acronym for these substances has been PFCs (perfluorinated compounds). This name is unfortunate since it also applies to perfluorocarbons without functional groups that are a family of greenhouse gases regulated by the Kyoto protocol [8].

A subdivision of PFASs is the perfluoroalkyl acids (PFAAs), this family consists of the perfluoroalkane sulfonic, carboxylic, sulfinic, phosphonic, and phosphinic acids, whereof the first two acids are the most well studied classes of PFAAs. The perfluoroalkane sulfonic acids (PFSAs) are dominated by perfluorooctane sulfonic acid (PFOS) while the perfluoroalkyl carboxylic acids (PFCAs) most prominent substance is perfluorooctane carboxylic acid (PFOA).

In **Paper II** we are using the terminology perfluoroalkyls (PFAs) instead of PFASs. However, in order to clarify and avoid confusion the new terminology of using PFAS for perfluoroalkyl and polyfluoroalkyl substances is expected to be welcomed and hopefully adopted in the future.

1.3 The big four in this thesis

The process of making perfluorinated organic chemicals was discovered during the 1930s and developed for larger scale production after the World War II via the Simons-electrochemical fluorination (ECF) process (discussed in Chapter 2). PFASs were at first mainly produced due to their stain resistant properties, but after a while more and more applications were employed, utilizing their ability to lower the surface tension and chemical resistance. Another subfamily, which is not further covered in this thesis, are the perfluoroalkyl phosphonates (PFPAs). They are surfactants used as levelling and wetting agents. The concentrations of PFPAs in human serum are in general similar with PFOS and PFOA [9], which warrants further interest [10]. The focus in this thesis has mainly been on four PFASs, i.e. perflurooctanesulfonic acid (PFOS), perfluoro-octanoic acid (PFOA), perfluorohexanoic acid (PFHxS), and perfluorobutanoic acid (PFBS). These PFASs have a diverse set of physicochemical properties and elimination half-life profiles. The things in common are their fully fluorinated backbone, their persistence in the environment, but also their unsurpassed characteristics that have been utilized in an enormous variety of consumer products and industrial applications.

1.3.1 Perfluorooctanesulfonic acid

The fully fluorinated eight carbon long molecule with its sulfonate group (Figure 1.1) is the most prevalent PFAS found in human serum and blood with a

serum elimination half-life ($T_{1/2}$) of 4.8 years [11]. PFOS is the degradation product from perfluorooctane sulfonamide derivatives, which includes perfluoroalkyl sulfonamido ethanols (PFASEs), chemicals that gave the 3M Company's Scotchgard its water- and stain resistant properties. Other use includes a variety of industrial and consumer products, i.e. fire-fighting foam, and as surface treatment to lower the surface strength in textiles, carpets and papers. Due to the unique properties of these compounds and the chemical strength they have also been used as floor polish, photo imaging, hydraulic fluids, and in pesticide control applications. Unfortunately, the end-product PFOS is extremely reluctant to undergo all types of transformation. PFOS is since 2002 considered a PBT (persistent, bioaccumulative, and toxic) chemical by the 34th OECD (Organisation for Economic Co-operation and Development) Chemical Committee [12]. PFOS and perfluorooctanoyl fluoride are since 2009 included in the Stockholm convention for persistent organic pollutants (POPs) under Annex B, which means it may only be used in certain applications [13]. The use of PFOS and related products may in most cases be substituted by other chemicals, however, in some applications there are no available alternatives. Photo imaging, anti-reflective coatings for semi-conductors, and aviation hydraulic fluid are examples of non-restricted purposes for PFOS- related chemicals [14]. Even though PFOS and its precursors have been phased out in the US and is classified as a POP, it is still manufactured in China [15].

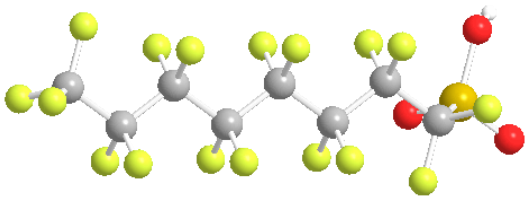


Figure 1.1 The structure of PFOS.

Experiments with PFOS-exposed mice demonstrates an increase in mortality shortly after birth (**Paper III**) [16,17]. This might be due the formation of ion-pair between pulmonary surfactants (e.g. dipalmitoyl phosphatidylcholine) and PFOS (investigation is undertaken) leading to inhibition or delayed lung maturation in the new-born pups [18,19].

1.3.2 Perfluorooctanoic acid

PFOA (Figure 1.2) is primary used as an intermediate, its salts are used to aid the syntheses of fluorinated polymers. These fluoropolymers have valuable properties since they will repel oil, water, grease and stains, they will provide non-stick surfaces on cookware, and fabrics are made waterproof and breathable thanks to these polymers.

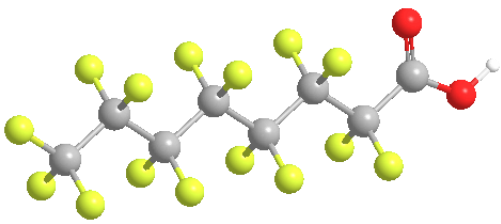


Figure 1.2 The structure of PFOA.

There are a variety of salts used to dissociate the PFO-anion, the most important one is the ammonium salt (APFO) that has been produced since the 1950s. PFOA is mainly used as an emulsifier in the process of making tetrafluoroetylene where it play a vital role in stabilizing the process and it is also a valuable chemical in controlling the exothermic reaction during this polymerization [20]. PFOA is persistent and bioaccumulative, in humans PFOA is the second most abundant PFAS after PFOS. The $T_{1/2}$ is estimated to be 3.5 years in humans [11]. Major efforts have been made in order to understand the environmental fate, the toxicity, and how humans are exposed to PFOA. To

protect human health and the environment, the emissions of PFOA from the industry have decreased during recent years. This is achieved either by altering the chemistry, e.g. by the use of other fluorochemicals (discussed in Chapter 2), or by recycling the APFO [21]. The United States Environmental protection agency (US EPA) has together with the fluorine industry launched a program aiming to eliminate PFOA and its related chemicals from the US market no later than 2015 [22].

1.3.3 Perfluorohexanesulfonic acid

PFHxS (Figure 1.3) is used in a variety of specialized applications. Its high surface activity has been exploited in applications like textile impregnation and fire-fighting foams. PFHxS and its precursors have never been produced in the same quantities as PFOS and PFOA. However, it is a abundant PFAS in human serum and milk [23-26] and it has a long $T_{1/2}$ in humans (7.3 years) [11]. Analysis of mothers' milk revealed the presence of PFHxS also in human milk. Even though the levels were lower in comparison with PFOS and PFOA, it is still an addition to the infants' body burden. PFHxS is not considered to be toxic to the reproductive or developmental system in rodents [27].

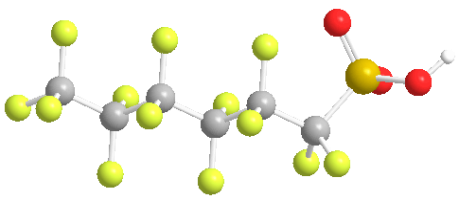


Figure 1.3 The structure of PFHxS.

1.3.4 Perfluorobutanesulfonic acid

PFBS (Figure 1.4) is the short-chain replacement homolog of PFOS. PFBS is the terminal degradation products of perfluorobutanesulfonyl fluoride, N-alkyl

derivatives of perfluorobutanesulfonamides and perfluorobutanesulfonamido-ethanols [28]. Together with its precursors, PFBS are used for treatment of carpets, in corrosion resistant paints, in coatings and in the metal processing industry [29]. Compared to the previous discussed PFASs, PFBS has a short $T_{1/2}$ in humans, 25.8 days [28].

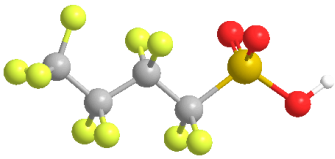


Figure 1.4 The structure of PFBS.

OECD has classified the PFASs into short and long PFASs. Short PFASs should not exceed five perfluorinated carbons, i.e. PFBS [7]. These short PFASs are not considered to bioaccumulate, instead they are regarded to be excreted quite efficiently from the body via the urine [28]. Like all PFASs described in this thesis, PFBS is still highly persistent but it is also highly soluble in water, a property that will make the chemical remaining in water columns, i.e. it will end up in the seas. Due to its non-bioaccumulative property, the four-carbon chemistry filled an important gap in the fluorine industry after the phase-out of the perfluorooctanoyl chemistry in 2000-2002. The eight-carbon chemistry could in many applications be replaced by the four-carbon PFASs without loosing its surface active property.

1.4 Toxicological profile of PFASs

Toxicological studies of humans are unethical and of course not allowed, this applies for environmental contaminants in general, including any PFASs. However, there are some epidemiological studies of humans living close to PFAS polluted sites. Olsen *et al.* [30] discovered that infants, toddlers and

children have a higher serum concentration of PFASs than adults, meaning they are either exposed to an increased level of PFASs, or the $T_{1/2}$ is longer in young children in comparison with adults [30]. So far no studies have revealed any adverse health effect in humans. However, people exposed to PFASs through e.g. drinking water at polluted sites demonstrates modest altered health outcomes but it is still not fully understood how these chemicals affect human health (reviewed in Andersen *et al.* [31]). There are indications that PFASs may cause developmental deficiencies like decreased birth weight [32-34] and also infertility in women [35].

Toxicological studies of PFASs have mainly been conducted in laboratory rats, mice, rabbits, and monkeys [36]. When comparing the serum elimination half-life of perfluorochemicals in different species it is apparent that the rate is very diverse, in some species it is just a couple of hours while in humans it could be several years. PFASs may undergo enterohepatic circulation and renal tubular reabsorption, which influence the long $T_{1/2}$ observed for these chemicals [37,38]. Several animal studies also show a difference in elimination half-life between the sexes within the same species [39,40] (**Paper V)**.

