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Evaluation of occurrence and toxicity of per- and polyfluoroalkyl substances in a skiing area

Utvärdering av förekomst och toxicitet av per- och polyfluorerade ämnen vid Vasaloppsstarten

Joakim Mesch

Abstract

Evaluation of occurrence and toxicity of per- and polyfluoroalkyl substances in a skiing area

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Per- and polyfluoroalkyl substances (PFASs) are a group of organic substances that are persistent in nature, and potentially bioaccumulative and toxic. For example, studies have associated elevated levels of PFASs to hormone disruption and cancer amongst other medical conditions. Furthermore, PFASs have the potential to be transported over long distances through the atmosphere or water phase, and are globally distributed in the environment. One of the most known PFAS is perfluorooctanesulfonate (PFOS), which has been classified as persistent organic pollutants (POPs) by the Stockholm Convention in 2009. PFASs have been used in ski waxes due to their ability to repel both water and dirt, which increases the glide. However, PFASs can be abraded from the base of the ski and can potentially enter the environment.

In this study, PFASs have been evaluated in terms of their fate in snow, water, soil and sediment and toxicity at a large skiing event in Sweden. The results showed that the samples from the area around the skiing race was contaminated with long chain (C_{10}, C_{13} and C_{16}) perfluoroalkyl carboxylates (PFCAs), probably originating from the skiing activities. However, the concentrations of long chain PFCA were generally low (maximum of 6.4 ng L^{-1} in snow from a hill at the skiing tracks). Similar concentrations of shorter chain PFCAs (C_4-C_8) were measured in the snow at the skiing area (in average, 7.2 ng L^{-1}) and at a reference site (6.4 ng L^{-1}) indicating atmospheric deposition of shorter chain PFCAs. In snow and surface water samples shorter chain PFCAs (C_4-C_8) were dominant (in average 80% and 86% of the ∑PFASs, respectively), whereas in soil and sediment PFAS precursors were dominant (in average 46% and 52% of the ∑PFASs, respectively) indicating that the distribution of PFASs in snow, water, soil and sediment depends on their physicochemical properties.

The toxicity of PFASs in water, soil and sediment was evaluated using a zebrafish embryo toxicity test. The fish embryos exposed to the three water samples developed normally with no toxicity compared to tap water from Uppsala. For sediment and soil samples, only one of the eight tested samples (i.e. forest podzol soil from the skiing area) affected the embryos (i.e. unhatched or coagulated embryo). It is important to note that the toxic effect can also originate from other organic pollutants which were not measured in this study. More studies are needed to investigate the fate and impact of PFASs at skiing areas.

Keywords: PFAS, PFCA, PFSA, zebrafish embryo test, skiing

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Referat

Utvärdering av förekomst och toxicitet av per- och polyfluorerade ämnen vid Vasaloppsstarten

Joakim Mesch

Per- och polyfluorerade ämnen (PFASer) är en grupp organiska ämnen som är persistenta i naturen, och potentiellt bioackumulativa och toxiska. Till exempel har studier associerat förhöjda halter av PFASer med bland annat hormonrubbningar och cancer. Utöver det har PFASer potentialen att färdas långa sträckor dels genom atmosfären, dels genom vattnet, vilket har gjort dem allmänt förekommande i miljön över hela Jorden. En av de mest kända PFASerna, perfluorooctanesulfonate (PFOS), klassades 2009 som en långlivad organisk förorening (POP) av Stockholmskonventionen. PFASer har använts i skidvalla för sina egenskaper att stöta bort både smuts och vatten, vilket ökar glidet. Dessvärre kan vallan nötas bort från skidans underlag och då kan PFASerna hamna i miljön.

I denna studie har PFASer utvärderats med avseende på deras toxicitet och förekomst i snö, vatten, jord och sediment kring Vasaloppsstarten. Resultaten visade att proverna från området kring skidspåret var förorenade med långkedjade (C_{10}, C_{13} and C_{16}) perfluoroalkyl karboxylater (PFCAer), som troligen härrör från skidverksamheten. Halterna av längre PFCAer var generellt låga (max. 6,4 ng L^{-1} i snö från en backe i skidspåret). I snö från skidområdet och från en referensplats uppmättes liknande koncentrationer av kortkedjade (C_4-C_8) PFCAer (7,2 ng L^{-1} respektive 6,4 ng L^{-1}), vilket indikerar atmosfärisk deposition. I snö- och ytvattenprover var kortkedjade PFCAer dominanta (i genomsnitt 80% respektive 86% av ΣPFASer), medan i jord och sediment var föregångare till PFAS dominerade (i genomsnitt 46% respektive 52% av ΣPFASer), vilket indikerar att fördelningen av PFASer i snö, vatten, jord och sediment beror på deras fysikalisk-kemiska egenskaper.

Toxiciteten för PFASer i vatten, jord och sediment utvärderades med hjälp av ett zebrafiskembryotest. Fiskembryon som utsattes för tre olika vattenprover utvecklades normalt utan toxicitet jämfört med kranvatten från Uppsala. För sediment- och jordprover, var det endast ett av de åtta testade proverna (dvs. skogsjord från skidområdet) som påverkade embryona (dvs. okläckt eller koagulerat embryo). Det är viktigt att notera att den toxiska effekten även kan härröra från andra organiska föroreningar, som inte mättes i denna studie. Fler studier behövs för att undersöka hur och effekterna av PFASs på skidorter.

Nyckelord: PFAS, PFCA, PFSA, Vasaloppet, zebrafiskembryotest

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Preface

This master thesis was the final part of my education in the Aquatic and Environmental Engineering Programme at Uppsala University and the Swedish University of Agricultural Sciences (SLU). The work was carried in cooperation between two departments at SLU, namely the Department of Aquatic Sciences and Assessment and the Department of Biomedical Sciences and Veterinary Public Health.

I would like to thank my supervisor Lutz Ahrens at the Department of Aquatic Sciences and Assessment for his excellent guidance throughout this thesis work and his colleague Sarah Josefsson for her help in sampling and laboratory work. I also want to thank my subject reviewer Stefan Örn at the Department of Biomedical Sciences and Veterinary Public Health and his colleague Gunnar Carlsson for their help in sampling and guidance in the zebrafish embryo test. Examiner was Professor Allan Rodhe at the Department of Earth Sciences at Uppsala University.

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Populärvetenskaplig sammanfattning

Utvärdering av förekomst och toxicitet av per- och polyfluorerade ämnen vid Vasaloppsstarten

Joakim Mesch

Per- och polyfluorerade ämnen (PFASer) är en stor grupp organiska ämnen som på mänsklig väg har ändrats till att bli mycket stabilare än de var tidigare. En anställd på företaget 3M upptäckte av en slump att PFASer var fläckavvisande. Men det var inte den enda nya egenskapen dessa ämnen erhöll. Vissa PFASer är flyktiga och sprids genom atmosfären medans andra är vattenlösliga och sprids via vattnet. Detta har gjort att PFASer nu kan hittas i stort sett över hela Jorden. De är även persistenta i naturen, toxiska och bioackumulativa, egenskaper som lett till att många PFASer sedan 2009 är klassade av Stockholmskonventionen som långlivade organiska föroreningar (POPs, från eng. Persistent Organic Pollutants). Trots att PFASer har använts sedan 1950-talet dröjde det tills strax efter millennieskiftet innan de första lagarna som reglerade deras användning dök upp. Tillverkningen av föregångare till de vanligaste PFASerna började fasas ut år 2000 av världens i särklass största tillverkare av dessa ämnen, 3M. Det ledde till en kraftig minskning av utsläppen till miljön av dessa ämnen. I och med att produktionen i Amerika och Europa upphörde började den samtidigt sakta men säkert öka i Asien.

PFASer har använts till bland annat olika ytbeläggningar, i till exempel stekpannor och matförpackningar, impregnering av kläder samt till brandsläckningsskum. De används även i skidvalla där deras egenskaper att stöta bort både smuts och vatten ökar glidet. Nackdelen är att vallan kan nötas av från belaget och då hamnar både den och PFASerna i miljön. PFASer beter sig lite annorlunda jämfört med klassiska POPs, där en stor skillnad är att PFASerna är proteinofila istället för lipofila som de andra. Detta gör att de ansamlas i blodet och levern hos de djur som fått i sig dem istället för i fettvävnader. Djurstudier har visat att vissa PFASer bland annat kan störa hormonsignaler i kroppen, påverka levern och orsaka cancer i levern, bukspottskörteln och testiklarna. Studier på människor har kopplat förhöjda halter av PFOS och PFOA i blodet till kronisk njursvikt, högt blodtryck och hjärt- och kärlsjukdomar. Förhöjda halter av dessa två ämnen i navelsträngsserum har kopplats till födselvikt och storlek.

Det här examensarbetet hade som mål att undersöka förekomsten och toxiciteten av per- och polyfluorerade ämnen i miljön kring Vasaloppet. Förekomsten av PFASer undersöktes genom att analysera halterna av dessa i olika medier. Två dagar efter Vasaloppet hade gått av stapeln i Berga by, den 4:e mars 2014, hämtades snöprover från skidspåren för analys. Efter snösmältningen samma år hämtades, den 29:e april, även vatten-, jord- och sedimentprover från samma område. Resultaten visade att snön var kontaminerad av långa perfluoroalkylkarboxylater (PFCAer) från skidvallan jämfört med referensplatsen, på västra sidan Västerdalälven, som inte innehöll dessa. Ett vattenprov som hämtades från en vattensamling i en sänka mellan sjön och backen på den östra sidan om Västerdalälven uppvisade samma mönster som snöproverna från skidspåren på samma sida. Resterande vattenprover innehöll endast låga halter av korta PFCAer. Troligen berodde det på att de vattendragen hade en kortare retentionstid än provet från sänkan och de längre varianterna

sköljts bort vid tiden för provtagningen. Jord- och sedimentproverna följde samma mönster där fyra av fem prover från västra sidan innehöll längre PFCAer medans ingen av de åtta proverna från referensplatsen innehöll dessa.

För att utvärdera toxiciteten utsattes grupper av zebrafiskembryon för några av de insamlade vatten-, jord- och sedimentproverna. Vid test med vatten utsattes totalt fyra grupper för kranvatten från Uppsala, kontrollvatten som samlats in från en referenssjö på motsatt sida om Västerdalälven, vatten från sjön strax norr om startområdet öster om älven respektive vatten från sänkan just norr om den sjön. Fiskembryona i de tre testade vattenproverna utvecklades normalt och visade sig trivas bättre än de embryon som tilläts utvecklas i kranvatten från Uppsala. I nästa test tilläts embryon indelade i totalt åtta grupper utvecklas i fyra sedimentprover, tre jordprover respektive en blank med endast kranvatten (till jord- och sedimentproverna tillsattes kranvatten för att inte torka ut embryona). Av alla dessa åtta grupper var det bara den som var utsatt för ett jordprov som var tänkt som referens som inte utvecklades normalt. Denna jord var främst tänkt som referens till analysen av PFAS-halterna och tros innehålla något ej undersökt här som påverkade embryona.

I alla typer av prov (snö, vatten, jord och sediment) var det tydligt att de som insamlats från Vasaloppssidan var kontaminerade av längre perfluoroalkylkarboxylater från skidvalla. Halterna var för låga för att för att påverka zebrafiskembryo när dessa tilläts utvecklas i insamlade vattenprover eller på insamlade jord- och sedimentprover.

Abbreviations

PFAS – Per- and polyfluoroalkyl substances

PFCAs – Perfluoroalkyl carboxylates

PFSAs – Perfluoroalkyl sulfonates

POP – Persistent organic pollutant

HPLC-MS/MS – High-performance liquid chromatography coupled with tandem mass spectrometry

K_d – Sediment-water distribution coefficient

K_{oc} – Organic carbon normalised coefficient

SD – Standard deviation

MDL – Method detection limit

For abbreviations of target compounds see table 1 in chapter 2.1.

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1 Introduction

There is a growing concern for per- and polyfluoroalkyl (PFASs) substances in the environment in recent years, because they have been found to be persistent in nature, potentially bioaccumulative and toxic. They have excellent surfactant properties as they repel dirt and water amongst other, which have made them attractive for use in ski waxes to increase the glide. Waxes containing a larger portion of PFASs are more expensive and are more commonly used at races rather than on practice. During a ski race the waxes can loosen from the ski base and end up in the snow. In the spring the waxes and PFASs get washed out into the surrounding environment. Since many PFASs tend to partition to the solid phase they could potentially accumulate in soils and sediments in the close vicinity of skiing track. The several thousands of participants in Vasaloppet could possibly have deposited a large quantity of PFASs along these tracks. Therefore, this area is of particular interest to investigate.

2 Background

2.1 Per- and polyfluoroalkyl substances

Per- and polyfluoroalkyl substances (PFASs) are a group of substances consisting of a hydrocarbon chain of various length (C_nH_{2n+2}) which have exchanged some (poly) or all (per) of their hydrogen atoms by a fluorine atom. Polyfluoroalkyl substances have thus at least one H atom substituted by one F atom, whilst a complete substitution of all $C_nH_{2n+2}X$ (X = functional group) moieties would result in a perfluoroalkyl with the structure $C_nF_{2n+2}X$ (Buck et al., 2011).

PFASs are known to be produced in nature by a number of plants and fungi (Key et al., 1997) but all of those substances contain only one fluorine atom, including some antibiotics made by fungi (Giesy and Kannan, 2002). Smaller molecules with only one fluorine atom stand in great contrast to the man-made PFASs of which several are normally fully or almost fully fluorinated. This fact reflects well just how unnatural these perfluoroalkyl substances really are.

Fluorine is the most electronegative of all the elements causing it to form stronger bonds with carbon than others. Fluorine is also a larger atom than hydrogen, resulting in a denser electron field around the carbon chain, which better shields the bonds. The combination of these two physical differences has made PFASs very reluctant to react with other chemicals, such as acids/bases, reducing/oxidising agents and has also made them resistant to degradation through biochemistry and heat (Faithful and Weers, 1998; Schröder, 2003; Schröder and Meesters, 2005).

There is a wide variety of PFASs and most studies focus on two groups: perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs). As their names suggest they have a functional group, either a carboxylic or a sulfonic, attached to a fully fluorinated carbon chain (Scheutze et al., 2010) giving them the chemical formula $C_nF_{2n+1}R$ with R being either COO^- or SO_3^-.

A fluorinated carbon chain is by itself not soluble in water. Even with the addition of a hydrophilic functional group some compounds do not mix well with water. At a critical concentration PFASs can form aggregates in water. This hydrophobia makes PFASs lower the surface tension of water to a greater extent than their hydrocarbon counterparts (Pabon and Corpart, 2002).

PFSAs and PFCAs have different properties depending on if they occur in its protonated or anionic state, e.g. perfluorooctanoate (PFOA) has been reported to have low water solubility and high enough vapour pressure to transition into gaseous phase while its anion has high water solubility and low vapour pressure (Buck et al., 2011). Furthermore, PFAS precursors and their final degradation products can also have very different properties. Perfluorooctanesulfonate (PFOS), for instance, has very low vapour pressure but high solubility in water (Giesy and Kannan, 2002) and its precursors (e.g. perfluorooctane sulfonamide (FOSA)) has a high vapour pressure and low water solubility (Stock et al., 2004) therefore PFOS is mainly transported via water and its precursors are mainly transported through the air. A precursor is, for example, 8:2 fluorotelomer alcohol (8:2 FTOH), it has two carbon atoms in its 10 carbon chain that is not fully fluorinated. Those two are more exposed to reactions than the rest of the chain which can lead to 8:2 FTOH degrading to PFOA (Hagen et al., 1981).

2.2 Production and usage

Due to PFASs unique properties they have been produced for more than 60 years for various applications such as refrigerant, surfactant and lubricant. Some products that contain PFASs include frying pans, fire-fighting foams and waxes for cross-country skiing among others. They have also become immiscible with common fluids such as water and oil, resulting in use as a coating agent as well as water- and oil-proofing of textile, leather and paper products (Key et al., 1997; Faithful and Weers, 1998; Prevedouros et al., 2005). The discovery of PFOS's extraordinary stain repellence was made by accident when a lab assistant spilled the compound on a shoe and the spot kept clean (Renner, 2006).

The most commonly used method for manufacturing is electrochemical fluorination. This method uses a hydrocarbon chain with a functional group at the end as raw material. The raw material is exposed to an electric current in anhydrous hydrogen fluoride to exchange the hydrogen atoms for fluorine atoms. Electrochemical fluorination is not a fully controlled process, the presence of free-radicals causes the carbon chain to deform or break down into smaller pieces. This results in a mix of linear and branched isomers and also different lengths of the fluorinated carbon chain (Buck et al., 2011). The method can result in different yields and ratios between linear and branched isomers depending on the reacting compound but it can also change between experiments. The presence of other anions than F^-, such as Cl^- or OH^-, and the concentration of the reacting compounds can also affect the purity of the product (Conte and Gambaretto, 2003). The ratio between linear and branched chain has been reported to 70-80% linear and 20-30% branched fluorinated carbon chains (Giesy and Kannan, 2002; Hekster et al., 2002; Buck et al., 2011).

Another common method is telomerisation. By reacting two perfluoroalkyl iodides together, it creates linear product every time if the reactants both have an even number of carbon atoms. If one of them is already branched the product will also be branched. Commonly pentafluoroethyl, C_2F_5I, and tetrafluoroethylene, $CF_2=CF_2$, is reacted together in a first step. The product can further react with ethylene to create the building blocks for the production of fluorotelomer surfactants and polymers (Buck et al., 2011). Telomerisation yield polyfluoroalkyl substances but the products can degrade to perfluoroalkyl substances.

Although the products from telomerisation have a greater purity and is easier to separate from the by-products than the products of electrochemical fluorination it is also more expensive (Hekster et al., 2002), which has led to the worldwide distribution and application of the electrochemical fluorination method.

Ever since the emergence of PFASs the production has steadily increased over the years as new applications were found. It has been estimated that almost 4500 metric tons of PFOS-related chemicals was produced in the year 2000 (US EPA, 2000). In 2000 negotiations between US EPA and 3M, the dominating producer on the market, led to the company's announcement to voluntarily face out its perfluorooctanyl chemistry (US EPA, 2000). In practice the decision resulted in a large decrease in production of PFOS in the following years. In addition, the emissions of ammonium perfluorooctanoate (APFO), a precursor to PFOA, greatly decreased in the USA from many tones to just kilograms per year when 3M discontinued its electrochemical fluorination manufacturing process (Prevedouros et al., 2006). Following the decrease of PFAS production in USA and Europe the production in China increased. From producing less than 50 tons in 2003 Chinas perfluorooctanesulfonyl fluoride (PFOSF) production grew to over 200 tons in 2006 (Yue, 2008).

Following 3M's voluntary phase out many legislators have acted against PFASs. The Environmental Protection Agency (EPA) in USA issued regulations on 75 PFASs in 2002 that implied mandatory notification to the EPA 90 days in advance of importing those PFASs or using them in a new production (US EPA, 2002). The list was extended in 2007 to include a total of 183 PFASs (US EPA, 2007). In 2006, the EU Commission amended a directive to restrict marketed product not to contain PFOS in concentrations equal to or higher than 0.005% by mass (EU Parliament, 2006). This limit was later lowered to 0.001% by mass in 2010 as a result of PFOS being recognised as a persistent organic pollutant (POP) and a part of the Stockholm convention in 2009 (Stockholm Convention, 2009). Exceptions to this law were made for older products already in use, photolithography processes, photographic coatings, hard chromium plating and hydraulic fluids for aviation. This is due to an absence of better alternatives. The exceptions are granted provided that the member state reports its progress in phasing out PFOS to the Commission every four years (EU Commission, 2010). Other countries, such as Canada (Environment Canada, 2013) and Australia (NICNAS, 2013) also have similar regulations.

2.3 Sources, transport and fate in the environment

The reason for the growing concern for PFASs in recent years is that they have been found to be persistent in nature, bioaccumulative and toxic (Giesy and Kennan, 2001; Giesy and

Kennan, 2002; Kennedy et al., 2004). The lack of analysing methods was a significant factor as to why the issue has not attracted awareness earlier (OECD, 2007), despite that PFASs and their precursors have been produced since the 1950s (Giesy and Kannan, 2001).

Sources of PFASs include manufacturing industry as well as the use of products containing PFASs (Prevedouros et al. 2006). Sewage treatment plants can also be considered a major source (Sinclair and Kannan, 2006). Another important point source that has recently brought attention to itself is fire-fighting training grounds, where exercises have been carried out with fire-fighting foams that contained PFASs (Carlsson, 2014). The PFASs can potentially leak into the soil of these sites and contaminate the nearby groundwater and drinking water supplies (Kärrman et al., 2011). Recently, PFAS-containing ski waxes has been identified as a potential source of PFASs in the nearby environment around ski tracks (Plassmann et al., 2011a). Studies on PFASs behaviour in snow are scarce though it has been shown that during snowmelt longer chained species binds more to surfaces and end up in the underlying soil while the shorter chained species either volatilized or washes away with the melt water (Plassmann et al., 2011b; Plassmann and Berger, 2013).

In remote areas diffuse sources can be of great concern (Ahrens et al., 2010a; Cai et al., 2012). As mentioned in the previous section, many precursors are volatile while their persistent degradation PFASs are not volatile. For example, the atmospheric lifetime of fluorotelomer alcohols (FTOHs) has been estimated to about 20 days, and is governed by the reaction with OH radicals as the wet and dry deposition of these substances are negligible (Ellis et al., 2003). These volatile precursors that get emitted into the atmosphere could be transported up to 7000 km (Stock et al., 2004) before they degrade to more persistent PFASs such as PFCAs or PFSAs and can therefore be deposited ubiquitous in the environment. Even though manufacturers of PFASs are concentrated to the northern hemisphere treated products may very well be exported and used elsewhere, which extends the reach of the atmospheric deposition.

In contrast to the FTOHs, for the PFCAs in the atmosphere the reaction with OH radicals is of minor importance. They are instead removed from the atmosphere mainly by particle adhesion combined with wet or dry deposition with an atmospheric residence time of 10 days (Hurley et al., 2004). Studies have shown that atmospheric deposition is a contributing factor for the distribution of PFASs. The PFCA precursors 8:2 and 10:2 fluorotelomer carboxylic acid (FTCA) and 8:2 and 10:2 fluorotelomer unsaturated acid (FTUCA) along with C_{3-9} PFCAs have been found in rain samples from several sites in Canada and USA (Loewen et al., 2005; Scott et al., 2006) and PFOS, perfluorohexanoate (PFHxA), PFOA and perfluorononanoate (PFNA) have been found in rain samples from Sweden and Finland (Kallenborn et al., 2004). Young et al. (2007) concluded that the levels of PFASs in the arctic snow caps are probably due to atmospheric deposition.

In coastal areas the oceanic transport may be of greater importance than atmospheric deposition (Yamashita et al., 2005; Ahrens et al., 2010a). Knowledge of regional currents or tidal movement is important for the transport of PFASs away from the coast to be fully understood. Long-range transport in the oceans is possible due to the structural stability of the

PFASs (Yamashita et al., 2005) but it is not certain how important this transport route is. In a comparison made by Prevedouros et al. (2006) the oceanic transport to the Arctic could be around one order of magnitude larger than that of atmospheric deposition, with estimated 2-12 tons per year and 0.1-1 tons per year respectively. In contrast Scott Mabury argued at a OECD-workshop on PFCAs and precursors (OECD, 2007) that the oceanic route was not of significance for the pollution of the Arctic. Furthermore Young et al. (2007) concluded that PFASs in coastal waters of the Arctic originated from melting snow and consequently the atmosphere. There are also studies supporting Prevedouros result, the modelling results of Armitage et al. (2006) and Stemmler and Lammel (2010) were in the same range, 8-23 tons per year transported to the Arctic. Armitage et al. (2009) suggested that PFCAs with chain length C_8 and C_9 were mainly transported via oceanic currents while PFCAs with chain lengths of C_{10}-C_{13} were deposited through atmospheric deposition of precursors.

PFASs has been found in animals all over the world, in polar bears in the Arctic (Kannan et al., 2001), in sea turtles of the coast of USA (Keller et al., 2005), and in penguins on Antarctica (Schiavone et al., 2009). With degradation of PFASs assumed negligible the only sinks for the most persistent PFASs is hypothesised to be sedimentation and deep ocean burial (Prevedouros et al., 2006). Therefore it is important to keep future emissions of these substances as low as possible. Seals from the Canadian Arctic have displayed decreasing trends for PFOS believed to be a direct effect of 3M's phase out (Butt et al., 2007). But in polar bears on Greenland PFOS and some PFASs have been observed still increasing up to 2005. These different trends are hypothesised to be correlated with how the PFASs are transported. It takes much longer time for PFASs to reach remote places by ocean currents than atmospheric transport (Dietz et al., 2008).

2.4 Toxicity

2.4.1 Exposure and toxicological effects of PFASs

After PFASs have been released into the environment they can potentially accumulate in biota (Martin et al., 2003; Haukås et al., 2007). Unlike other bioaccumulating substances that often are lipophilic and tend to accumulate in fat tissues (Petersen and Kristensen, 1998), PFASs are instead proteinophilic and accumulate primarily in the liver and blood (Conder et al., 2007). It has been hypothesised that PFASs can be recycled back to the liver from the intestines through the entero-hepatic circulation in both fish and rats (Martin et al., 2003; Houde et al., 2006; Environment Canada, 2001). Consequently, the entero-hepatic circulation may increase the retention time for PFASs in biota.

Once inside the body PFASs could disturb the hormone signals as some of them are considered to be endocrine disruptors, for example PFOS and PFOA (Austin et al., 2003; Jensen and Leffers, 2008). There are also PFASs that are considered to induce peroxisome proliferation, for example perfluorohexane sulfonate (PFHxS), PFOS, perfluoropentanoate (PFPeA) and PFNA (Ishibashi et al., 2011), which can lead to cancer (Feige et al., 2006). The three compounds PFHxA, PFOS and perfluorooctane sulfonamide (PFOSA) have shown to be inhibitory on the gap intercellular communication, which for instance may lead to abnormal cell growth, whereas the shorter chained perfluorobutanoate (PFBA) did not (Hu et al., 2002).