PFASs accumulate in the body by binding to albumin in the blood [41]. The molecular structure of e.g. PFOS do resemble the structure of the fatty acid chain and both PFOS and PFOA are known to bind to β-lipoproteins [42] and also to liver fatty acid-binding protein (L-FABP) causing the chemical to be retained and accumulated in the liver [43]. PFOA and PFOS cause hepatomegaly and both chemicals are weak exogenous peroxisome proliferator - activated receptor-alpha (PPARα) agonists. This means they will bind to and inhibit the function of the receptor, causing changes in biochemical and morphological processes, e.g. giving rise to increased β-oxidation of fatty acids and cause inhibition of the secretion of cholesterol and lipoproteins from the liver. This process gives a decrease of cholesterol in the serum and an accumulation of lipids in the liver (fatty liver) [44]. The fact that some of the

PFAS are PPARα agonists does not only affect the lipid metabolism; the energy homeostasis and inflammatory responses are also influenced (reviewed in DeWitt *el al*. [45]). Wolf and coworkers conducted a *in vitro* study where they concluded that a chain length of eight carbon gave rise to the most activated PPARα, while less activity were seen for both shorter and longer perfluoroalkylated chains [46].

PFASs have in animal studies also been associated with tumors in the testis (Leydig cells) and adenomas of the pancreatic acinar cells [44,47].

Studies have showed that *in utero* exposure of certain PFASs may cause obesity later in life. Hines *et al*. [48] studied the health outcome on mice after developmental exposure to PFOA and observed that low environmental doses during the developmental stage caused decreased body weight of the newborn pups (on postnatal day 1). After ten weeks the pups started to gain weight and at 20 – 29 weeks of age they weighted 11–15% more than the control pups. Mice exposed to a high dose of PFOA decreased in body weight early in life, however, this decrease was apparent also in the mid-life [48]. This phenomenon with a low environmental relevant dose that increases the risk of obesity in the female mice during adulthood while high dose gives weight loss, is similar to other known environmental obesogens like bisphenol A and diethylstilbestrol as reviewed by Hines *et al*. [48]. Similar obesogenic effects have been seen in humans in a recent study by Halldorsson *et al*. where they studied pregnant women and followed up their children twenty years later. They concluded that exposure to low-dose PFOA *in utero* is associated with obesity in the female offspring later in life [49].

There are a large number of PFASs released into the environment and all of these chemicals have different physicochemical properties and toxicological profiles. Slow progress is made in order to extrapolate the toxicological effects observed in laboratory animal to humans. However, uncertainties remain due to the species differences [31].

2 The Chemistry of PFASs

PFASs have unique chemical properties, they behave neither as hydrocarbons nor halocarbons. This chapter discusses the characteristics of PFASs as well as the industrial processes for manufacturing PFASs.

2.1 Industrial production of PFASs

The main global industrial processes for manufacturing of PFAS are via electrochemical fluorination (ECF) with hydrogen fluoride (HF), or via telomerization of tetrafluoroethylene [7]. PFASs containing an ether bridge are usually manufactured either through oligomerization of hexafluoropropene oxide or oxetanes, or through photooxidation of tetrafluoroetylene or hexafluoropropene [7,50]. The ECF pathway has been utilized since 1947 by Minnesota Mining and Manufacturing Company (the 3M Company) for the production of PFSAs (mainly PFOS) and PFCAs (like PFOA). The ECF process was the dominant pathway in the production of PFAAs for a long time. Nevertheless, in 2002 there was a shift in production of surfactants, production shifted from that date to involve telomerisation to a greater extent. However, ECF is still the major procedure in the industrial syntheses of perfluorobutyl-based chemicals [51].

2.1.1 Electrochemical fluorination

Simons-electrochemical fluorination technique was developed during the Manhattan Project in the 1940s with the aim of producing an inert chemical compound that could withstand the aggressive conditions in uranium hexafluoride diffusion tanks during separation of uranium isotopes [52]. The starting material in ECF is hydrocarbons with equal number of carbons (4, 6, 8, or 10 carbons), yielding perfluorobutane sulfonyl fluoride (PBSF),

perfluorohexane sulfonyl fluoride (PHxSF), perfluorooctane sulfonyl fluoride (POSF), or perfluorodecane sulfonyl fluoride (PDSF), respectively. POSF is the starting material for PFOS, i.e. it has been produced in large quantities (4650 tonnes in 2000) in the past [53]. Today the production is based more on PBSF, which after further reaction gives PFBS derivatives.

The ECF is a process where the starting material, i.e. octane sulfonyl fluoride, is subjected to electrolysis in hydrogen fluoride, a process that replaces all hydrogen atoms with fluorine (Figure 2.1).

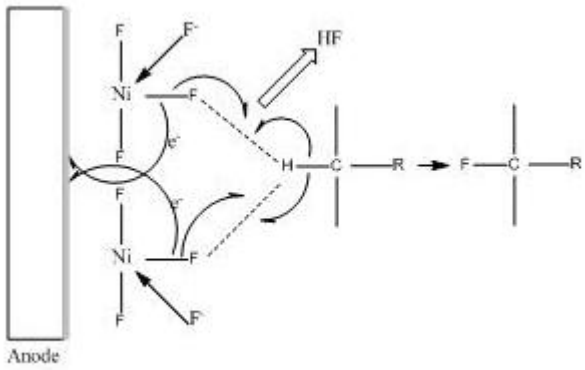
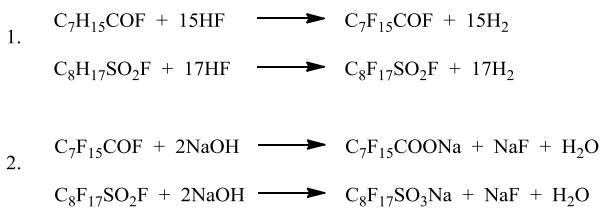


Figure 2.1 Proposed ECF process. The substrate is oxidatively fluorinated at the anode surface [54].

The 3M Company was for a long time the only larger producer utilizing the Simons-electrochemical fluorination technique process for producing PFAS salts. The potential between the anode and the cathode is kept between 4.5 and 6 V since higher potential would cause breakdown of the substrate. The organic substrate is dissolved in anhydrous hydrofluoric acid at 0ºC; the solubility at this temperature is low for alkenes lacking any functionalized groups. As a result, the ECF process is suitable particularly for ethers, carboxylic and sulfonic acid derivatives. The fluorination of the substrate is taking place at the

anode (Figure 2.1), while hydrogen is evolved at the steel cathode. As the fluorination proceeds, the solubility of the substrate decreases in the hydrofluoric acid. The product is thus separated as a second denser phase at the bottom of the reaction vessel. ECF is a powerful process, leading to rearrangements and a variety of branched products and by-products.



Scheme 2.1 ECF gives the fluorides (1) that after hydrolysis yields the carboxylates and sulfonates (2).

Consequently, the product will consist of both branched and linear perfluorinated alkyl chains. However, the predominant product will consist of the same carbon skeleton arrangement as the starting stock [55]. Although the overall low yields of pure product, ECF is considered a cost-effective process due to the relatively low cost of electricity and hydrogen fluoride [7,54,56]. The fraction of linear PFOS and PFOA chains in the ECF-process are usually 70 - 80%. The remaining products are branched to different degrees. By further reactions with functionalized hydrocarbons it is possible to synthesize a variety of perfluoroalkyl derivatives e.g. FOSA [51,55]. The procedures for the synthesis of PFOS and PFOA are illustrated in Scheme 2.1.

2.1.2 Telomerization

Telomerization is the second most important process for elongation of the perfluorinated chain. The process involves a free-radical reaction between a

telogen and a taxogen which gives a telomere. This telomere is a perfluoroalkyl iodide with an even-numbered carbon chain. The perfluoroalkyl iodides may be hydrolyzed to perfluoroalkyl carboxylate salts [57], used in Grignard reactions as in **Paper I,** or further reacted with ethylene to give perfluoroalkylethyl telomere iodide which could be converted into sulfonyl chlorides, acids, alcohols, or thiols. Due to the termination with ethylene, telomerisation-produced PFAS have a non-fluorinated signature, as in the case with telomere alcohols ($CF_3(CF_2)_nCH_2CH_2OH$) [58].

2.2 Properties of PFASs

Even though PFASs share lots of characteristics with each other, there are still properties that separate them. First thing to be mentioned is the carbon chain length. PFOS with its eight-carbon long chain have proven to accumulate in humans, a property it does not share with its shorter homologue PFBS. The functional head group also influences the features, e.g. PFOS does not share the same properties, i.e. does not have the same $T_{1/2}$ in human serum, as PFOA or perfluorononanoic acid (PFNA) even though they share the same number of carbons (or perfluorinated carbons). PFOA is in its acidic form rather volatile and may sublime at room temperature [59]. Depending on the acid strength, the acid will be dissociated at physiological pH. Most of the PFAAs are very strong acids, i.e. they occur as anions in biological systems and in the environment.

The fluorine atoms give the fluorinated compound unique properties due to its electronegativity, its small size, and its tightly non-bonding electron pair. The covalent carbon-fluorine bond is the strongest known bond with a bond length of 1.35Å, a value that decreases with increasing number of fluorine atoms [60]. This property is exploited in the formulation of PTFE which is tightly packed polymers that are almost chemically inert.

Fluorinated sulfonic acids are very strong Brønsted acids. A valued fluorinated acid in the industry is Nafion-H (a perfluororesin sulfonic acid). This acid is unique since it combines an ionic character with a stable polymer tail of tetrafluoroethylene. The polymer is linked together with the sulfonic acid through perfluorovinyl ether groups. The acidic head group will react with inorganic and organic bases while the polymer tail will remain inert and highly hydrophobic [61,62]. One of the most well-known and used fluorinated sulfonic acid is the trifluoromethanesulfonic acid (CF_3SO_3H, triflic acid), this acid is one of the strongest superacids known, highly valued due to its stability as an acidic catalyst in carbocationic reactions. It is also superior aluminum chloride in Friedel-Crafts acylations of aromatic substances [63].