Animal studies have linked PFASs with hormonal disturbances, alterations of the liver, different kind of cancer (liver, pancreas and testes) and even death shortly after birth in rats and mice (Lau et al., 2007; Kennedy et al., 2004). Even though those results may not be directly transferable to humans there have been studies showing effects of PFAS on humans as well (Shankar et al., 2010; Min et al., 2014). Elevated serum levels of PFOS and PFOA have been correlated with chronic kidney disease and higher risk of cardiovascular disease and high blood pressure in adults (Shankar et al., 2010; Min et al., 2014). PFOS and PFOA have been found in cord serum and could have an effect on birth weight and size (Apelberg et al., 2007). A few studies have shown a correlation between PFSAs (PFOA, PFNA, PFDA, PFHxS and PFOS) and impulsivity and ADHD in children (Hoffman et al., 2010; Gump et al., 2011; Stein and Savitz, 2011) but opposite results have also been obtained (Liew et al., 2014).

2.4.2 Zebrafish embryo testing

Testing different chemicals for their toxicity on aquatic life has conventionally been carried out through acute fish test. However, this has become ethically questioned with modern animal rights legislations in many countries (Nagel, 2002). The issue has been centred on the acute fish test exposing fully developed, juvenile or adult, fish to potentially harmful substances that could put them in distress or pain (Lammer et al., 2009). Toxicity testing with zebrafish (*Danio rerio*) embryos is in many countries, including Sweden, not considered as an animal test as long as they do not develop further from the embryo stage, i.e. when the fry has used up its yolk and started eating as an adult (Embry et al., 2010). The test is standardised within OECD with a test period of 96 hours (OECD, 2013), however it can be extended to 144 hours to allow consideration of more endpoint (Carlsson et al., 2009).

In the OECD zebrafish test four lethal endpoints are evaluated during development: coagulated embryos, lack of somite formation, non-detachment of the tail and lack of heartbeat. Testing the toxicity of a chemical on zebrafish embryos has been equated with acute testing on juvenile or adult fish (Braunbeck et al., 2005; Embry et al., 2010). However, a disadvantage of zebrafish embryo test compared to the acute fish test was considered to be the lack of analysing chronic effects (Wedekind et al., 2007). By assessing sublethal endpoints this issue could be solved (Embry et al., 2010). Therefore, some studies (Hallare et al., 2005; Carlsson et al., 2009; Ulhaq et al., 2013) have expanded the test to include sublethal endpoints such as pigmentation, edema and deformations of tail, eye or head among other.

The use of zebrafish embryos in toxicology testing has many advantages. The species is small, with adults around four centimetres long in general and they have a short development time, six days from fertilised egg to fry. The eggs themselves are transparent allowing for examination through light microscopy, which is both cheap and simple. The maintenance is easy and they breed almost every day at the first light, providing a reliable source of test subjects all year around. The chorion could pose as a barrier for non-ionic surfactants with high molecular weight (Lammer et al., 2009). However, studies have shown that the chorion can be passed by at least PFASs up to the size of PFOS and perfluorodecanoate (PFDA) (Hagenaars et al., 2011; Wang et al., 2011; Zheng et al., 2012; Ulhaq et al., 2013).

2.5 Objectives and hypothesis

Some PFASs are known to be toxic, bioaccumulative and persistent in nature. Because of their water and dirt repellent properties they are used in glide waxes for cross-country skiing. The waxes applied to the ski base can loosen due to abrasion against the snow. However, there is a lack of knowledge of the environmental fate and toxic effects of PFASs originating from ski waxes.

This master thesis aims to map the toxicity of PFASs and where they end up in nature by analysing samples of snow, water, soil and sediment collected near the start of the largest ski race in the world (Vasaloppet). Snow samples were collected from the bottom of the skiing tracks from various locations and a reference location shortly after Vasaloppet. After snowmelt, surface water, soil and sediment were collected at various locations along the skiing tracks and reference locations. In addition, surface water samples were collected at three locations in the nearby river. The toxic effect of PFASs was evaluated using zebrafish embryo tests by exposing them to natural surface waters, soil and sediment samples from the Vasaloppet area.

The following hypotheses were tested:

- PFASs can be released from ski-waxes into the nearby environment
- The pattern of individual PFASs in snow, water, soil and sediment is different and depends on the chain length and functional group
- Zebrafish embryo tests can be used to evaluate the effects of PFASs in the environment

3 Method

3.1 Target compounds

In this study, 26 PFASs were investigated (Table 1). The target analytes included 4 PFSAs (i.e. PFBS, PFHxS, PFOS and PFDS), 13 PFCAs (i.e. PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFHxDA and PFOcDA), and 9 PFAS precursors (i.e. FOSA, *N*-MeFOSA, *N*-EtFOSA, *N*-MeFOSE, *N*-EtFOSE, FOSAA, *N*-MeFOSAA, *N*-EtFOSAA and 6:2 FTSA). Additionally, 13 mass labelled PFASs were used as internal standards, including one PFSA (i.e. $^{18}O_2$ PFHxS), seven PFCAs (i.e. $^{13}C_4$ PFBA, $^{13}C_2$ PFHxA, $^{13}C_4$ PFOA, $^{13}C_5$ PFNA, $^{13}C_2$ PFDA, $^{13}C_2$ PFUnDA and $^{13}C_2$ PFDoDA) and five precursors (i.e. d_9-N-EtFOSE, d_5-N-EtFOSAA, $^{13}C_8$-FOSA, d_3-N-MeFOSAA and d_7-N-MeFOSE) and one injection standard (i.e. $^{13}C_8$ PFOA).

Table 1. List of target compounds

Abbreviation	Full name
PFSAs	
PFBS	Perfluorobutane sulfonate
PFHxS	Perfluorohexane sulfonate
PFOS	Perfluorooctane sulfonate
PFDS	Perfluorodecane sulfonate
PFCAs	
PFBA	Perfluorobutanoate
PFPeA	Perfluoropentanoate
PFHxA	Perfluorohexanoate
PFHpA	Perfluoroheptanoate
PFOA	Perfluorooctanoate
PFNA	Perfluorononanoate
PFDA	Perfluorodecanoate
PFUnDA	Perfluoroundecanoate
PFDoDA	Perfluorododecanoate
PFTriDA	Perfluorotridecanoate
PFTeDA	Perfluorotetradecanoate
PFHxDA	Perfluorohexadecanoate
PFOcDA	Perfluorooctadecanoate
PFSA and PFCA precursors	
FOSA	Perfluorooctanesulfonamide
N-MeFOSA	*N*-methylperfluorooctansulfonamide
N-EtFOSA	*N*-ethylperfluorooctanesulfonamide
N-MeFOSE	*N*-methylperfluorooctanesulfonamido-ethanol
N-EtFOSE	*N*-ethylperfluorooctanesulfonamido-ethanol
FOSAA	Perfluorooctanesulfonamidoacetic acid
N-MeFOSAA	*N*-methylperfluorooctanesulfonamidoacetic acid
N-EtFOSAA	*N*-ethylperfluorooctanesulfonamidoacetic acid
6:2 FTSA	6:2 fluorotelomer sulfonate

3.2 Sampling

3.2.1 Snow sampling

Snow samples were collected in the Vasaloppet area (latitude: N 61° 6′ 52″, longitude: E 13° 17′ 30″) on the 4th of March, 2014. In total 14 snow samples were collected in 12 L stainless steel POP-cans using a stainless steel scoop. Surface snow samples were collected from the ski tracks in the starting area (A1, A2 and A3), at ski tracks in the north end of a lake (A4, A5-1, A6), at ski tracks at a hill (A7, A8 and A9) and at a reference site by a lake on the other side of the river (A10, A11, A12) (*Figure 1*). At all sites surface snow samples were collected (top 2-4 cm). In addition, below A5-1 two deeper snow samples were collected between 8 and 13 cm depth (A5-2) and between 13 and 18 cm depth (A5-3). For a description of every sampling site see table A1 in appendix.

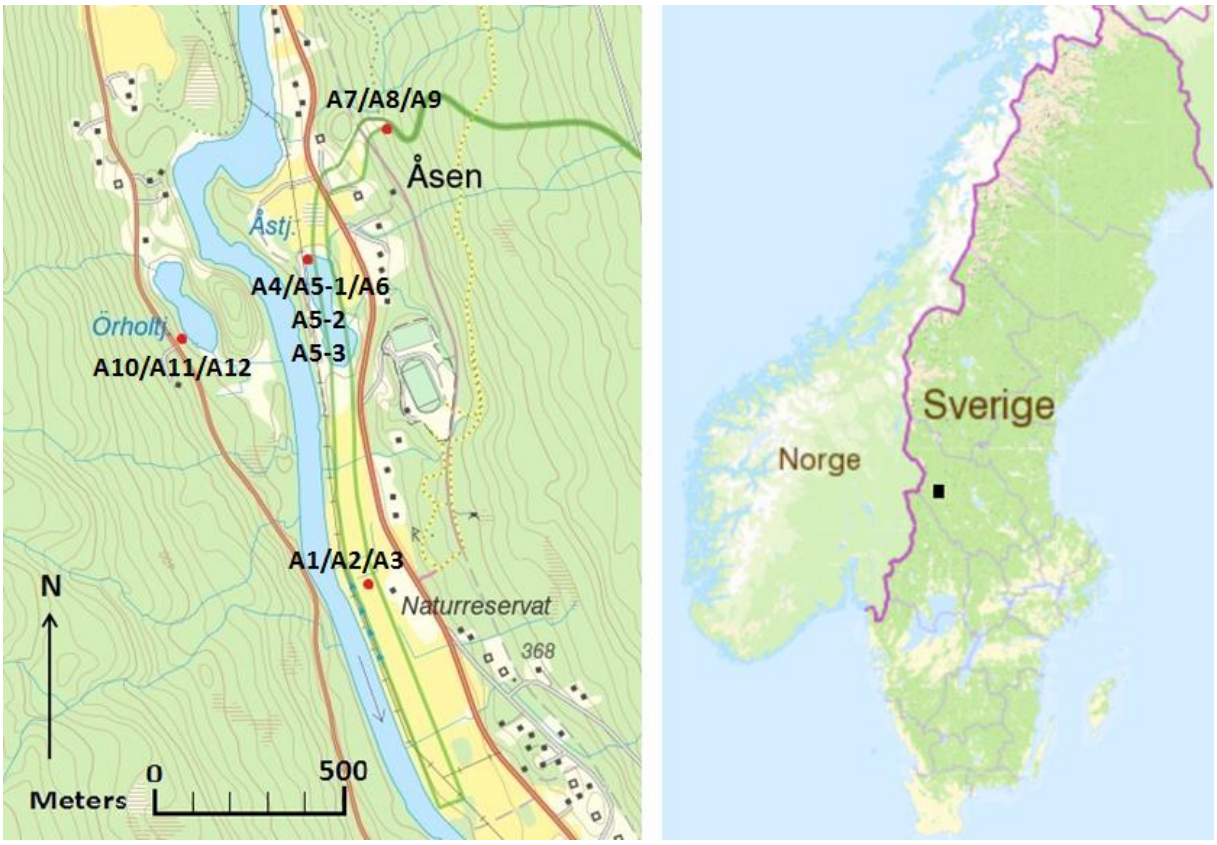

Figure 1. Locations where the snow samples were collected around the starting area (left) and location of the starting field in Sweden (right). © Lantmäteriet, i2014/764

3.2.2 Surface water sampling

Surface water samples (~0.5 m depth) were collected in the Vasaloppet area on the 29th of April, 2014. In total 10 samples were collected in 1 L polypropylene bottles (PP-bottles). Surface water was sampled from the reference lake (B1, B2 and B3), the potentially contaminated lake under the skiing tracks (B4, B5 and B6), from a depression in the field between the potentially contaminated lake and the hill (B7) and from the river downstream (B8), upstream (B10) and next to the starting area (B9) (*Figure 2*). For a description of every sampling site see table A2 in appendix.

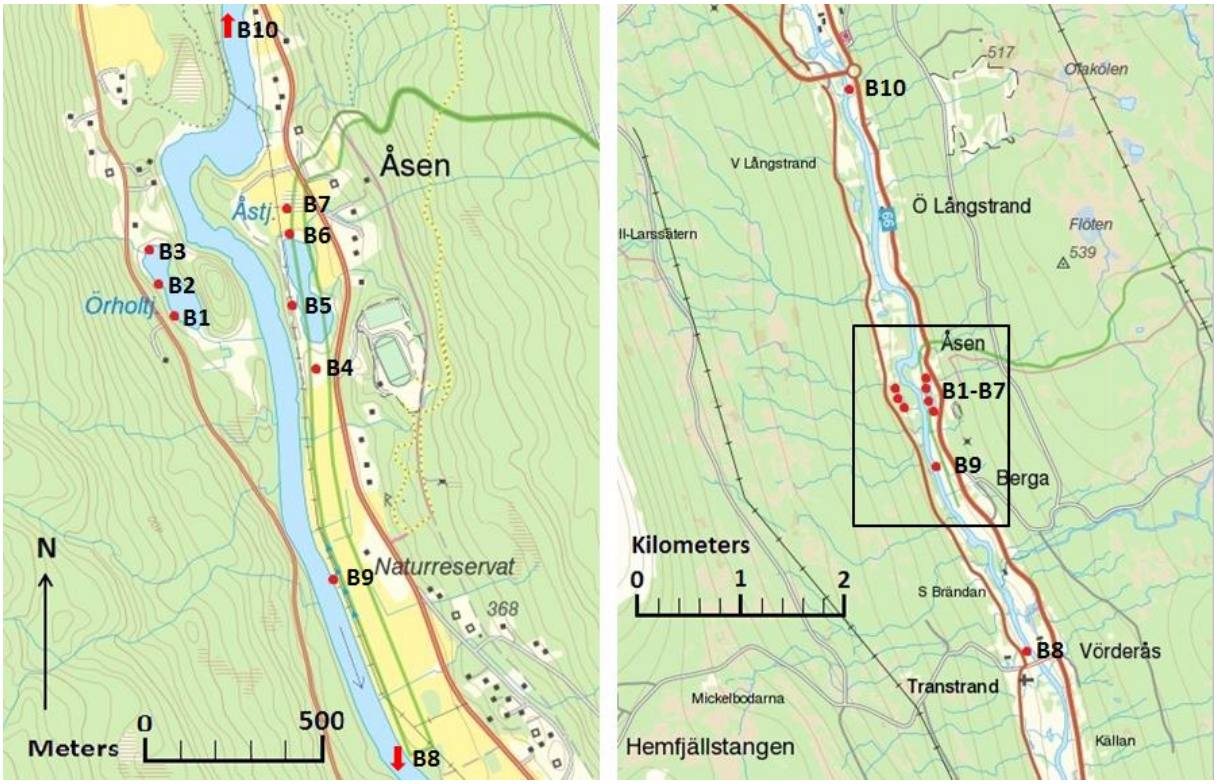

Figure 2. Locations where the surface water samples were collected around the starting area. The box in the right map represents the area of the left map. © Lantmäteriet, i2014/764

3.2.3 Soil and sediment sampling

Soil and sediment were collected in the Vasaloppet area on the 29th of April, 2014. In total 4 soil samples and 9 sediment samples were collected in 100 mL PP-jars. Surface soil samples (0-3 cm) were collected from the ground along the first kilometer of ski track, one just after the starting field (D1) and two in the first hill (D2 and D3) using a stainless steel scoop. The organic layer had to be removed to reach to soil when sampling D1 and D3. Surface sediment (0-3 cm) was collected at the north end of the lake under the ski tracks (C9), in a depression on the field just north of that lake (C10) and at the reference lake (C1, C2, C3, C4, C7 and C8) using a Wilner-grabber. In addition, below C4 two deeper sediment samples were collected from between 3 and 6 cm of depth (C5) and between 6 and 9 cm depth (C6) (*Figure 3*). For a description of every sampling site see table A3 in appendix.

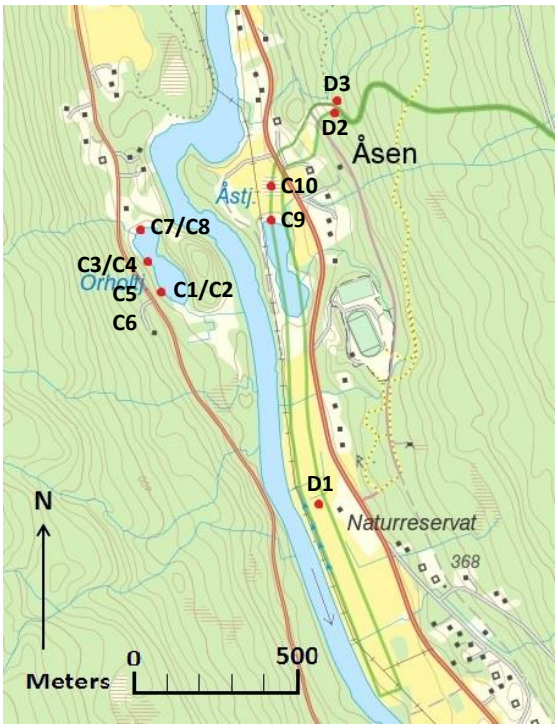

Figure 3. Locations where sediment (C) and soil (D) samples were collected around the starting area. © Lantmäteriet, i2014/764

3.3 Extraction and instrumental analysis

3.3.1 Snow and water extraction

The water and snow samples were analysed by the same method, although the snow samples were left in the fridge to melt for a couple of days before transferred to 1 L PP-bottles and further analysis could be performed. All samples were filtered through a glass fibre filter (GFF, GC/C, Whatman, ø 47mm, >1.2 µm) using vacuum. The bottles were weighted before and after filtration to determine the amount of water used in upcoming procedures. Before extraction, each sample was spiked with 100 µL internal standard mixture of 20 pg μL^{-1}. The internal standard along with other reagents have been previously described by Ahrens et al., (2009). Samples were extracted by solid-phase extraction (SPE) using Oasis WAX cartridges (Waters, 150 mg, 6 cm^3, 30 µm) as described by Taniyasu et al. (2005). The cartridges were preconditioned with 4 mL 0.1% ammonium hydroxide (prepared with ammonium hydroxide with purity 28.0-30.0% from Sigma-Aldrich) in methanol (purity ≥99.9% LiChroSolv® from Merck KGaA), 4 mL methanol and 4 mL MilliPore water (filtered through Milli-Pak® 0.22 µm filter at the laboratory). After preconditioning the cartridge was loaded with 0.5 L water sample with a rate of around one drop per second. Afterwards the cartridges were washed with 4 mL 25 mM ammonium acetate buffer (prepared with acetic acid with purity ≥99.7% from Merck KGaA and ammonium acetate with purity ≥99.0% BioUltra® from Sigma-Aldrich) and dried in a centrifuge with 3000 rpm for 2 minutes. The dry cartridges were eluted with 4 mL methanol and 4 mL 0.1% ammonium hydroxide down into 15 mL PP tubes. The samples were concentrated with nitrogen gas down to 1 mL and then 10 µL injection standard (c = 200 pg μL^{-1}) was added before analysis using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) (Ahrens et al., 2009).

3.3.2 Soil and sediment extraction

An arbitrary amount of soil or sediment was initially transferred to 50 mL PP-tubes and freeze-dried for four days. For the extraction, 2.5 to 5 g soil or sediment was used. The extraction method used here has been described and used before (Ahrens et al., 2009) with some small changes. To each sample 2 mL of 50 mM sodium hydroxide (purity 99% pro analysis® from Merck KGaA) in 80%/20% methanol/MilliPore water was added and soaked for 30 minutes. 20 mL of methanol and 100 µL of an internal standard mix of 20 pg μL^{-1} were added before the samples were placed in a wrist-action shaker on 200 rpm for 60 minutes. After shaking, the tubes were centrifuged at 3000 rpm for 15 minutes. The supernatant was then decanted into another 50 mL PP-tube. The process was then repeated but with 1 mL of 50 mM sodium hydroxide in 80%/20% methanol/MilliPore water, 10 mL of methanol but without an extra addition of internal standard. The time in the wrist-action shaker was halved from the previous iteration and the supernatant was decanted into the same 50 mL PP-tube. Afterwards 0.1 mL of 4 M hydrochloric acid (prepared from purity of 30% Suprapur® from Merck KGaA) was added and the tubes were shaken by hand for about 5 seconds and centrifuged at 3000 rpm for 5 minutes. One eighth of the sample, 4.15 mL, was transferred into a 15 mL PP-tube and blow-dried down to 1 mL with nitrogen stream. The 1 mL was transferred to a 1.7 mL PP-microcentrifuge tube containing 25 mg ENVI-Carb (Supelclean® from Sigma-Aldrich) and 50 µL glacial acetic acid (purity 100% from Merck KGaA) and vortex-mixed before centrifuged at 4000 rpm for 15 minutes. Exactly 0.5 mL of supernatant was transferred to a brown glass vial, 10 µL injection standard (c = 200 pg μL^{-1}) was added and finally analysed using HPLC-MS/MS (Ahrens et al., 2009).

3.3.3 Partitioning coefficients

To determine the partitioning of PFASs between water and sediment the sediment-water distribution coefficient (K_d) was calculated accordingly (Schwarzenbach et al., 2003):

$$K_d = c_{sediment}/c_{water} \quad (1)$$

where $c_{sediment}$ was the concentration in ng g^{-1} dw of the specific PFAS in sediment and c_{water} was the corresponding concentration in ng cm^{-3} in water. The pairing of sediment and surface water samples can be seen in table A4 in appendix.

From K_d and the fraction of organic matter in the sediment samples (f_{oc}) the organic carbon normalised coefficient (K_{oc}) can be calculated (equation 2) to determine the tendency for the PFASs to sorb to organic matter (Schwarzenbach et al., 2003).

$$K_{oc} = K_d/f_{oc} \quad (2)$$

3.4 Zebrafish embryo test

3.4.1 Testing the surface water samples

Two 48-well plates were filled with 1 mL of water from the samples B1, B6 and B7 from around the starting area in Berga (*Figure 2*) and carbon filtered tap water from Uppsala (T). One egg was added to each cell, resulting in a total of 24 wells for each water sample. The four waters were distributed in diagonal lines across the plates and shifted one position to the left between the plates (*Figure 4*) to minimise the risk that outer wells could possibly be affected by an unknown parameter.

A)

T	B1	B6	B7	T	B1	B6	B7
B7	T	B1	B6	B7	T	B1	B6
B6	B7	T	B1	B6	B7	T	B1
B1	B6	B7	T	B1	B6	B7	T
T	B1	B6	B7	T	B1	B6	B7
B7	T	B1	B6	B7	T	B1	B6

B)

B7	T	B1	B6	B7	T	B1	B6
B6	B7	T	B1	B6	B7	T	B1
B1	B6	B7	T	B1	B6	B7	T
T	B1	B6	B7	T	B1	B6	B7
B7	T	B1	B6	B7	T	B1	B6
B6	B7	T	B1	B6	B7	T	B1

Figure 4. Distribution of four different waters, B1, B6, B7 and carbon filtered tap water (T), in 48-well plate A (left) and plate B (right).

Newly laid zebrafish eggs were collected and screened with a light microscope to make sure only fertilised eggs were used in the test. The eggs were then transferred into one well each and the exposure began approximately 1 hour after fertilisation. The temperature was kept constant at 26°C.

The embryos were screened for five endpoints 24 hours post fertilisation (hpf): 1. coagulation of the embryos, 2. normal head development, 3. normal eye development, 4. Tail growth and detachment from the yolk, 5. movement of the embryo (measured by setting a timer to thirty seconds and see if it move within that time).

At 48 hpf the same endpoints were screened again, except for movement as they move significantly less in this phase. In addition, the pigmentation of the embryos was graded on a scale of 1-4, where 1 meant normal dark eyes and normal pigments on body, 2 was normal dark eyes but reduced pigments on body, 3 was lighter eye colour and reduced pigments on body and 4 meant lack of pigmentation on eyes and body. They were also screened for heartbeats, circulation and edema, in addition heart frequency was checked for plate A.

The last screening of endpoints was done 144 hpf (six days). Normally they should have hatched at this time so this was checked as an endpoint. If they had died it was noted whether it had occurred before or after hatching. As for the previous screening the embryos were checked for normal head and eyes, and if edema had developed. Their spine was checked if it was straight. Last endpoint to be listed was if an embryo was lying idle on its side on the bottom.

Between 48 and 144 hpf the two plates were placed on a glass plate with a camera rigged under it to take pictures once every hour to allow for hatching time of each embryo to be determined.

Most endpoints were measured binary (yes or no). Heart rate and hatching time were measured as continuous data and pigmentation according to a ordinal scale (1-4, where 1 represents the normal whereas a 4 represents total lack of pigmentation). All data were tested for differences between the control water (B1) and the respective water sample using various methods. Fischer's exact test with Bonferroni correction of p-values was used to see if the binary data from different test waters at 144 hpf differed statistically, in terms of number of embryos showing lethal or sublethal endpoints. Bonferroni correction means multiplying the p-value with the number of comparisons that is made, this is done to prevent false positives to occur. Differences in heart frequency and hatching time between groups were evaluated with a one-way ANOVA test. The variances of the groups were tested with Levene's test and had

to be equal at the p≥0.05 level before the one-way ANOVA test. Therefore the heart frequency data was transformed according to equation 3.

$$(transformed\ heart\ frequency) = 1/(heart\ frequency)^2 \quad (3)$$

Pigmentation was tested using Kruskal-Wallis test.