Fluorine is a strong electron-withdrawing atom which has an acidic effect of fluorinated acids, alcohols etc. The pK_a (logarithmic acid dissociation constant) values for the PFAAs are so far inconsistent, e.g. the value ranges from 1 to 3.8 for PFOA [64]. However, at physiological and environmental pH the PFAAs will be dissociated. The physicochemical properties differ between the acid and the ionic form of the PFAAs, it is also dependent upon the concentration, and whether the chain is branched or straight [65]. The acidic form of PFOA has a considerable high vapor pressure, whereas the salt is in practical non volatile [7,59].

2.2.1 Partitioning in biota

The standard octanol-water partition coefficient (K_{ow}) is a measure of a compounds ability to either distribute in the fat organic phase or in the water column. A log K_{ow} value equal, or over three, meaning at least one thousand times more of the compound is present in the octanol phase than in the water, the compound is thus considered to be bioaccumulative in animals and humans. Persistent organic pollutants like polybrominated diphenyl ethers (PBDEs) and

polychlorinated biphenyls (PCBs) are well-known environmental pollutants with a log $K_{ow} \geq 3$. Halogenation of a compound increases in general the lipophilicity. However there are some exceptions, the lipophilicity of i.e. saturated alkanes decreases by mono- or trifluoromethylation [60]. The partition of PFASs is a little more problematic since the presence of the functional group (carboxylate or sulfonate) will make the molecule partly hydrophilic while the long fluorinated chain is lipophilic and will partition into the octanol. These properties may cause a third interlayer to develop, or in some cases the formation of emulsions makes it difficult to determine the log K_{ow} (not published observations). In **Paper I** we determined the solubility and partition for PFOS, PFOA and PFBS in common laboratory organic solvents and buffers by lowering the temperature and employ centrifugation. Classic POPs are known to accumulate in the fat reservoir in the body, i.e. they do not share the same properties as PFASs. Even though PFASs binds to proteins in the body, instead of lipids they do share one property, i.e. the shorter carbon chain the lower tendency to accumulate in the body. For hydrocarbon chains this is due to higher water solubility while in the case of PFASs this is accomplished by decreased protein binding potency. In some studies PFASs are denoted proteinophilic since they are strongly associated with proteins, in particular serum albumin [66]. Longer chains of PFASs bind more efficient to the proteins in comparison with shorter chains [67].

3 The PCBs of the twenty first century

PFASs may be found far away from manufacturing sites, meaning that they or their precursors are accessible for long-range transport. Due to their ability to undergo long range transport, having high persistence, being bioaccumulative and toxic, PFOS is since 2006 regulated in the European Union under Directive 2006/122/ECOF [68]. PFOS is also included in the Stockholm Convention on persistent organic pollutants since 2009 [13].

Humans are exposed to PFASs either through *direct* exposure via inhalation of air, through house dust or food and water or *indirect* sources which includes inhalation or digestion of PFAS precursors. These precursors, e.g. perfluorosulfonamide, may after metabolism in the body be transformed to PFASs [69]. Reviewing the levels in indoor and outdoor air, house dust, food and water, Fromme *et al*. found dietary exposure to be the predominant source for PFOS and PFOA, accounting for 91% and 99%, respectively, of the plasma levels. They concluded the average daily intake to be 1.6 and 2.9 ng per kilogram body weight, respectively [69]. Among other non-occupational sources are surface treated carpets and waterproofed clothes [70-73].

3.1 Cease in PFAS production

PFASs have with its unique structure an exceptional stability. They possess high surface activity and they are not prone to undergo chemical reactions. These properties have made the PFASs very useful in a variety of industrial and consumer product applications. Unfortunately, these properties also cause problems, since certain PFASs have been found to bioaccumulate [36]. Giesy and Kannan [2] detected PFOS in wildlife and Hansen *et al.* [3] performed analysis of non-occupational American citizens and found several PFASs in their blood. This was worrying news for the fluorine industries; so severe that the major producer decided to cease its production of perfluorooctanesulfonyl

fluoride together with all PFOS-, PFHxS-, and PFOA- related chemistries, in 2000-2002 [4,74].

3.2 What has happened since then?

Following the voluntary phase-out, by the principal worldwide manufacturer, the production of perfluorooctanoyl fluoride chemistry was considerably decreased [53]. The US EPA launched, together with eight companies in the fluorine industry, the PFOA Stewardship Program in 2006. The goal with the program was to reduce the emissions of PFOA with 95% until 2010 and to come to a complete stop in PFOA-emissions by 2015 [22]. The participating companies are obliged to annually report their emissions of PFASs to the US EPA. So far the emissions seem to have decreased substantially [23-25]. A comparison of the serum levels of PFOS prior and post the phase-out indicates a 50-60 % decline in concentrations in the general US population [75]. This is in agreement with another study of the general population in the US that indicated a 60 % decrease in serum concentration from 2001-2002 [25]. This decreasing trend is also apparent in the human milk study presented in this thesis (**Paper II**). Still there are companies that do not participate in the stewardship to reduce the emissions of PFASs, but actions are taken to include also these companies in the program [22].

3.3 The future of PFASs

Efforts are made to find environmentally friendly alternatives that could replace PFOS and PFOA in industrial and consumer product applications. The four-carbon (C4) chemistry (PFBS derivatives) is one alternative that is exploited [76], incorporation of ether bridges into the chemicals, is another alternative that will make the molecule more prone to degrade into shorter chain compounds, i.e. chemicals that do not bioaccumulate [77]. Short carbon chains, as in the case with the C4 chemistry, means they will not accumulate in humans or animals. However, the C4 chemicals are still persistent and will not be

degraded under normal environmental conditions. The insertion of ether bridges in the carbon chain will make the molecule less persistent, this is utilized in the synthesis of ADONA (ammonium 4,8-dioxa-3H-perfluorononanoate) that was announced in 2008 to be the possible replacement for PFOA [20].

3.4 Mother - Child Exposure to PFASs

It is known that at least certain PFASs can cross the placenta [78] (**Paper III**) and thus expose the fetus. Prenatally exposed mice demonstrate neonatal mortality, developmental adverse effects such as delayed eye opening and early onset of puberty in males [79,80].

Even though the PFASs have diverse properties and effects depending on species, this indicates the possibilities of exposure in utero even for humans. There is a possible correlation between the levels of PFOA in human maternal plasma/umbilical cord blood and the fetus birth weight [32,33]. The serum levels of PFASs in women and their newborn babies, living in a PFAS contaminated site in the Mid-Ohio Valley, were analyzed. The analysis revealed a weak correlation between maternal levels of PFASs with the risk for the offspring to be born with birth defects and a lower than normal birth weight [81]. However, in a study conducted by Hamm *et al.* they did not see any association between maternal PFAS levels and the newborns' weight or length [82]. Lopez-Espinosa *et al*. conducted a study at the contaminated site in the Mid-Ohio Valley and found a correlation with PFOS and PFOA concentrations with a later onset of puberty in both girls and boys [83].

Foreign substances may cross the placenta either by diffusion or by active transport from the maternal to the fetal circulation. PFASs are transferred from maternal serum via the umbilical cord to the fetus. Umbilical cord blood (UCB) samples collected at delivery represent the surrogate for *in utero* exposure. The PFAS profile is not the same in UCB samples and maternal blood samples. The concentration of PFASs is also in general decreased in the UCB, i.e. the fetus

will have some protection against exposure towards PFASs in the uterus [84-87]. The transfer efficiency of the PFASs decreases from the maternal to the UCB with increasing chain length. Short-chain PFASs have greater ability to be transferred to the UCB, i.e. to the neonate [84,85,88]. PFOS is the most prominent PFAS in most of the maternal serum samples followed by PFOA. The PFOS isomeric pattern differs between the two matrices, the branched isomers of PFOS have a tendency to cross the placenta more efficient than the linear isomer [84,89]. The functional group plays an important role in the PFASs ability to cross the placenta. Comparing groups of carboxylates and sulfonates it is apparent that the passage of sulfonate groups over the placenta barrier is inhibited, indicating different transport efficiencies of PFASs. Gützkow *et al*. discovered an increase in the proportion of PFOA in UCB in comparison with the maternal plasma [84]. A similar increase was observed in a study by Liu *et al*. [86]. Both these studies concluded that approximately 30% of the PFAS content in UCB arises from PFOA.

Newborn children may be further exposed through PFASs through the mothers' milk. The concentrations of PFASs in human milk is low (up to 130 pg/mL) [69,85]. However, the body burden may be higher for small children in comparison to adults. Investigators have measured the levels in maternal serum with matched milk samples and the concentrations correlates, in most cases, quite well with each other [90,91], while some studies could not detect any significant correlation [85]. There are some indications that PFOA could be transported more efficient through lactation in comparison to other PFASs [86].

3.5 PFASs in mothers' milk

The protein content of human milk varies during the lactation period, shortly after delivery the milk consists of 1.5 % of protein, while the content decreases by half after six months [92]. PFASs associates primarily to serum albumin, i.e.

the levels of PFASs in milk are expected to be low. However, even though the concentration of PFASs in milk is low, it is a route of exposure. Lactating babies may be subjected to a considerable amount of chemicals via the mothers' milk, as well as the general population consuming dairy products.

Lipophilic substances like PCBs, PBDEs, and DDT are more prone to accumulate in the milk than PFASs. Milk consists of three to five per cent of lipids [93]. The PCBs, PBDEs and DDT diffuse along with fats from the plasma into the mammary glands and accumulates in the milk [94-97].

In **Paper II** we evaluated the concentrations of PFOS, PFHxS and PFOA in milk obtained 1972 to 2008 from Stockholm mothers. Since our samples covered the 1970s it was possible to follow the changes over time due to the large-scale fluorochemical production that began around that time [53]. Our milk samples also allowed us to study the abrupt change in production during the phase-out in 2000-2002.

Studies from Norway and the United States conclude an apparent decrease of PFOS levels in human serum after 2000. For PFOA there is a slow decrease as well from this time [23-26,75,98]. However, the longer perfluorononanoic acid seems to increase, which indicate a continuous use [75,98]. The decreasing levels in human serum are most likely due to the phase-out of PFOS, PFHxS and PFOA from the European and American market [53].