3.4.2 Testing the soil and sediment samples

Four different sediment samples (i.e. C2, C4, C9 and C10) and three soil samples (i.e. D1, D2 and D3) were tested with a modified version of the zebrafish embryo test along with a control of only carbon filtered tap water (Nagel, 2002). In addition to the original test, the bottom of the cells was covered with 0.2 g of soil or sediment before 1 mL of carbon filtered tap water was carefully added to avoid turbidity. One egg was then placed in each cell directly on top of the soil or sediment. The sediment samples C2 and C4 from the reference lake, C9 and C10 from the potentially contaminated lake and all three soil samples (i.e. D1-D3) were tested (*Figure 3*). The eight samples were diagonally distributed in the 48-well plates with an off-set of four wells between the plates (*Figure 5*).

A)

C2	C4	T	D1	C9	C10	D2	D3
D3	C2	C4	T	D1	C9	C10	D2
D2	D3	C2	C4	T	D1	C9	C10
C10	D2	D3	C2	C4	T	D1	C9
C9	C10	D2	D3	C2	C4	T	D1
D1	C9	C10	D2	D3	C2	C4	T

B)

C9	C10	D2	D3	C2	C4	T	D1
D1	C9	C10	D2	D3	C2	C4	T
T	D1	C9	C10	D2	D3	C2	C4
C4	T	D1	C9	C10	D2	D3	C2
C2	C4	T	D1	C9	C10	D2	D3
D3	C2	C4	T	D1	C9	C10	D2

Figure 5. Distribution of seven different sediments with carbon filtered tap water and one group with only tap water (T) in 48-well plates A (left) and B (right).

Just as for the test with surface water samples, fresh zebrafish eggs were screened to find the ones that were fertilised. Due to few eggs being visible in the spawning tanks there was a delay between fertilisation and exposure. The exposure began after about 2 hpf at 16-cell stage.

After 24 hpf, the embryos were screened for coagulation, movement and, if they moved, tail-detachment. Those were the only three endpoints that could be checked for all subjects as particles had settled on some embryos. The screening at 48 hpf only considered pigmentation and coagulation for the same reason as before. The last screening at 144 hpf was carried out as for the testing of the surface water samples. However, since the bottoms of the wells were covered with soil or sediment the hatch time analysis could not be done.

After the experiment was done and all the endpoints were measured the water from the same group was collected for analysis of nitrate and nitrite levels using test strips (Reflectometric, Merck KGaA) and pH using a calibrated pH-meter at the laboratory to see if these parameters could be correlated to any effects. Fischer's exact test was used to see if the test waters differed statistically, in terms of number of embryos showing lethal or sublethal endpoints and the p-value was Bonferroni corrected.

4 Results

4.1 Quality assurance/quality control

To avoid losses of PFASs during the extraction process only glass and polypropylene equipment was used. Contamination from the laboratory was minimised by rinsing all equipment, which were to come in contact with the samples, three times with methanol. The recovery of the 13 internal standards was calculated based on the concentration of the internal standard in the spiked samples compared to the concentration of the internal standard in the calibration curve (*Table 2*).

Table 2. Recovery of the 13 the internal standards used for the extraction of the snow, water and soil and sediment samples

	Recovery [%]		
Compound	Snow (n=31)	Water (n=13)	Soil and Sediment (n=23)
$^{18}O_2$ PFHxS	54 ± 14	76 ± 18	85 ± 11
$^{13}C_4$ PFBA	86 ± 7	95 ± 5	57 ± 14
$^{13}C_2$ PFHxA	95 ± 5	96 ± 10	53 ± 12
$^{13}C_4$ PFOA	82 ± 9	83 ± 12	59 ± 9
$^{13}C_5$ PFNA	92 ± 5	94 ± 5	63 ± 12
$^{13}C_2$ PFDA	94 ± 6	98 ± 5	62 ± 9
$^{13}C_2$ PFUnDA	86 ± 7	98 ± 7	67 ± 10
$^{13}C_2$ PFDoDA	80 ± 12	94 ± 7	66 ± 11
d_9-N-EtFOSE	66 ± 14	87 ± 11	112 ± 31
d_5-N-EtFOSAA	48 ± 16	70 ± 21	80 ± 12
$^{13}C_8$-FOSA	100 ± 31	91 ± 9	85 ± 12
d_3-N-MeFOSAA	76 ± 11	89 ± 8	99 ± 30
d_7-N-MeFOSE	101 ± 26	93 ± 7	77 ± 12

Obtained recoveries were in an acceptable range and the mean recoveries for all PFASs for the snow, water, soil and sediment samples were 74%, 90% and 82%, respectively. These recoveries were similar to what has previously been reported for snow (Plassmann et al., 2011b; Plassmann and Berger, 2013), water (Naile et al., 2010; Cai et al., 2012) and soil and sediment (Naile et al., 2010; Plassmann and Berger, 2013).

The method detection limit (MDL) was calculated for every target compound from the mean concentrations and standard deviations (SD) of the laboratory blanks (triplicates for all three sample matrices) (equation 4).

$$MDL = Mean_{Blanks} + 3 * SD_{Blanks} \quad (4)$$

If the MDL was lower than the lowest calibration point, the lowest calibration point was defined to be the MDL (i.e. 0.05 ng L^{-1}) (*Table 3*). Concentrations that were not detected or detected at lower concentration than its corresponding MDL were set to half MDL (i.e. 0.025 ng L^{-1}).

Table 3. Average blank values and standard deviations calculated for each target compound, along with corresponding MDL for snow, water and soil and sediment samples

	Snow [ng L^{-1}] (n=3)		Water [ng L^{-1}] (n=3)		Soil and sediment [ng g^{-1} dw] (n=3)	
Compound	Mean ± SD	MDL	Mean ± SD	MDL	Mean ± SD	MDL
PFBS	0.5 ± 0.2	1.1	nd	0.05	nd	0.05
PFDS	nd	0.05	nd	0.05	nd	0.05
PFHxS	0.01 ± 0.004	0.02	nd	0.05	nd	0.05
PFOS	0.3 ± 0.1	0.7	nd	0.05	0.06 ± 0.05	0.2
PFBA	0.06 ± 0.02	0.1	nd	0.05	nd	0.05
PFPeA	0.01 ± 0.01	0.02	nd	0.05	2.8 ± 0.1	3.2
PFHxA	nd	0.05	nd	0.05	0.4 ± 0.01	0.5
PFHpA	nd	0.05	nd	0.05	nd	0.05
PFOA	0.03 ± 0.02	0.1	nd	0.05	nd	0.05
PFNA	0.3 ± 0.1	0.7	nd	0.05	nd	0.05
PFDA	0.6 ± 0.3	1.4	nd	0.05	nd	0.05
PFUnDA	2.1 ± 0.9	4.9	nd	0.05	nd	0.05
PFDoDA	4.5 ± 1.9	10	nd	0.05	nd	0.05
PFTriDA	0.02 ± 0.01	0.04	nd	0.05	nd	0.05
PFTeDA	5.7 ± 2.5	13	nd	0.05	nd	0.05
PFHxDA	nd	0.05	nd	0.05	0.2 ± 0.2	0.7
PFOcDA	nd	0.05	nd	0.05	nd	0.05
EtFOSA	nd	0.05	nd	0.05	0.1 ± 0.02	0.2
EtFOSAA	nd	0.05	nd	0.05	nd	0.05
EtFOSE	0.1 ± 0.01	0.2	nd	0.05	0.5 ± 0.2	1.2
FOSA	0.01 ± 0.002	0.02	nd	0.05	0.1 ± 0.1	0.3
FOSAA	0.0003 ± 0.003	0.001	nd	0.05	0.2 ± 0.1	0.6
MeFOSA	0.03 ± 0.003	0.04	nd	0.05	0.1 ± 0.02	0.2
MeFOSAA	nd	0.05	nd	0.05	nd	0.05
MeFOSE	1.0 ± 0.1	1.2	0.5 ± 0.2	1.3	0.9 ± 0.4	2.0
6:2 FTS	nd	0.05	nd	0.05	nd	0.05

4.2 PFASs in snow, water, soil and sediment

4.2.1 PFASs in snow

Within the different sites the pattern of PFASs in the snow samples was similar to each other (*Figure 6A*). 10 PFASs were detected in the snow samples (i.e. PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFTriDA, PFHxDA, MeFOSA). The dominant PFASs were PFOA, PFHxA, and PFHpA with an average contribution of 44%, 15%, and 8.6%, respectively for the ∑PFASs. The only detected PFAS precursor was MeFOSA. MeFOSA was found in two samples (A6 and A9) from different sites and did not contribute more than a few percent (6% and 3%, respectively) to the ∑PFASs in those samples. The PFAS profile in the western lake (i.e. samples A5-1, A5-2 and A5-3 at 0-3 cm, 3-6 cm, and 6-9 cm depth, respectively) diverged from the pattern in the other snow samples. The pattern in these

samples showed a higher portion of the shorter chain PFASs (i.e. PFBA and PFPeA) and decreasing content of the longer chained homologues (i.e. PFHxDA and PFTriDA respectively) with increasing depth. The snow samples from the reference lake (i.e. A10-A12) did not contain any PFASs with a longer perfluorocarbon chain length than C_8. In contrast, the other sampling sites had samples containing longer chained homologues (except of sample A5-3). The statistically higher ∑PFAS levels were found at the sample site at the hill (i.e. A7-A9) in comparison to the other sampling locations (T-test: two sample assuming equal variance, α=0.01) (*Figure 6B*). The samples from the hill (i.e. A7-A9) contained about twice as much ∑PFASs (30-31 ng L^{-1}) as the other sampling locations (1.7 to 16.4 ng L^{-1}). ∑PFAS levels in samples collected at the reference lake (i.e. A10-A12, 4.8 to 8.5 ng L^{-1}) were not statistically different from levels in samples from the starting area (i.e. A1-A3, 3.8 to 16.4 ng L^{-1}) or the potentially contaminated lake (i.e. A4-A6, 6.0 to 11.3 ng L^{-1}) (T-test: two sample assuming equal variance, α=0.05).

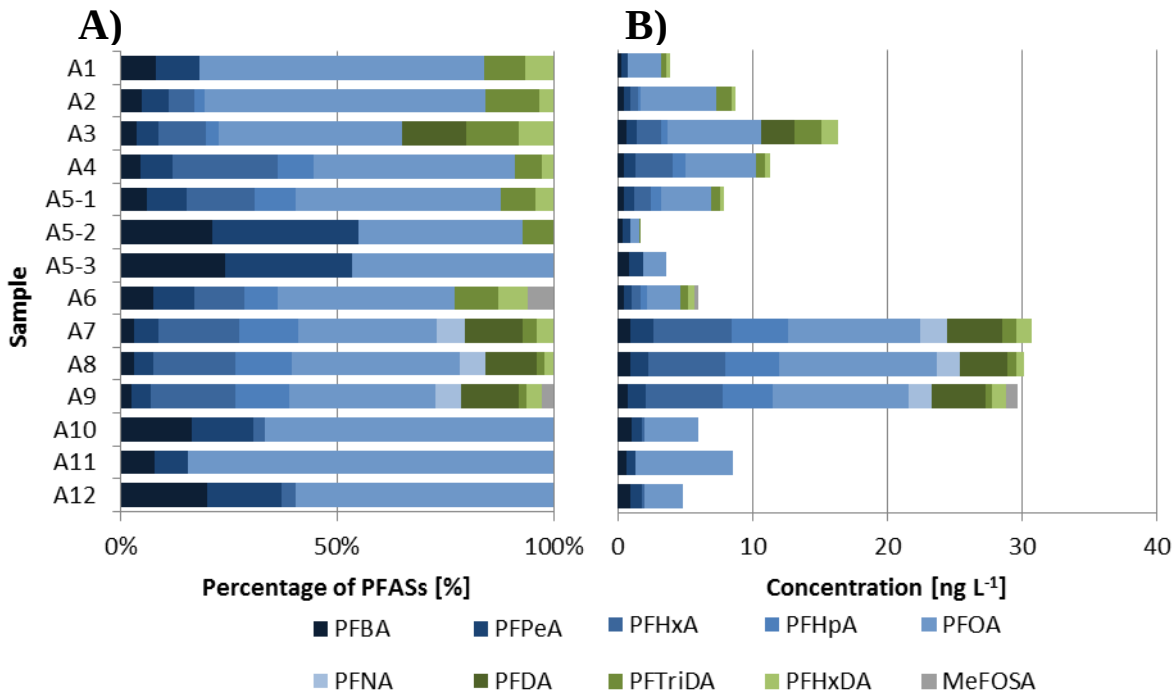

Figure 6. A) *The distribution of the PFASs found in each snow sample. B) The total amount of PFASs found in the snow samples as well as the individual contributions from the different PFASs*

4.2.2 PFASs in surface water

In total, 9 PFASs were detected in the surface water samples (i.e. PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA and PFDoDA). The dominant PFASs were PFOA, PFPeA, and PFBA with an average contribution of 31%, 22%, and 18%, respectively for the ∑PFASs. No PFSAs or PFAS precursors were detected in the samples. The surface water samples collected furthers away from the starting area (i.e. B1-B3, B8 and B10) only contained the shorter chained PFCA homologues PFBA and PFPeA (*Figure 7)* Closer to the starting area samples (i.e. B4-B6 and B9) also contained PFOA. Surface water sample B7 diverged from all the other samples and contained PFCA homologues of chain lengths from C_4 up to C_{12}. Low ∑PFAS levels were detected in the surface water samples from the

reference lake (i.e. B1, B2 and B3, 0.9 to 2.3 ng L^{-1}), the potentially contaminated lake (i.e. B4, B5 and B6, 0.6 to 1.5 ng L^{-1}) and from the river (i.e. B8, B9 and B10, 0.9 to 1.93 ng L^{-1}) and were not significantly different from each other (T-test: two sample assuming equal variance, α=0.05). The highest ∑PFAS level (21.9 ng L^{-1}) was detected in the depression (B7).