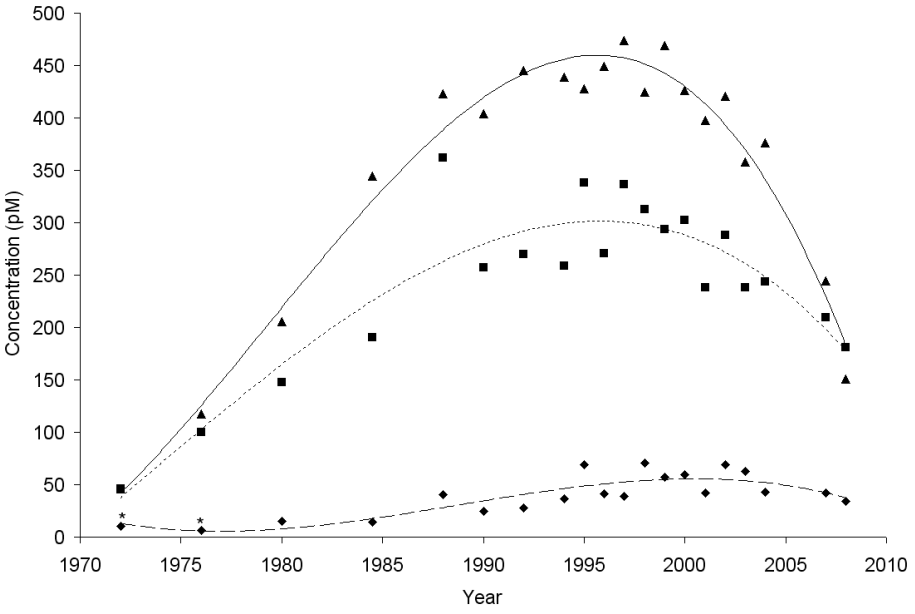


Figure 3.1. Time trend of PFOS (▲), PFOA (■), and PFHxS (♦) on a molar basis (pM) in human milk from Stockholm.

In a Swedish study conducted by Kärrman *et al*. [90] they found that PFOS and PFHxS concentrations in human serum correlates with levels found in human milk. The decreasing trend of PFASs we observed in the Swedish human milk in **Paper II**, did not come as a surprise since similar decreasing trends in human serum have previous been reported from Norway and the US.

The results from our study indicated an increasing trend from 1972 through the late 1990s, rising almost one order of magnitude. Thereafter the trends leveled off and a decrease is observed beginning around 2001 (Figure 3.1). These observations are consistent with the production patterns (Figure 3.2) as well as regulatory and voluntary exposure controls by the producers [53].

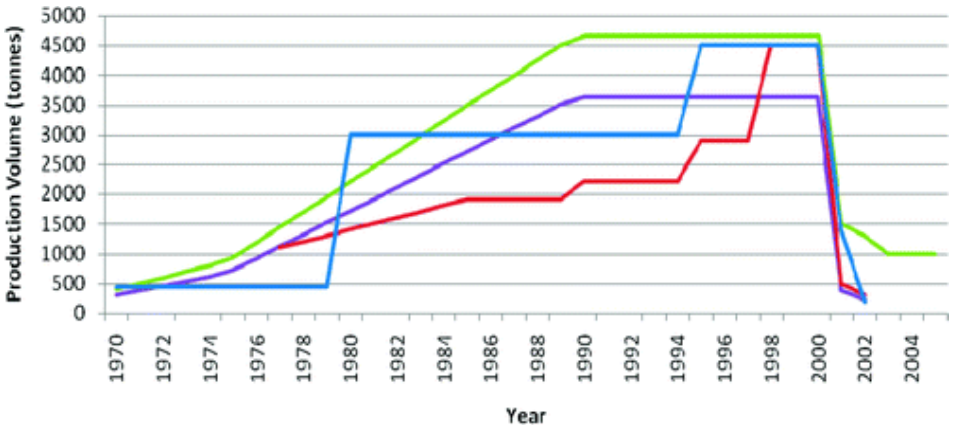


Figure 3.2 .The global production of POSF during 1970 to 2002. Green line is the total production, purple is 3M's production. The blue and red line corresponds to estimates made by other research groups. The figure is adopted from Paul *et al*. [53]

3.6 Contamination of PFAS in solvents and reagents

In order to quantify low levels of analytes extensive methodological development are crucial. The levels of PFASs analysed in **Paper II** were expected to be in the low picomolar (pM) range. To successfully perform these analyses there was a need to reduce all potential sources of background contamination. Our target was to reach a lower limit of quantification (LLOQ) of 5 pg / mL (5 ppt).

It is a well known fact that instruments as well as solvents may contribute to an undesirable background levels of PFASs [99]. Indeed, we found measurable amounts of PFASs in the formic acid, potassium hydroxide, and in the ammonium sulfate solutions. To obtain reliable and accurate results these contamination issues had to be addressed. Our target was to obtain water blanks with a maximum level of 5 ppt of PFASs, which was achieved by repeated liquid–liquid extractions of the solutions and reagents with ethyl acetate and methyl *tert*-butyl ether.

Young *et al*. experienced similar contamination issues during their analysis of bovine milk [100]. They analyzed the formic acid from three different vendors and found similar PFOA levels in all bottles. This problem made them develop another analytical procedure, without the use of formic acid. It is therefore of great importance to analyze all individual reagents and solvents separately prior starting the sample analysis. It is the only way to ensure that reliable analytical results will be obtained.

4 Radiolabeled PFAS - a tool for improved understanding

A radiolabeled compound gives the opportunity to explore the distribution and effect in animals at a low dose. Animals are often treated at high doses of chemicals to see whether or not there are any adverse effects caused by the chemical, i.e. the dose exceeds by far what humans are exposed to. In order to assess the toxicity of the chemical this procedure may be necessary, but to see whether a low dose causes any health effects at an environmental relevant exposure the dose needs to be decreased. The analytical methodologies for analyzing PFASs today, are well developed with good LLOQs. But the question always arises; have we managed to extract "all" PFASs from the matrix? It is a well-known fact that PFASs may adsorb to surfaces and may be difficult to extract. This is a problem that may not always be solved even though the analytical procedure nowadays includes the use of internal surrogate standards for compensating the loss of analytes during the extraction. Isotopic radioactive chemical compounds like the ^{35}S-PFSAs allow us to perform detailed distribution studies e.g. in pregnant mice and also to conduct further studies by use of autoradiography or liquid scintillation counting techniques. Metabolites of PFCAs or PFSAs have not been found in exposed animals due to extreme molecular stability of the C-F bond [36]. Consequently, the radioactivity found in ^{35}S-PFSAs- or ^{14}C-PFCA- dosed animals could therefore only be ^{35}S-PFSA or ^{14}C-PFCA as far as hitherto understood. A major drawback of using radioactive isotopes is otherwise to distinguish between the original substance and its metabolites. In order to confirm the structures of the metabolites further analysis is needed, e.g. by HPLC-MS/MS.

4.1 How to synthesize radiolabeled PFASs?

There are numerous questions to consider before the radiosynthesis can start. When choosing proper synthetic procedures for the radiolabeled PFASs one needs to consider which the possible routes for the synthesis are and which isotopes for doing the synthesis are available on the market. Further questions that need to be addressed are: Does the synthetic procedure work well? Is it a safe synthetic procedure with good reproducibility? Is the chemical and radiochemical purity of the product high enough?

Sulfur-35 has a physical half-life of 87.39 days which is quite short in comparison with carbon-14 with its half-life of 5730 years. Considering this, it would be more beneficial to only work with carbon-14. The problem with the synthesis of carbon-14 labeled PFASs is the insertion of labeled carbon in the perfluorinated carbon chain. Johnson and Behr [40,101]conducted synthesis of ^{14}C-PFOS where they used ^{14}C-labeled POSF after an ECF reaction of the hydrocarbon analogue. Employing ECF in an ordinary laboratory is both difficult and risky. Nevertheless, it is possible to synthesize ^{14}C-PFOS by applying e.g. the method by Shtarov and coworker [102]. The method starts with perfluoroheptyl iodide that will react with ethylmagnesium bromide to create a Grignard reagent in diethyl ether and tetrahydrofuran, which after reaction with ^{14}C-carbon dioxide gives ^{14}C-perfluorooctanoic acid. The reaction is proceeded by introducing N,N-diethyl aminosulfur trifluoride (DAST) or bis (2-methoxyethyl) aminosulfur trifluoride (DEOXO-FLUOR). The produced acid fluoride can be purified through small-scale distillation before reaction with hexafluoropropylene oxide (HFPO) and potassium iodide in tetraglyme which creates a HFPO adduct that may react further with lithium iodide and subsequently rearrange to the primary halide ^{14}C-perfluorooctyl iodide. This iodide could then be used in the ^{14}C-PFOS synthesis as described in **Paper I** but with native sulfur dioxide in large excess. However, synthesis of ^{35}S-PFOS

involves less synthetic steps in comparison with the synthesis of ^{14}C-PFOS which is favorable when working with radiochemicals.

In the year 2006, when the present work began, there were two suitable and commercially available ^{35}S-labeled starting materials applicable for the PFOS synthesis, whereof the first being elemental sulfur. The idea was to let ^{35}S-sulfur dioxide react with the reagent in a Grignard reaction. Experiments to oxidize non-labeled sulfur with oxygen were undertaken, and found to be a safety risk due to the need of a rather high temperature to oxidize the sulfur. The idea of oxidizing sulfur was abandoned and the focus was set on ^{35}S-sulfuric acid for the syntheses of PFSAs. Sulfuric acid may be reduced in the presence of copper, a process which would produce the desired sulfur dioxide but it would also give equivalent amount of copper sulfate and water. This means we would lose half of the starting material due to the formation of sulfate, but an even greater problem would be the formation of water since Grignard reactions are extremely sensitive towards moisture.

Another difficulty was the sulfuric acid itself. For the reduction to occur at high yield it was necessary to use concentrated sulfuric acid (98%), since even small amounts of water would affect the reduction negatively. Since sulfuric acid is hygroscopic it will attract and retain water which was another problem to consider. However, all this considered, the reduction of sulfuric acid was found to be the most suitable approach for synthesis of radiolabeled PFOS, based on pilot syntheses.

The second obstacle was how to introduce the sulfur dioxide into the Grignard reagent. Since the ^{35}S-sulfur dioxide would be the limiting factor in the synthesis, efforts were made to allow as much sulfur dioxide to react with the regent as possible. Attempts were made where the sulfur dioxide were bubbled together with argon gas into the reagent mixture. The reactions were always held below -20ºC, which is below the boiling point for sulfur dioxide (-10ºC). However, this procedure with a carrier gas was not a successful method

since the yields were repeatedly low. A customized vacuum manifold (Figure 4.1) was designed to overcome the need of a carrier gas. By using vacuum and cold traps it was possible to safely transfer gases between the reaction vessels. The sulfur dioxide was led over calcium chloride to dry before introducing the gas into the reaction mixture.

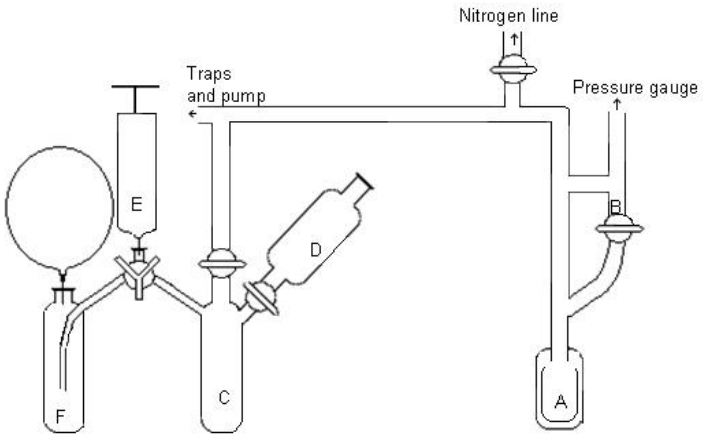


Figure 4.1. The vacuum manifold for transfer of ^{35}S-sulfur dioxide into reaction vessel. A – vial with sulfuric acid, B – stopcock for controlled addition of granular copper, C – cold trap, D - oil funnel, E – syringe for controlled addition of sulfur dioxide, F – reaction vessel. The manifold was designed in house and manufactured for the ^{35}S-PFSAs syntheses.

There have been incidents where Grignard reagents of fluorochemicals have detonated after observation of a deep blue to purple color in the reaction mixture prior oxidation (Schultz, J., personal communication). It is not clear whether this is caused by condensation of liquid oxygen (if liquid nitrogen is used as refrigerant) or if it is due to the reagent itself. However, the observed purple color observed after quenching and oxidation of the ^{35}S-PFOS synthesis is caused by a large amount of iodine, which is a byproduct formed after oxidation of the remaining perfluorooctyliodide.

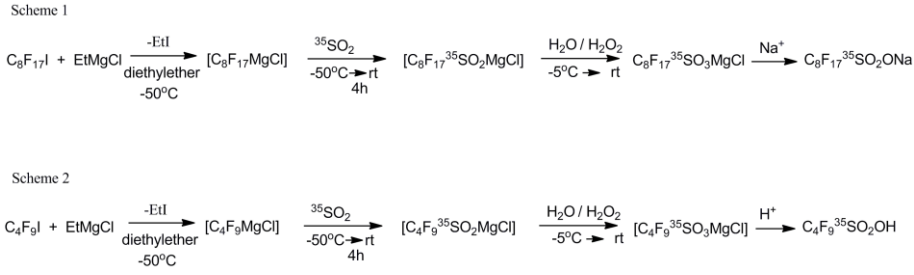
The first attempts to synthesize ^{35}S-PFOS were performed with trimethyl phosphate as solvent. One major drawback with this solvent was its high boiling point (197ºC) which makes it very difficult to evaporate. Trimethyl phosphate is most often used as a methylating agent, which also means it is harmful to exposed humans because of its possible carcinogenic properties. The reason for choosing trimethyl phosphate as solvent is its high ability to dissolve and maintain sulfur dioxide. It is more prone to dissolve gases in comparison with the more commonly used solvent dimethyl sulfoxide (DMSO). However, trimethyl phosphate was abandoned since the yields were low, even though the solubility of the gas was believed to be rather good in this solvent.

To achieve a highly reactive reagent, a zinc-copper couple powder was used to create a complex with the perfluorooctyl iodide. The solvent and reagent were kept under an inert atmosphere to minimize interference from e.g. water that would otherwise quench the reagent. There are a number of published articles describing the reactive character of this zinc-copper reagent [103-106]. Hence, it was expected to work well in the synthesis of the ^{35}S-PFSAs. Nevertheless, the reagent showed low reactivity in the experiments. Since the limiting factor in these radiosyntheses is the ^{35}S- sulfur dioxide, it is essential to obtain a highly reactive reagent that is prone to react with all of the sulfur dioxide that is introduced into the reaction vessel. Focus was at this point shifted to a more classic Grignard reagent in order to achieve higher reactivity of the reagent. Solutions of ethyl magnesium bromide/chloride in diethyl ether were used to create reagents with the perfluoroalkyl iodides. Due to decreased solubility of sulfur dioxide in diethyl ether (compared to trimethyl phosphate), the temperature of the reaction vessel was lowered. Together with the customized design manifold this approach was indeed found to be successful, giving ^{35}S-PFOS in 20 % yield (Scheme 4.1).

The same methodology was used for the synthesis of ^{35}S-PFBS, resulting in 18 % yield (Scheme 4.1). The synthesis of ^{35}S-PFOS was developed during

two years of intensive experimental work. The critical limiting factor in this synthesis is the use of sulfur dioxide. Previous published methods describing similar reactions always use the reacting gas in large excess.

Further, another unexpected problem was the oxidation of the unreacted perfluorooctyl iodide, forming PFOA upon oxidation with hydrogen peroxide. Attempts were made to isolate the raw product (the perfluorooctanesulfinic acid) prior oxidation, something that would prevent the formation of PFOA, unfortunately those attempts were unsuccessful. During reactions with good results there was a small amount of un-reacted perfluorooctyl iodide left, which in turn gave low amounts of PFOA after oxidation. During reactions with low yields of PFOS, the formation of PFOA was considerably larger.



Scheme 4.1. Synthesis of ^{35}S-PFOS (1) and ^{35}S-PFBS (2).

To purify PFOS from PFOA contamination we did some experiments with C18 SPE-columns (solid phase extraction), an extraction method suitable when handling analytical samples but not really applicable in radiosynthetic work. The best way to separate these compounds was found to be extraction with n-hexane. PFOA is more soluble in hexane than PFOS. Even though PFOA is not easily dissolved in hexane, this extraction procedure still seems to be the most practical process to separate the two compounds.

4.1.1 Synthesis of ^{14}C-PFOA

The synthesis of ^{14}C-PFOA was developed from the method published by Shtarov and Howell [102], who synthesized a variety of ^{13}C- and ^{14}C-labeled derivatives of perfluorocarboxylates in good yields. A major and crucial difference between the syntheses of labeled PFOA is the scale in which they are performed. Shtarow and Howell performed their synthesis in an approximately 30 times larger scale than I allowed the reaction to go. The smaller scale was required to achieve a synthesis of ^{14}C-PFOA with a high specific activity. High specific activity is beneficial when it comes to distribution studies e.g. autoradiography. A larger scale is always easier to practically handle than the small scales dealt with in this thesis. Due to the scale of the present synthesis of ^{14}C-PFOA, I used repeated liquid-liquid extractions for the purification of ^{14}C-PFOA. A larger scale would have allowed me to distill the product, a procedure that is expected to have given a higher purity of the ^{14}C-PFOA prepared.

Synthesis of ^{14}C-PFOA was accomplished by introducing a solution of ethyl magnesium bromide (3 M, 1.0 mmol, 0.33 mL) at -50ºC into the reaction flask containing diethyl ether (dried, 10 mL) and perfluoroheptyl iodide (0.93 mmol, 0.22 mL). The Grignard reagent was stirred and left at -50ºC for one hour before the temperature was decreased to approx. -100ºC. Tetrahydrofuran (10 mL) was added to the reaction mixture to increase the solubility of carbon dioxide in the reaction mixture. The ^{14}C-carbon dioxide (50 mCi, 59 mCi/mmol) was delivered in a break-seal flask which was opened and transferred under vacuum according to the supplier's guidelines using a similar manifold as demonstrated in Figure 4.1. After the transfer of all of the carbon dioxide into the reaction flask the temperature was allowed to increase to -78ºC during four hours, and thereafter slowly to room temperature over night. The reaction was cooled to -30ºC and carefully quenched with water (0.5 mL) and sulfuric acid (10 M, 1 mL). The ether phase was transferred and collected in a specialized designed flanged evaporation beaker. This beaker prevented to some degree the

formation of micelles (excessive foaming) during evaporation. The raw product was extracted with diethyl ether (4 × 5 mL) that gave, after evaporation of the solvent, an orange-colored oil to which potassium hydroxide (2 M, 2 mL) was added. The formed colourless and gel-like mass was extracted with n-pentane (3 × 8 mL). Hydrochloric acid (37%, 1 mL) was carefully added to the water phase before extraction with dichloromethane (8 × 6 mL) followed by evaporation on a rotary evaporator (placed in a fume hood). The synthesis yielded 20 mCi of ^{14}C-PFOA with a specific activity of 59 mCi/mmol.

4.2 Handling of gaseous radioactive isotopes in the laboratory

Transfer of gaseous radioactive isotopes like ^{35}S-labeled sulfur dioxide and ^{14}C-labeled carbon dioxide are easiest achieved using vacuum manifold and cold traps. For efficient transfer between vial and reaction vessel it is necessary to have a sealed system without any intake of air that would otherwise condensate in the cold traps.