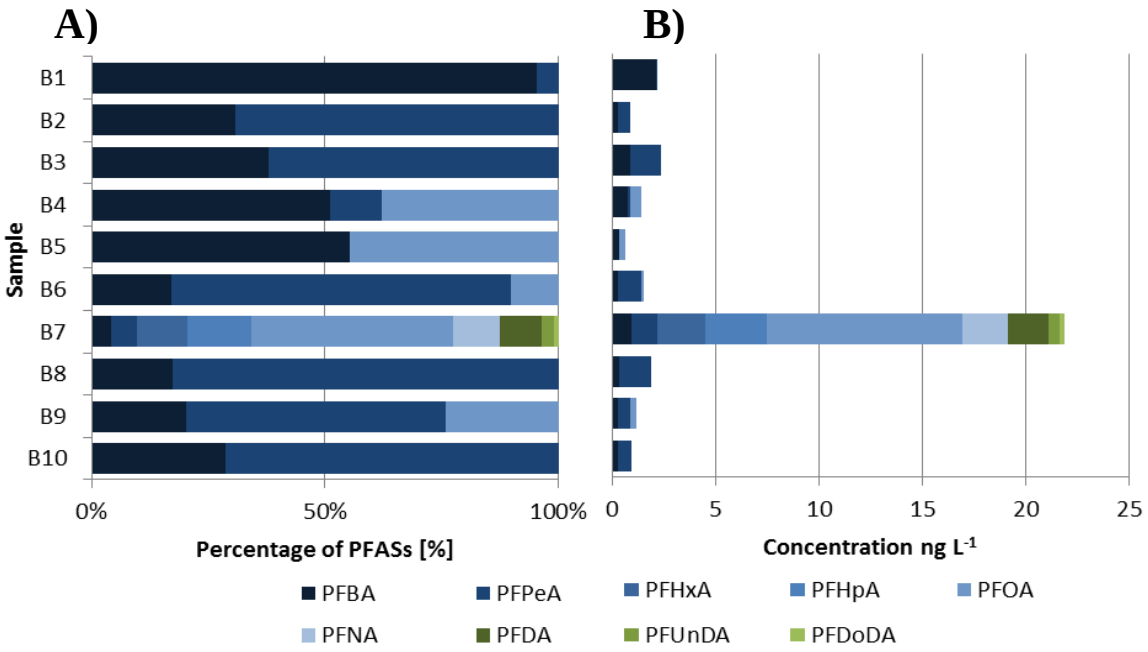

Figure 7. A) The distribution of the PFASs found in each surface water sample. B) The total amount of PFASs found in the surface water samples as well as the individual contributions from the different PFASs.

4.2.3 PFASs in soil and sediment

In both soil and sediment samples all three targeted PFAS groups (PFCAs, PFSAs and PFAS precursors) were represented (*Figure 8A*). All but four target compounds (i.e. 6:2 FTSA, MeFOSAA, PFDS and PFHxS) were detected in the soil and sediment samples. The dominant PFASs were MeFOSE, PFPeA and EtFOSE with an average contribution of 20%, 14%, and 11%, respectively for the ∑PFASs.

The samples collected from the potentially contaminated lake (C9), the starting area (D1) and from under the tracks in the hill (D2) contained a larger proportion of longer chained PFCAs (57%, 21% and 65% respectively) than the other soil and sediment samples (not detected to 1.9 %). The PFAS precursors was the most abundant PFAS group (in average 49% of the ∑PFASs) and in almost half of the samples, with 49% in C1 and over 50% in C2, C3, C7, C8 and D3. PFCAs had an average contribution of 47% of the ∑PFASs, whereas PFSAs were scarcely distributed (in average 4%) and PFBS and PFOS were found in two and eight samples, respectively.

∑PFAS levels were the highest in D3 and D1 with 13.7 and 8.3 ng g^{-1} dw respectively (*Figure 8B*). The sample from the forest in the hill next to the tracks (D3) contained 7 PFAS precursors which made up 73% of the ∑PFAS levels. The sample from under the tracks in the

hill (D2) contained 4.7 ng g^{-1} dw, only about a third of what D3 contained, of which only 2% was precursors (i.e. MeFOSE 0.1 ng g^{-1} dw).

In three sediment samples from the reference lake (C4, C5 and C6) low ∑PFAS levels were detected (<1 ng g^{-1} dw), while the other samples (C1, C2, C3, C7 and C8) contained ∑PFAS levels (3.3 to 5.5 ng g^{-1} dw) more comparable with the rest of the samples (C9, C10 and D1-D3, 2.3 to 13.7 ng g^{-1} dw).

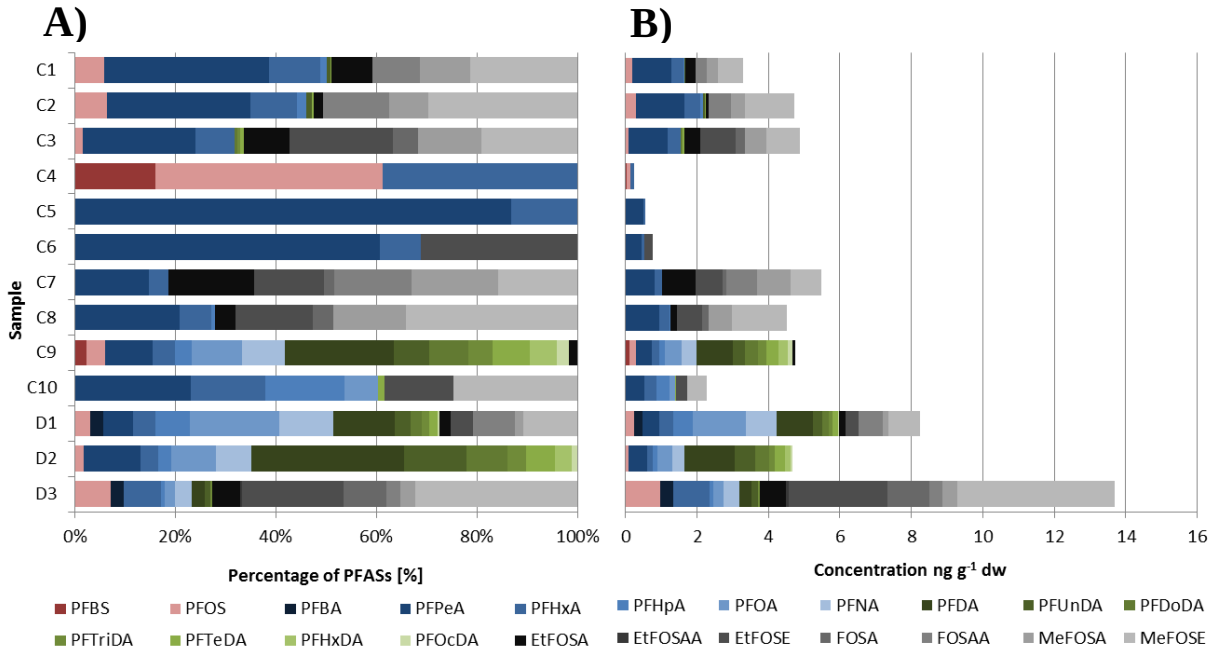

Figure 8. A) The distribution of the PFASs found in each soil and sediment sample. B) The total amount of PFASs found in the soil and sediment samples as well as the individual contributions from the different PFASs.

4.3 Sediment-water partitioning coefficients

Four PFCAs (i.e. PFPeA, PFHxA, PFHpA and PFOA) were detected in both sediment and surface water samples from the same location and the sediment-water distribution coefficient (K_d) and the organic carbon normalised coefficient (K_{oc}) was therefore calculated for only these PFASs (*Table 4*). PFHxA and PFHpA were only detected simultaneously in one sample, consequently no standard deviation could be calculated. The K_d-values ranged between 2.1 cm^3 g^{-1} (PFHpA) and 3.1 cm^3 g^{-1} (PFPeA), while the K_{oc}-values ranged between 2.7 cm^3 g^{-1} (PFHpA) and 3.8 cm^3 g^{-1} (PFPeA). In this study no increase of K_d or K_{oc} could be found with increasing perfluorocarbon chain length for PFPeA, PFHxA, PFHpA and PFOA which is not in line with previous studies (Higgins and Luthy, 2006; Ahrens et al., 2010b).

Table 4.The sediment-water distribution coefficients (K_d) and organic carbon normalised coefficient (K_{oc}) between sediment and water samples for PFPeA (n=5), PFHxA (n=1), PFHpA (n=1) and PFOA (n=2).

Compound	Log K_d [cm^3 g^{-1}]	Log K_{oc} [cm^3 g^{-1}]
PFPeA	3.1 ± 0.6	3.8 ± 0.5
PFHxA	2.2	2.8
PFHpA	2.1	2.7
PFOA	2.2 ± 1.4	3.0 ± 1.5

4.4 Zebrafish embryo test

4.4.1 Testing of water samples

The average heart frequency was around 140 beats per minute (bpm) for all four groups with variances between 6 and 18 bpm (*Figure 9*). The variances in the groups of raw heart frequency data were statistically different (p=0.037, Levene's Test) but the one-way ANOVA requires similar variances. Transforming the heart frequency data made the variances amongst the groups not statistically different (p=0.057, Levene's Test). In the following one-way ANOVA test the heart frequency in the four groups was not statistically different from each other (p=0.05).

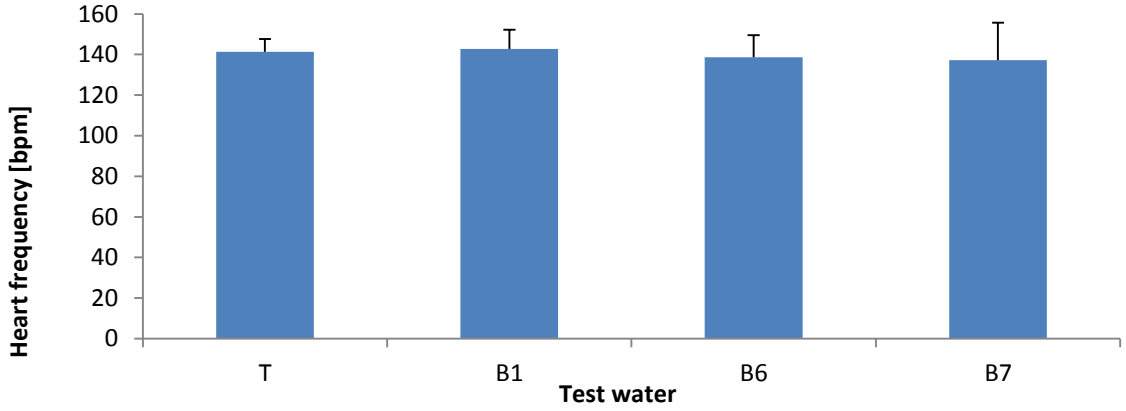

Figure 9. Average heart frequency with standard deviation for tap water (T, n=11) and the surface water samples B1 (n=12), B6 (n=12), and B7 (n=11).

Hatch time for plate A was consistent between the groups, with averages spanning 76-82 bpm (*Figure 10*). Three embryos (i.e. two from the tap water and one from B1) had longer hatch times of 118, 141 and 141 h which explains the longer whiskers for these two samples in the in the Box-whisker plots in *Figure 10*. The variances of hatch time between the groups were not statistically different (p=0.45, Levene's Test), thus no transformation was needed. The following one-way ANOVA concluded that none of the four groups was statistically different from another (p=0.23).

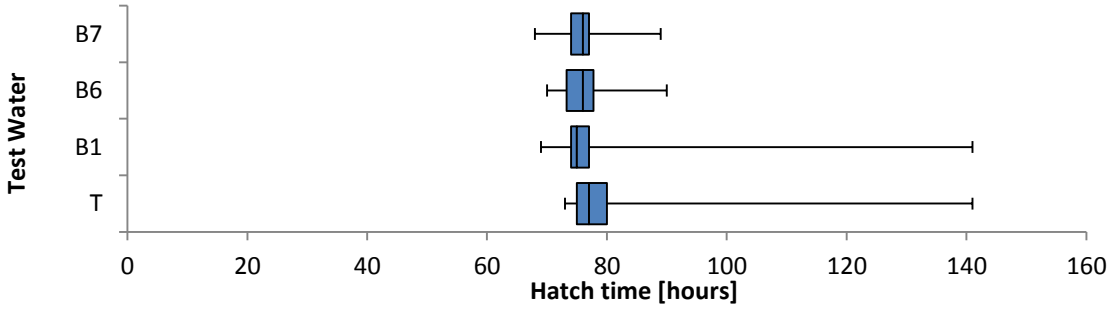

Figure 10. Box-whisker plot of hatch time for embryos in tap water (T, n=22), and the surface water samples B1 (n=24), B6 (n=23) and B7 (n=22). The surface water sample B1 was collected from the reference site. The boxes consist of first and third quartile and median value and the whiskers marks the maximum and minimum values.