Before using the manifolds they were first rinsed with acetone before applying vacuum and nitrogen line repeatedly. The pressure in the manifold was measured and the system was checked for leaks after a couple of hours of pumping. The average pressure, received after approx. 5 hours of pumping vacuum, was between one and ten millibars depending on vacuum pump and the glassware that was used. Even though a careful and thorough effort was made to get the system free from leaks, the pressure in the manifold did raise to 20 millibars after one and a half hour. Considering this increase in pressure, i.e. intake of moist air, together with the estimated one hour it will take for the gas to transfer between the vial and the reaction flask, the manifold was closed to the reaction flask after one hour to prevent further intake of moist air.

4.3 Safety aspects

Besides ordinary safety precautions such as well-ventilated fume hoods and personal safety equipment like lab coat and goggles we have installed an active-carbon filter from the air outflow above the fume hoods. Due to the large surface area in the activated carbon filter it would adsorb any gaseous ^{35}S-sulfur dioxide released from the reaction setup. It should be noted that the reaction setup with the manifold is a sealed system, limiting the risks of any radioactive discharges. After the reaction is completed the manifold is flushed with nitrogen gas which is bubbled through sodium hydroxide solution traps. Nevertheless, we have the filter if a situation occurs that we cannot predict or control, e.g. an unexpected implosion. In our lab all radiosyntheses are preceded with a substantial number of pre-syntheses to assure good knowledge and safe handling of the reaction and the equipment. Nevertheless, workings with gases in vacuum manifolds at high and low temperature always pose a certain risk. To avoid implosions it is essential to always work with glass in best possible condition, i.e. without any visible cracks. All work with the manifold and radioactive material was performed in a fume hood behind a Plexiglas shield, thus providing both shielding and protection from ionizing radiation and glass shatter in case of an accident. The pre-syntheses are crucial since any mistake during handling of radioactive material may have severe health effects not only on the person performing the synthesis but also to colleagues working in the same laboratory. Our isotope-laboratory and students working within this area follows the regulation established by the Swedish Radiation Safety Authority (paragraph SSMFS 2008:28).

5 Tissue distribution and pharmacokinetics of the four PFASs

In the present thesis, a series of studies were undertaken to examine the distribution of PFOS and PFBS in C57BL/6 mice (**Papers III, IV, VI**). Female mice were dosed with ^{35}S-PFOS at gestational day 16 and the distribution of the radioactivity was determined in the dams, fetuses and pups by whole-body autoradiography and liquid scintillation counting (**Paper III**). In **Paper IV**, we administered male mice with an environmentally relevant dose (0.031 mg/kg/day) as well as an experimentally dose (23 mg/kg/day) of ^{35}S-PFOS. The experiment was performed to study the uptake and distribution of ^{35}S-PFBS that was essentially similar to the study undertaken for ^{35}S-PFOS, thereby allowing for a comparison of the pharmacokinetic distribution properties of the two homologues **(Paper VI**). The use of radioisotopic PFASs facilitates the study of pharmacokinetics, allowing for whole-body imaging and rapid determination of tissue concentrations. The stability of PFASs to metabolic and environmental degradation obviates the need to account for radiolabeled metabolic or degradation products. Several previous pharmacokinetic studies of radiolabeled PFASs have exploited these advantages [38,40,107-111].

The tissue distribution and the pharmacokinetics of the PFASs are quite diverse. It is also apparent that pharmacokinetic parameters are different depending on species and also between the genders of the same species. This is apparent in **Paper V**, where we determined the pharmacokinetics for PFHxS.
Independent of species, all four PFASs have volumes of distribution (V_d) in a range (~200 mL/Kg body weight) that suggests predominant extracellular distribution [28,40,112], i.e. uptake of these PFASs into tissues is limited.
To be able to conduct and establish these pharmacokinetic parameters it is often necessary to use laboratory animals. Commonly the laboratory animals that have been used to study the pharmacokinetics of PFASs are rats, mice, and monkeys. Establishing pharmacokinetic parameters in laboratory animal models

used in toxicological research decreases the uncertainty in extrapolating results from toxicological studies in non-human animal models to humans in the process of risk assessment.

5.1.1 PFOS and PFOA

The pharmacokinetics of PFOS and PFOA have been studied extensively [36,113]. These compounds are readily absorbed orally, they do not metabolize, they are only slowly eliminated from the body, and their $T_{1/2}$ in humans are in the order of several years (Table 5.1).

Table 5.1 Average serum elimination half lives for humans, monkeys, rats, and mice. The PFASs are either administered orally or by *iv* injections. The dose is in the range of 2 - 30 mg PFAS per kg body weight.

	Average serum elimination half-lives ($T_{1/2}$)			
	PFOA	**PFOS**	**PFHxS**	**PFBS**
Human *	3.5 yrs [i]	4.8 yrs [i]	7.3 yrs [i]	25.8 days [k]
Monkey - Male - Female	iv [b] 20.9 days 32.6 days	[e] 132 days 110 days	**, iv [j] 141 days 87 days	iv[k] α: 0.80 h, β:13.2 h, γ: 95.2 h α: 1.28 h, β: 11.3 h, γ: 83.2h
Rat - Male - Female	o [c] 4.4 – 15 days 1 day	o [e] 41.2 days 71.1 days	iv [j] α: 0.96 days, β: 29.1 days α: 1.64 days, β: N/A	o[k] α: 0.79 h, β: 4.68 h α: 0.53 h, β: 7.42 h
Mouse - Male - Female	o [d] 21.7 days 15.6 days	o [e] 36.4 days 30.5 days	**, iv [j] 28 days 26.8 days	N/A

* Geometric mean half-life. ** The $T_{1/2}$ is not statistically significant between the sexes. iv - intravenous injection, o - oral dose, N/A – not available, [b] [112], [c] [110,114], [d] [115], [e] [40], [i] [11], [j] **Paper V,** [k] [28].

PFASs have an affinity for blood proteins, in particular serum albumin, and this may explain, in part, their V_d values. As a result, larger proportions of the body burdens of PFOA and PFOS are found in blood and in highly-perfused tissues like the liver, kidneys, and spleen. PFOS and PFOA have the ability to cross the placenta and accordingly they will reach the fetus [84].

In the work described in **Paper III,** we utilized ^{35}S-PFOS to determine the distribution of PFOS in dams, fetuses and pups after exposure *in utero.* High levels of PFOS were found in the liver and the lungs of the dams. The fetuses had substantially higher concentrations in their lungs, liver, and kidneys in comparison with the maternal levels in respectively tissue.

Compared to the levels of PFOS found in the lungs of the fetuses, the newborn pups had higher levels of PFOS in their lungs. To correct for the radioactivity derived from the blood in the tissues, the haemoglobin content in each tissue was determined and consequently the radioactivity in each tissue were corrected depending on the haemoglobin levels.

As described in **Paper IV** we exposed C57BL/6 mice for ^{35}S-PFOS either during 1, 3 or 5 days for two experimental doses. Further studies were conducted on two male mice that were given a single dose (12.5 mg/kg) of ^{35}S-PFOS via oral gavage. They were sacrificed 48h post dose and examined through whole body autoradiography (Figure 5.1).

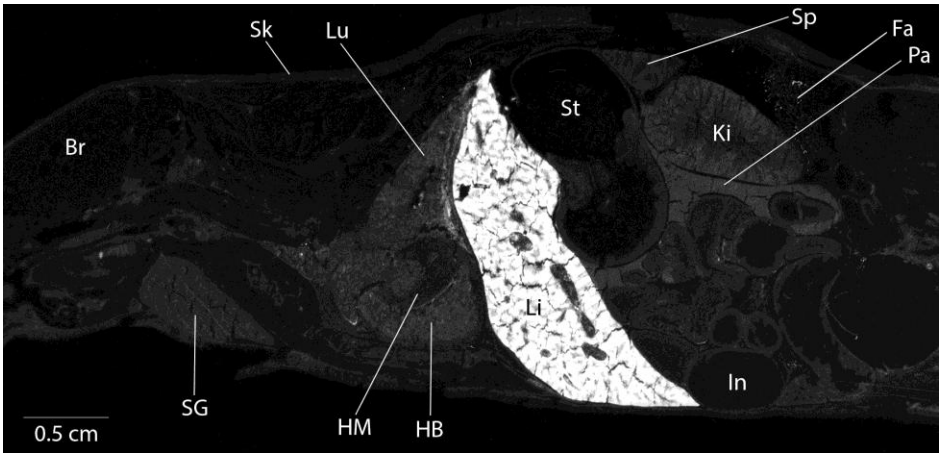


Figure 5.1 Whole body autoradiogram of a male mouse at 48 h after administration an oral dose of 35S-PFOS (12.5 mg/kg, single dose). Brighter areas correspond to higher levels of radioactivity. Br = brain, Fa = fat, HB = heart blood, Hm = heart muscle, In = intestine, Ki = kidney, Li = liver, Lu = lung, Pa = pancreas, Sk = skin, SG = salivary gland, Sp = spleen, and St = stomach.

^{35}S-PFOS was found in all body compartments, lowest concentrations were observed in fat depots and in the brain. 20-45% of the recovered dose was found in the liver after low dose treatment. Similar levels of PFOS were found in the whole bone at this low dose. After high dose was 40-50% of the recovered dose found in the liver. This is in agreement with Chang *et al.* [40] who established the pharmacokinetics and the tissue distribution of PFOS in rats by using radiolabeled and unlabeled PFOS. However, the high per cent of the recovered dose in the whole bone presented in **Paper IV** have not previously been reported. The PFOS levels in the skin (20% of the recovered dose) were the second highest level after giving the mice the high dose of ^{35}S-PFOS. These observations are inconsistent with Chang *et al*. who only found a recovery of 0.1 % in the skin. It is not yet possible to explain the observed difference.

The pharmacokinetics for PFOA exhibit species and gender differences in elimination rates [37]. Early studies by Gibson and Johnson [107,108] indicated slow excretion of PFOA in male rats. This was confirmed by Hundley *et al.*

who concluded blood, liver and kidneys to be the major compartment for PFOA. They noticed a huge difference in excretion between female and male rats, with females excreting PFOA substantially faster [116]. This might most likely be explained by differences in the expression of organic anion transport processes [37].

There is an inconsistency in the distribution pattern of PFOS and PFOA in tissues between different species, even among the rodents. This implies the difficulties when it comes to extrapolating animal data to humans and to do a proper risk assessment.

5.1.2 PFHxS and PFBS

The work described in **Paper V** led to the establishment of various pharmacokinetic parameters for PFHxS in rats, mice and monkeys. Analysis of rat serum 96 h post-dose revealed a non-linear relationship with the given dose of PFHxS. There was a clear distinction between the sexes, female rats had a much lower serum and liver PFHxS concentrations in comparison with the males, i.e. females eliminated PFHxS more efficiently. Male rats excreted PFHxS in a dose-dependent manner. After a high dose of PFHxS, 30% of the dose was excreted in the urine 96 h post-dose. For the low dose, only 6-7% of the recovered dose was excreted via the urine. To study the serum uptake and elimination in mice, we dosed CD-1 mice with 1 or 20 mg potassium PFHxS per kilogram body weight. Analysis of tissues revealed highest concentrations in the serum, liver, and kidneys. Cynomolgus monkeys (*Macaca fasicularis*) received a single *iv* dose of PFHxS in order to provide pharmacokinetic parameters for non-human primates.

The $T_{1/2}$ was the pharmacokinetic parameter that varied the most in the species studied. Female rats eliminated PFHxS most efficiently, followed by male rats, and mice. Monkeys had the longest $T_{1/2,}$ and there were indications of a sex-difference in serum elimination rate, with females excreting faster than

males. However, the gender-dependent elimination rates were not statistically significant for mice and monkeys.

The pharmacokinetics of PFHxS is similar to PFOS [40]. Both these compounds, together with PFOA, exhibit gender differences in the elimination profile of rats. The differences in elimination may be due to different expression of renal organic anion transporters (further discussed below) [37] (Butenhoff, JL, personal communication).

Olsen *et al*. have presented the pharmacokinetics of PFBS in humans, monkeys, and rats. Compared to the other three PFASs (PFOA, PFHxS, and PFOS), PFBS is rather rapidly eliminated from the body, thus it has a low tendency to bioaccumulate [28]. Rats and monkeys excreted 30-87% of the PFBS 24 h post a single dose.

In **Paper VI** a study is described in which mice were fed a diet containing ^{35}S-PFBS. The mice were sacrificed either 1, 3, or 5 days after been given the daily dose of 16 mg/kg/day of ^{35}S-PFBS. Besides whole-body autoradiography we examined twenty different tissues in mice by liquid scintillation counting techniques. The levels of ^{35}S-PFBS in tissues were between 4 and 50 fold lower in comparison with PFOS (**Paper IV**), confirming its fast elimination profile in rodents [28]. Both PFOS and PFBS are well absorbed (>95% within 24h) in rodents (Butenhoff, JL, personal communication). An indication of accumulation of PFBS was seen up to day 3, where after the levels leveled off and a steady-state was reached at day 5. The whole-body autoradiography confirmed highest levels in the gastrointestinal content, liver, cartilage, blood, stomach, and intestinal walls. Moderately high levels were present in the kidneys and lungs. Interestingly, the autoradiograms showed concentrations of PFBS in the salivary glands and male genitals that were similar to those in the liver and the kidneys, respectively.

There were no time-dependent increases in serum levels of PFBS during 5 days of exposure via diet in mice, and this was likely due to efficient elimination, as previously demonstrated for rats, monkeys, and humans [28].

5.2 Excretion of PFAS

Urinary and fecal elimination are major routes of removal of toxicants and waste products from the body, often facilitated by metabolism/conjugation of parent compounds to more polar and water soluble substrates. However, the strong carbon-fluorine bonds of PFASs render them impossible for the body to metabolize/conjugate. Although the aqueous solubility of PFASs varies considerably depending on structure, urinary has been identified as the major route of excretion for PFBS, PFHxS, PFOS, and PFOA, while biliary excretion (i.e. excretion via feces) is the second most abundant elimination pathway [28,40,112].

The great species and sex-difference observed for PFOA, PFOS, and PFHxS may *not* be explained by different ability of binding to plasma proteins [117], instead it is thought to depend on species, developmental age, and sex specific expression of organic anion transporters [37] (Butenhoff, JL, personal communication). Gender differences in elimination due to differential expression of transporters based on hormonal regulation have been illustrated clearly in rats in the case of PFOA [37,110,118-120]. In addition, differential expression of transporters during development may explain the ontological development of the gender difference in PFOA elimination during sexual development in the rat [121].

There are several organic anion proteins involved in the renal organic anion transport system for secretion and reabsorption of PFASs. In rats the organic anion transporting polypeptide (OATP) 1a1 is thought to be the major cause of renal reabsorption of PFOA. Compared to the female kidney, the

protein expression of OATP1a1 is favoured in the male kidney. Furthermore, the expression of OATP1a1 is regulated by the levels of testosterone, castrated rat males have lower levels of OATP1a1 [111,120,122]. There are species-specific renal transporters, i.e. rats and humans have different renal transporter protein that may contribute to the species-related differences in the elimination of PFASs (reviewed in Han *et al*. [37]).

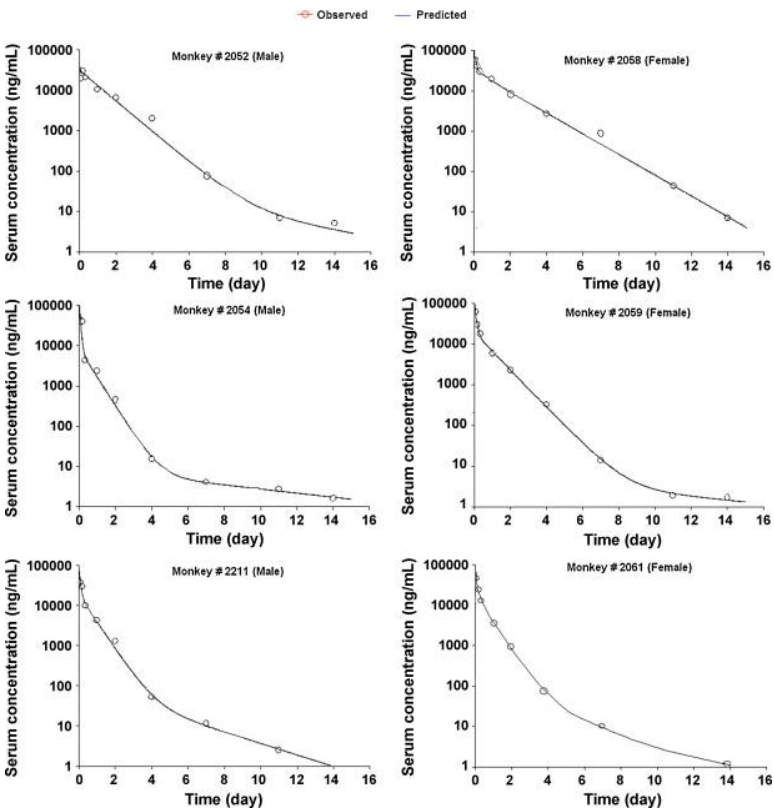


Figure 5.2 Serum concentrations for male (left column) and female (right column) Cynomolgus monkeys after receiving a single iv dose of PFBS (10mg/kg body weight). Adopted from Olsen *et al*. [28].

The serum concentration profile of PFASs in dosed animals exhibit in some studies a rather fast elimination phase, where after a steady-state phase is reached and the elimination rate is severely reduced. This is exemplified in

Figure 5.2 where Olsen *et al*. [28] studied the serum levels of PFBS in male and female Cynomolgus monkeys after a single *iv* dose (10 mg/kg body weight). The elimination phase in their study appears to follow a multi-phasic mechanism. The first phase (α) corresponds to the distribution in the body while the β-phase is the elimination period. The last excretion phase (γ) is slow due to reabsorption, enterohepatic circulation, or tissue release of the PFAS (schematic overview in Figure 5.3).

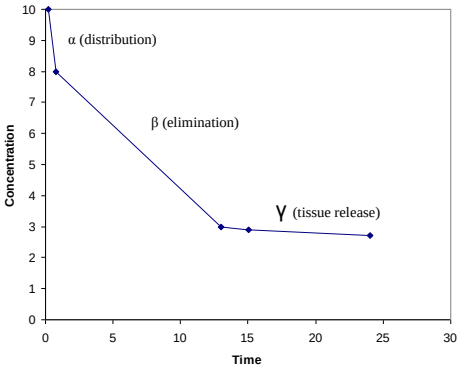


Figure 5.3 The first phase (α) corresponds to the distribution in the body while the β-phase is the elimination period and γ is the reabsorption, enterohepatic circulation, or tissue release phase.

Fecal excretion is most often accomplished via the biliary route. Nutrients and foreign substances in the gastrointestinal tract will be absorbed and transferred with the blood to the liver where it may be metabolized or withheld to avoid further distribution in the body. Whether a compound is secreted with the urine or bile depends mostly on the species.

Once the substance is excreted into bile and enters the intestines it may either undergo enterohepatic circulation or be eliminated with feces. PFOA and PFOS are known to undergo enterohepatic circulation as evidenced by the ability of cholestyramine to facilitate the plasma clearance of PFOA and PFOS [38,123].

Fecal elimination via the bile is not the prominent excretion pathway for PFASs in rats, meaning the gender difference in elimination rate is not due to differences in biliary excretion (reviewed in Han *et al.* [37]). For humans the situation is another, we are very poor renal eliminators [123-125] when it comes to PFASs, meaning biliary excretion is in favour. However, for the highly water soluble PFBS, urine excretion has been found to be an important elimination pathway in humans [28].Whitworth *et al*. [126] found pregnancy to be an important clearance route for PFASs in humans. As observed by Liu *et a*l., the same is true for pregnant mice exposed to PFOS. It is also likely that menstruation will lower the body burden of PFAS in women [125]. However, artificial regular bleeding of female mice did not cause a reduction of the serum levels of PFOS. Male mice on the other hand did show significant lower levels of PFOS after artificial bleedings [127], this might possible be explained by differences in uptake and elimination rates of PFASs in mice.