In rating the pigmentation at 48 h only 2 of 22 embryos in the tap water received a rating lower than 2, namely a 3. However, this was not statistically different from the other groups (p=0.42, Kruskal-Wallis test).

At 24 hpf one embryo had coagulated and one did not move in the tap water as well as in water sample B7. In water sample B6 three embryos did not move. All embryos in B1 had normal development.

At 48 hpf one embryo in tap water and B7 was still coagulated. In addition, one embryo in the tap water had edema on its yolk and one in B7 had bad circulation. One embryo in B1 had developed a small edema below its heart, the same for two embryos in B6 along with two that had bigger edema. Since at 24 and 48 hpf, three or less embryos of 24 showed harmful endpoints, no statistics were performed on that data.

At 144 hpf, 10 of 24 embryos had harmful endpoints (i.e. coagulated, not hatched but alive and lying idle on its side on the bottom) in the tap water (*Figure 11*). Two embryos (n=24) had coagulated whilst six had not hatched and two were lying on its side. In B1 (n=24), one embryo had died after hatching, the same happened to one in B6 (n=24) were another one had edema. In B7 (n=24), one embryo had coagulated and one had edema on yolk near the heart and its spine was bent sideways. Statistically the number of lethal of sublethal endpoints at 144 hpf for the tap water was significantly higher compared to B1 using Fischer's exact test with Bonferroni correction (p=0.013). No significant difference was found when comparing B1 against B6 and B7 (p=1, Fischer's exact test).

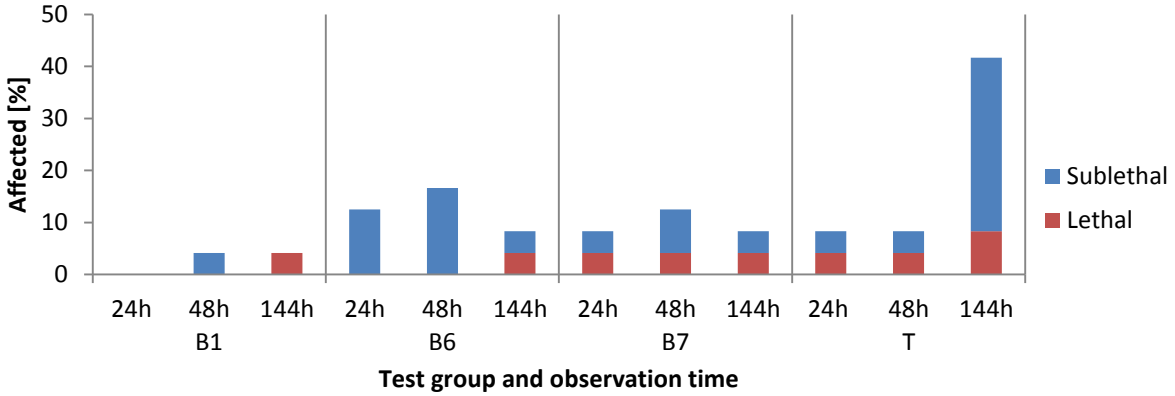

Figure 11. Percentage of embryos (n=24 in all groups) in carbon filtered tap water (T) and three surface water samples (i.e. B1, B6 and B7) showing either lethal or sublethal endpoints at the three screenings times (i.e. 24 hpf, 48 hpf, and 144 hpf).

4.4.2 Testing of soil and sediment samples

At 24 hpf, 5 of 12 embryos in soil D3, while only 1 of 12 from sediment C9 did not show movement and 1 of 12 from soil D2 had coagulated. All embryos in the other samples (tap water, C2, C4, C10 and D1) developed normally.

At 48 hpf, 8 of 12 embryos (i.e. 75%) in soil D3 were clearly affected, either coagulated or very poor pigmentation. In D2, 1 of 12 was still coagulated. In the other groups 2 of 12 from

sediment C9 and 1 of 12 from both sediments C2 and C10 had reduced pigments on body. All embryos in the other samples (tap water, C4 and D1) developed normally.

At 144 hpf, 9 of 12 of the embryos in soil D3 had coagulated (*Figure 12*). The remaining three were alive but not hatched and with weak pulse, one of them also had edema behind the yolk. One in D2 was still coagulated and one in C9 was alive with weak pulse but not hatched and its head was deformed and edema had developed along most of its body. All embryos in the tap water and the other samples (C2, C4, C10 and D1) developed normally. Groups with one affected embryo (i.e. C9 and D2) and groups with no affected embryos (i.e. C2, C4, C10 and D1) differed significantly from D3 (*Figure 12*), that had 12 affected embryos, when using Fischer's exact test (p=0.0000288 and p=0.0000021 respectively). The two low affected group clusters did not differ significantly between each other (p=1, Fischer's exact test).

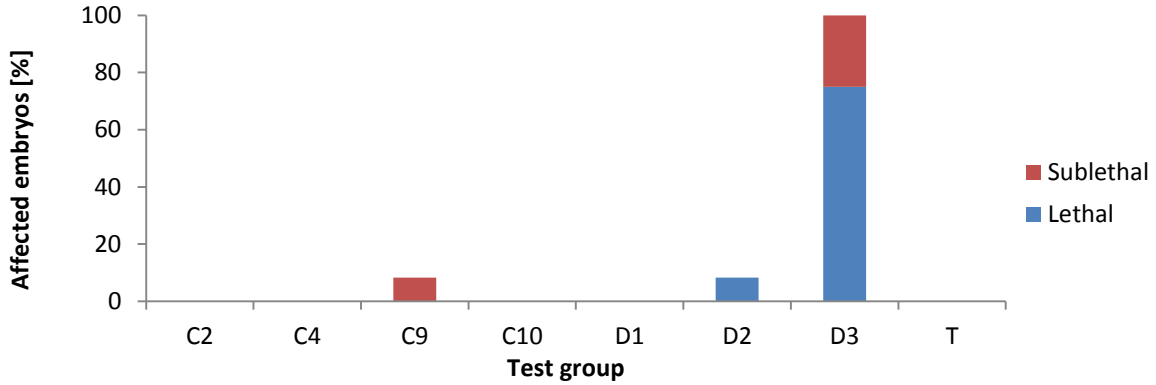

Figure 12. Percentage of embryos (n=12 in all groups) exposed to four different sediment samples (i.e. C2, C4, C9 and C10), three different soil samples (i.e. D1, D2 and D3) and carbon filtered tap water (T) showing either lethal or sublethal endpoints at 144 hpf.

The pH of water from most of the soil and sediment samples (pH = 4.3-7.5) was close to that of the control with only tap water (pH = 4.9) (*Table 5*). The pH in soil C10 and sediment D2 was 7.5 and 5.9 respectively. These were the only waters that had higher pH than the tap water (pH = 4.9). Water from soil C2 had the lowest pH of 4.3. There was not much nitrate in the waters, sediment C2, sediment C9 and soil D1 contained most with 9, 11 and 11 mg L^{-1}, respectively. The nitrite levels were under the detections limit of 0.5 mg L^{-1} in all waters.

Table 5. pH, nitrate (NO_3^-) and nitrite (NO_2^-) for the water from the soil and sediment test

Sample	pH	NO_3^- [mg L^{-1}]	NO_2^- [mg L^{-1}]
D3	4.8	<5	<0.5
D2	5.9	6	<0.5
D1	4.4	11	<0.5
C10	7.5	<5	<0.5
C9	4.6	9	<0.5
C4	4.9	6	<0.5
C2	4.3	11	<0.5
Tap water	4.9	<5	<0.5

5 Discussion

5.1 Distribution of PFASs in snow during the winter time

The ∑PFAS levels in snow samples from the starting area (i.e. A1, A2 and A3, 3.8 to 16.4 ng L^{-1}) did not differ significantly (T-test: two sample assuming equal variance, α=0.05) from the levels in samples from the reference lake (i.e. A10, A11 and A12, 4.8 to 8.5 ng L^{-1}) and neither did the samples from the potentially contaminated lake (i.e. A4, A5-1 and A6, 6.0 to 11.3 ng L^{-1}). However, three samples (i.e. A2-A4) contained higher ∑PFAS levels (8.7 to 16.4 ng L^{-1}) than the highest value from the reference lake (A11, 8.5 ng L^{-1}). Based on the ∑PFAS levels in the snow samples it cannot be concluded that the potentially contaminated lake (A4-A6) and the starting area (A1-A3) is higher contaminated due to PFAS-containing ski-waxes from skiing activities compared to the reference lake (A10-A12). The detected PFASs at the potentially contaminated lake and the starting area and reference lake can originate from atmospheric deposition of PFASs (Ahrens et al., 2010a; Cai et al., 2012). The samples from the hill (i.e. A7-A9) contained more than three times higher ∑PFAS levels (29.6 to 30.7 ng L^{-1}) than the highest level from the reference lake (8.5 ng L^{-1}), which indicates a pollution from PFAS-containing ski-waxes from skiing activities since the abrasion at the uphill track might be higher compared to the flat starting area.

The pattern of PFASs in the samples differed between the sites, as some longer chained PFCAs (PFDA, PFTriDA and PFHxDA) were detected in the surface samples from the potentially contaminated lake (A4, A5-1 and A6, 9 to 17% of the ∑PFASs) and the starting area (A1, A2 and A3, 16 to 35% of the ∑PFASs), but not in samples from the reference lake (i.e. A10-A12). These findings imply that the skiing area is contaminated with PFCAs of perfluorocarbon chain length longer than C_8. This is supported by Plassmann and Berger (2013) who found a majority of longer chained PFCAs in their snow samples (74 to 93% of the ∑PFASs) but one (42% of the ∑PFASs) and only shorter perfluorocarbon chained homologues (C_8 and shorter) in their reference sample. The contamination is likely to be due to the skiing activity as analysed ski waxes contain these longer chained PFCAs (Freberg et al., 2010; Plassmann and Berger, 2013).

Although no apparent human activity was observed in the close vicinity of the reference lake (i.e. A10-A12), PFCAs were found at low levels (4.8-8.5 ng L^{-1}). The most probable reason for these findings is that the site has been contaminated through atmospheric deposition. Previous studies have shown that PFCAs can be transported through the atmosphere. In Arctic snow Young et al. (2007) found levels for PFOA and PFNA up to 147 pg L^{-1} and 246 pg L^{-1} respectively, but levels of the longer chained PFDA and PFUnDA was only found up to 22 and 27 pg L^{-1} respectively. In snow from Antarctica, Cai et al. (2012) found ∑PFAS at 1129-2491 pg L^{-1}, concluding that about half of it was due to atmospheric deposition. These levels found at the poles were lower that what has been found at our reference lake (A1-A3). However, the closer a site is to industrialised areas it is expect to receive more PFASs from atmospheric deposition which could account for our ∑PFAS levels at 4.8-8.5 ng L^{-1} (A10-12).

Plassmann and Berger (2013) collected snow samples along the whole length of the track and found levels of PFCAs up to 1380 ng L^{-1} at the same area as this study (Vasaloppet) but 3 km

after the starting area and then lower levels (PFCAs < 360 ng L^{-1}) at five locations between 26 km and 82 km after the start. Compared to our highest levels of 30.7 ng L^{-1} (A7), in the hill at approximately 1 km after the start, it indicates that abrasion of ski wax off of the skis has a maximum after a few kilometres. This could mean that there is an area further down the tracks from our sampling site in the hill that has a greater need for evaluation than the starting area.

5.2 Distribution of PFASs in surface water, soil and sediment after snow melting

5.2.1 Surface water

The surface water samples generally contained low ∑PFAS levels ranging between 0.6 and 2.3 ng L^{-1} (B1-B6 and B8-B10), although B7 contained 21.9 ng L^{-1}. The reference lake showed low levels of ∑PFASs (B1-B3, 0.6 and 2.3 ng L^{-1}), as well as the potentially contaminated lake (B4-B6, 0.6 and 2.3 ng L^{-1}). The low levels at the potentially contaminated lake might be due to short retention time in the lake and dilution effects. In contrast, the water in the depression (B7) has probably a longer retention time than the potentially contaminated lake to which it drains. This might explain the high level of ∑PFASs (21.9 ng L^{-1}) in the depression (B7). This would mean that the potentially contaminated lake was diluted to some degree already at the time of sampling. Furthermore, this would mean that B7 probably was a more accurate representation of the PFAS contamination from ski waxes than the rest of our surface water samples

The ∑PFAS levels in the water samples from the river (i.e. B10, B9 and B8) were low and did not differ by much, but the further downstream the sample was collected the ∑PFCA levels increased from 0.9 ng L^{-1} (B10) to 1.9 ng L^{-1} (B8). This trend could be explained by water percolating in the starting area allowing for PFSAs and precursors to partition to the particle phase whereas the PFCAs follow the water into the river to elevate the levels. However, as this trend could not be tested statistically due to only one sample each for the three locations it could be a coincident. Furthermore, the increasing trend was small, which might be due to the short retention time of the water in the river and dilution effects. The water samples from the reference lake (i.e. B1, B2 and B3), the potentially contaminated lake (i.e. B4, B5 and B6) and the river (i.e. B8, B9 and B10) contained ∑PFAS levels, below 2.5 ng L^{-1}. Comparing those levels with an Arctic lake in Canada (Stock et al., 2007) and a Norwegian lake (Kallenborn et al., 2004), with detected levels of ∑PFAS at 12 ng L^{-1} and 6-10 ng L^{-1}, respectively, shows that the PFAS levels detected in this study represent background levels.