6 Conclusions and future perspectives

To understand the behaviour of a chemical and to be able to foresee its distribution in living organisms are important matters for hazard identification of a chemical. Both radiosyntheses and distribution studies of common PFASs have been presented in this thesis. Also the temporal trends of PFASs in Swedish human milk have been studied. A decreased level of PFOS and PFOA were determined in the human milk, beginning around 2001. This trend is in alignment with studies from the US and Norway [23-26,75,98].

Radiolabeled substances are unique material since it behave and distribute just as the stable isotopic form of the compound. One important advantage by using a radiolabeled compound is the possibility to then follow and detect the compound at very low concentrations. Still, since it is the radioactivity that is being followed it may be metabolites that are observed. This is however highly unlikely with the very stable PFASs studied herein.

The first one out is PFOS, a chemical substance which today are regulated both within EU and the US due to its persistence, toxicity, and pharmaco-kinetics. The synthesis of ^{35}S-PFOS was accomplished through a Grignard reaction of perfluorooctyliodide and ^{35}S-sulfur dioxide. Transfer of gaseous sulfur dioxide into the reaction mixture was achieved through a vacuum manifold, designed in house. The synthesised ^{35}S-PFOS was promptly utilized in distribution studies of mice as well as in solubility experiments. PFOS exposures *in utero* are associated with developmental disorders and reduced neonatal survival [128]. Our mouse dams were dosed *iv* or *po* with ^{35}S-PFOS at gestational day 16. Autoradiography and liquid scintillation counting revealed high levels of PFOS in the fetuses' lungs, liver, and kidneys. The pups had remarkable high levels in their lungs. In the second paper of PFOS distribution in mice we gave male mice both a low environmental relevant and a high dose. The study demonstrated that PFOS distribute in the body in a dose dependent

manner. High doses cause PFOS to leave the blood stream and enter tissues to a larger extent.

The only drawback with the ^{35}S-PFOS is the short half-life of sulfur-35. Utilizing ^{14}C-labeled PFASs may have been preferable, especially for long term studies. Radiosynthesis of ^{14}C-PFOS is difficult but not impossible, as described in Chapter 4. We have already designed the proper glass equipment for the synthesis and it would be interesting to perform the synthesis in the future. However, the challenge lies in the scale of the synthesis. High specific activity of the desired product demands low quantities of the radiolabeled starting material.

The second most abundant PFAS is PFOA, for which regulatory and industrial efforts are being made to minimize the use and release to the environment. The method of synthesizing ^{14}C-PFOA is based on a methodology developed by Shtarov and Howell [102] and implies the use of a highly reactive Grignard reagent, perfluorohepyliodide, and ^{14}C-carbon dioxide. So far the ^{14}C-PFOA has been used in solubility tests and distribution studies in zebrafish (unpublished results).

PFHxS is the two carbon shorter analogue of PFOS and it has in humans a long $T_{1/2}$ (7.3 years), that is even longer than PFOS (4.8 years) [11]. Lately, concerns have been raised due to its former use in fire fighting foams. Analyses of drinking water have revealed highly elevated levels of PFHxS close to fire fighting practice sites at some locations in Sweden [129]. The pharmacokinetics of PFHxS in mice, rats, and monkeys established in this thesis are important parameters in order to make a proper risk assessment. The pharmacokinetic characteristics for PFASs are in general that they are highly diverse between species.

Industries have made efforts to replace, or at least minimize the environmental release, of the perfluorooctane based chemistry (PFOS and PFOA derivatives) due to its persistence and toxicological profiles. One

replacement is the perfluorobutane-based products with PFBS as the ultimate degradation product. PFBS seems to exert less toxicity in mammals and it is a compound that is rapidly excreted from the body [28,76,130]. I utilized the same methodology to synthesize ^{35}S-PFBS as I did for ^{35}S-PFOS. In order to compare the distribution of PFBS in mice we gave the mice ^{35}S-PFBS spiked feed and collected the tissues after one to five days. The majority of PFBS were found in whole bone, liver, blood, skin, and in the muscles. The levels of PFBS were overall 5-40 fold lower in the tissues compared to the levels found after exposure to ^{35}S-PFOS.

The distribution studies presented in this thesis may complement the understanding of PFASs. It is of utmost importance to study the long term pharmacokinetic parameters to assess whether a chemical substance is hazardous to humans, wildlife or the environment, and consequently avoid the release of future environmental pollutants.

The work done and presented in this thesis is mainly focused on three PFASs. Future studies are necessary for the inclusion of the very large number of different PFASs. The extreme stability of the carbon – fluorine bond in the PFASs family must be addressed in order to obtain a safe environment. There will be a future need of radiolabeled PFASs as well as extensive work on pharmacokinetic and toxicological studies, including the identifications of the metabolites of the novel PFASs.

7 Svensk sammanfattning

Att kunna förstå hur ett kemiskt ämne beter sig och därmed kunna förutse hur den kommer att fördelas i miljön och i organismer är grundläggande förutsättningar för att göra faroanalyser och fördjupande riskbedömningar av ämnet. I denna avhandling presenteras både radioaktivsynteser och distributionsstudier av några av de vanligast förekommande perfluor-alkylföreningarna (PFAS). Vidare presenteras en tidstrendstudie av halterna av tre PFAS i modersmjölk från Stockholm. Prover från 1970-talet fram till 2008 analyserades och visade på en nedåtgående trend i PFAS koncentrationen från omkring år 2001. Denna nedåtgående trend överensstämmer väl med resultaten från andra studier utförda i USA och i Norge [23-26,75,98] och speglar utfasningen av PFOS och PFOA som inleddes mellan år 2000 och 2002.

Radioaktivt inmärkta föreningar är unika eftersom de beter sig och distribueras på samma sätt som de stabila icke-märkta föreningarna. En väsentlig fördel med att använda en radiaktivt inmärkt förening är möjligheten att följa och detektera föreningen även vid mycket låga koncentrationer. Man ska emellertid komma ihåg att det är radioaktiviteten som mäts, det är därmed alltså fullt möjligt att man även mäter metaboliter. Med de PFASs som studerats i denna avhandling är det dock föga troligt då de är mycket stabila och kommer inte att brytas ned under normala fysiologiska betingelser.

Perfluoroktansulfonsyra (PFOS) är en av de mest välkända PFAS, den regleras idag både inom EU och i USA på grund av dess persistens, toxicitet samt dess farmakokinetik. Syntes av radioaktivt märkt ^{35}S-PFOS genomfördes via en Grignard reaktion mellan perfluoroktanjodid och ^{35}S-svaveldioxid. Svaveldioxiden förekom i gasfas och överfördes till reaktionskärlet med hjälp av en vakuummanifold, specialdesignad för ändamålet. Den syntetiserade ^{35}S-PFOS användes direkt till distributionsstudier av möss samt i löslighetsexperiment. Exponering av PFOS under fostertiden är i gnagare

associerad med ökad fosterdödlighet och utvecklingsstörningar [128]. Vi doserade honmöss med ^{35}S-PFOS under dräktighetsdag 16 antingen via intravenös injektion eller genom sondmatning. Autoradiografi samt vätskescintillation avslöjade höga halter i fostrens lungor, levrar och njurar, två till fyra dagar efter exponering. De nyfödda ungarna hade höga halter av PFOS i lungorna. Vi genomförde även en studie av PFOS där vi exponerade hanmöss antingen för en låg dos ^{35}S–PFOS eller en högre toxikologiskt relevant dos. Den lägre dosen gav ungefär samma serumkoncentration som personer som arbetar med produktion av PFOS uppvisar. Studien visade att PFOS distribueras på ett dosberoende vis. Hög dos medför att kvoten mellan PFOS i blodet och i vävnaderna förskjuts. Vid en hög exponering så kommer PFOS att lämna blodet och förekomma i vävnaderna i högre utsträckning.

Den näst vanligaste PFAS är perfluoroktansyra (PFOA), den är liksom PFOS reglerad och det görs ansträngningar för att minska användningen ytterligare. Metoden som användes för att syntetisera den radioaktivt märkta ^{14}C-PFOA bygger på en syntes av Shtarov och Howell [102], vilket involverar användning av ett reaktivt Grignardreagens, perfluorheptyljodid samt ^{14}C-koldioxid. Hittills har den syntetiserade ^{14}C-PFOA används för att fastställa en del löslighets parametrar samt för att studera distributionen i zebrafisk (ej publicerade resultat).

Perfluorhexansulfonsyra (PFHxS) har en lång halveringstid i människa (7,3 år), vilket kan jämföras med PFOS på 4,8 år [11]. På senare tid har frågor väckts på grund av att PFHxS har hittats i dricksvatten på flera orter i Sverige. Analyser av dricksvatten har på vissa orter visat höga halter av PFHxS, dessa tros bland annat härröra från brandsläckningsskum [129]. Farmakokinetiken för PFHxS som tas upp i denna avhandling för mus, råtta och apa är viktiga parametrar för att kunna göra en korrekt riskbedömning av denna kemikalie. I allmänhet uppvisar PFAS en stor variation i farmakokinetiken mellan arter. Ofta

föreligger det även en olikhet, främst i halveringstiden för serum-elimination, mellan könen.

Ansträngningar görs för att ersätta, eller åtminstone minimera utsläppen, av den perfluoroktan-baserade kemin (PFOS och PFOA, samt deras derivat). Ett alternativ som används är perfluorbutanderivat, vilket ger perfluorbutansulfon syra (PFBS) som den slutliga nedbrytningsprodukten. PFBS uppvisar lägre toxicitet och utsöndras snabbt från kroppen [28,76,130]. För syntesen av ^{35}S-PFBS användes en liknande metod som för syntesen av ^{35}S-PFOS. För att studera distributionen i kroppen gav vi möss ^{35}S-PFBS via fodret och insamlade vävnader efter 1– 5 dagar. Majoriteten av PFBS återfanns i skelettbenen, levern, blodet, huden och i musklerna. Halten av PFBS var totalt 5-40 gånger lägre i vävnaderna i jämförelse med de halter som återfanns i de ^{35}S-PFOS exponerade mössen.

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