The PFAS pattern in B7 was similar to patterns in the snow samples from the contaminated sites (i.e. A1-A9). They contained PFCAs with a perfluorocarbon chain length longer than C_8, in addition to the PFCAs of perfluorocarbon chain lengths C_4-C_7 that every sample contained. As the snow and water samples from the reference lake did not contain any of those longer homologues it is most probable that the contamination of longer chained PFCAs in B7 does come from ski wax abraded to the snow during the winter.

5.2.2. Soil and sediment

In the soil (n = 21 PFASs) and sediment samples (n = 20) more target compounds were found compared to the snow (n = 10) and water samples (n = 9). Unlike the snow and water samples, the soil and sediment samples were not filtrated during the extraction procedure. As

PFSAs, precursors and long chained PFCAs have higher partitioning coefficients (K_d and K_{oc}) than short chained PFCAs they tend to partition to the solid phase in greater extent. This could explain why PFSAs and precursors were detected in the soil and sediment samples and not in the snow and water. Thus, the dissolved phase contains a lower number of PFASs compared to the soil and sediment samples.

The ∑PFAS levels in the sediment samples were ambiguous. The sediment profile from the reference lake (i.e. C4, C5 and C6) contained low ∑PFAS levels (0.2, 0.6 and 0.8 ng g^{-1} dw) but the other samples from the reference lake (i.e. C1-C3, C7 and C8) contained ∑PFAS levels of 3.3 to 5.5 ng g^{-1} dw which were higher than from the depression (C10, 2.3 ng g^{-1} dw) and similar to the level of the sample from the potentially contaminated lake (C9, 4.7 ng g^{-1} dw). The soil samples from the starting area (D1) and the hill (D2) both contained lower ∑PFAS levels (4.7 and 8.2 ng g^{-1} dw, respectively) than the intended reference soil from the forest next to the tracks (40 m distance) in the hill (D3, 13.7 ng g^{-1} dw). That sample contained 73% precursors whereas the ∑PFCAs+∑PFSAs (without the precursors) was 3.8 ng g^{-1} dw, which is not higher than that of the sample from the tracks in the hill (D2, 4.7 ng g^{-1} dw). The origin of the high levels of PFAS precursors in the reference sample for soil next to the tracks (D3) is not known and further investigations are needed.

The pattern in most of the sediment samples from the reference lake (C1, C2, C3, C7 and C8) consisted of a large portion of precursors (EtFOSA, EtFOSE, FOSA, FOSAA, MeFOSA and MeFOSE) and those ∑PFAS levels (3.3 to 5.5 ng g^{-1} dw) could have come from atmospheric deposition. Furthermore, the sample from the starting area (D1) and the hill (D2) contained more of the longer perfluorocarbon chained C_9-C_{18} PFCAs (1.7 and 3.0 ng g^{-1} dw respectively) than the sample from the forest in the hill (D3, 0.6 ng g^{-1} dw). As for the snow samples and water sample B7 this follows the same pattern indicating that the starting area (D1) and the hill (D2) are contaminated with PFCAs probably from ski waxes used during the winter. This was also evident in the sediment samples, where the potentially contaminated lake (C9) contained more of the longer perfluorocarbon chained C_9-C_{18} PFCAs (3.1 ng g^{-1} dw) than what the samples from the reference lake did (C1-C8, not detected to 0.09 ng g^{-1} dw).

Plassmann and Berger (2013) found ∑PFCA levels at 5 to 14 ng g^{-1} dw in soil samples from the starting area and 18 to 19 ng g^{-1} dw in samples collected at 3 km down the tracks (Plassmann and Berger, 2013). In D1 the ∑PFCA levels were just under 6 ng g^{-1} dw but at the hill D2 only contained 4.7 ng g^{-1} dw. This did not follow the same pattern as in the previous study with the highest concentrations in samples succeeding the starting area. The ∑PFAS levels in the soil reported here (4.7 to 13.7 ng g^{-1} dw) are elevated compared to less industrialised countries like Nepal (0.0 to 1.2 ng g^{-1} dw) (Tan et al., 2014). However, compared to the soil around a fluoropolymer manufacturing facility in Germany (64 to 416 ng g^{-1} dw) the soil at Vasaloppet is in the low contaminated region (Hangen et al., 2010).

5.3 Potential Effects of PFASs on aquatic organisms using zebrafish embryo tests

In using water samples from around Vasaloppet in the zebrafish embryo test one surprising observation was that the embryos seemed to develop better in all tested water samples than in

tap water from Uppsala. Now this does not directly have to imply that the tap water is contaminated with organic pollutants. There are many parameters that determine if a species can live in a specific water. The drinking water in Uppsala is artificial groundwater. The processes involved in producing this water can alter the ionic composition of the water. Therefore it may not be suitable for the zebrafish. It could also be due to the tap water temporarily having a lower quality. However, we included a field reference (B1) in the test and in that water the embryos thrived. Thus, the water from the reference site was used for comparison instead of the tap water.

For the zebrafish embryo tests using surface water (B1, B6 and B7), B1 was used as control water. There were no significant differences between the control and B6 or B7 for any of the screened endpoints, heart frequency or hatch time. B7 had 10 and 14 times higher levels of PFASs than B1 and B6, respectively, but still that were not enough to have a significant effect on the embryos. Ulhaq et al. (2013) obtained EC50 values for PFBA, PFOA, PFBS and PFOS at 3000, 100, 1500 and 1 mg L^{-1} respectively. EC50 is defined as the level of a substance that causes 50% of the test group to displayed lethal or sublethal endpoints. The lowest of their EC50 values, 1 mg L^{-1} for PFOS alone, was about 45 000 times higher than that of the ∑PFAS level in B7, which was the highest in our water samples.

There is a difference in the EC50 for the zebrafish test and exposing an environment of a long period of time. In 2008 the Swedish Environmental Protection Agency proposed a limit for PFOS in drinking water around 350-1000 ng L^{-1} (Swedish Environmental Protection Agency, 2008). That interval was partly based on a two-year rat study where rats had been given PFOS in their diet. Similar agencies in other countries have recommended similar limits. The British Health Protection Agency advised a maximum concentration for PFOS and PFOA of 300 ng L^{-1} and 1000 ng L^{-1} respectively (British Health Protection Agency, 2007). The US EPA recommended stricter limits in 2009 for PFOA and PFOA at 200 ng L^{-1} and 400 ng L^{-1} respectively (US EPA, 2014). But the effect PFASs have on aquatic life and land living mammals may differ and therefore it is still important to screen environmental matrices with the zebrafish test.

For the zebrafish embryo tests using soils (D1, D2 and D3) and sediments (C2, C4, C9 and C10), a control group with only carbon filtered tap water was used. In contrast to the testing of water the tap water group did not display any adverse effects now, indicating that it could have been a temporary dip in the quality at the time for the water sample test. The group that had been exposed to soil D3, which had the highest ∑PFAS levels (13.7 ng g^{-1} dw) of all the soil and sediment samples, showed adverse effects in all embryos in the group such as coagulated or unhatched embryo. However, the levels of PFASs in D3 (14 ng L^{-1}), were lower than in the water B7 (22 ng L^{-1}). And since only 2 of 24 embryos in B7 displayed effects while all did it in D3 it is more likely that something else in the soil or the soil itself was not suitable for aquatic life. Common parameters to measure along with the zebrafish test are pH, nitrate and nitrite. Both nitrate and nitrite, which could be harmful at higher levels, were below detection limit in water from group D3. The pH in D3 was 4.8, which is outside of the recommended pH-interval of 6.5-8.5 for water during zebrafish embryo test (OECD, 2013). But that was also the case in five other groups (D1, D2, tap water, C2, C4 and C9) which, as

mentioned, did thrive in their waters and three of those groups had even lower pH than D3. Results from all parameters analysed cannot explain why the embryos in D3 developed as they did. D3 was a soil and not a sediment, more specifically a podzol. In the top layer of podzols aluminium and iron are common and can form complexes with humus which could be the cause of the negative effects. It might be possible that this soil sample was contaminated with other PFASs not analysed in this study or other organic pollutants which might potentially be harmful.

6 Conclusion and outlook

PFSAs were ubiquitously detected in all collected snow, water, soil and sediment samples. The ∑PFAS levels were generally higher in the snow samples (in average 12.1 ng L^{-1}, median 8.2 ng L^{-1}, A1-A12, Figure 6) compared to surface water samples (in average 1.4 ng L^{-1}, median 1.4 ng L^{-1}, B1-A6, B8-B10, Figure 7), except of surface water site B7 (21.9 ng L^{-1}). Sample B7 was collected from a depression in the field between the potentially contaminated lake and the hill. The contamination of the snow at the reference lake (samples A10-A12) indicates atmospheric deposition of PFASs and the higher ∑PFAS levels in the hill points to an influence from skiing activities (samples A7-A9). The longer chained PFCAs seem to be connected to the skiing tracks and might originate from ski wax. These PFCAs included C_{10}, C_{13} and C_{16} PFCAs for snow samples (A7-A9), $C_{10\text{-}12}$ PFCAs for surface water samples (B7), $C_{10\text{-}14}$, C_{16} and C_{18} PFCAs for soil and sediment. In snow and surface water samples C_4-C_8 PFCAs were dominant (in average 80 % and 86 %, respectively), whereas in soil and sediment the precursors were dominant (in average 46 % and 52 %, respectively). The different PFAS pattern in the water phase (snow and surface water) compared to the solid phase (soil and sediment) can be explained by the different solid-water partitioning characteristics of the investigated PFASs. Hence, short chain PFASs are more water soluble whereas longer chained PFASs adsorb more to particles.

Generally all tested water samples in the zebrafish embryo test were not toxic enough to have an effect on a developing embryo. Almost all the zebrafish embryos developed normally, even in the water with the highest ∑PFAS level (B7). In the soil sample with the highest ∑PFAS level (D3) embryos did not develop normally, probably due to the soil itself being unsuitable for that kind of test. Parameters not analysed here such as other PFASs or POPs can also have affected the embryos.

Future studies should focus on the environment around the tracks between 1 and 26 km, where the contamination maximum probably is located. Snow and soil samples could preferably be collected directly from the tracks and a few meters away from them. Future studies of the starting area should collect samples at a time when the lakes are not flooded, to be able to reach the sediment further out in the lakes. In addition, another sampling occasion could be added in the summer to collect and analyse plants and water living organisms. Furthermore, the forest in the hill with high ∑PFAS levels needs to be investigated in future studies.

References

Ahrens, L., Yamashita, N., Yeung, L., Taniyasu, S., Horii, Y., Lam, P. and Ebinghaus, R. (2009). Partitioning Behavior of Per- and Polyfluoroalkyl Compounds between Pore Water and Sediment in Two Sediment Cores from Tokyo Bay, Japan. *Environmental Science & Technology, Vol. 43. 6969-6975.*

Ahrens, L., Gerwinski, W., Thebald, N. and Ebinghaus, R. (2010a). Sources of polyfluoroalkyl compounds in the North Sea, Baltic Sea and Norwegian Sea: Evidence from their spatial distribution in surface water. *Marine Pollution Bulletin, Vol. 60, 255-260.*

Ahrens, L., Taniyasu, S., Yeung, L., Yamashita, N., Lam, P. and Ebinghaus, R. (2010b). Distribution of polyfluoroalkyl compounds in water, suspended particulate matter and sediment from Tokyo Bay, Japan. *Chemosphere, Vol. 79, 266-272.*

Apelberg, B., Witter, F., Herbstman, J., Calafat, A., Halden, R., Needham, L. Goldman, L. (2007). Cord Serum Concentrations of Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoate (PFOA) in Relation to Weight and Size at Birth. *Environmental Health Perspectives, Vol. 115, n. 11, 1670-1676.*

Armitage, J., Cousins, I., Buck, R., Prevedouros, K., Russel, M., MacLeod, M. end Korzeniowski, S. (2006). Modeling Global-Scale Fate and Transport of Perfluorooctanoate Emitted from Direct Sources. *Environmental Science & Technology, Vol. 40. 6969-6975.*

Armitage, J., MacLeod, M. and Cousins, I. (2009). Comparative Assessment of the Global Fate and Transport Pathways of Long-Chain Perfluorocarboxylic Acids (PFCAs) and Perfluorocarboxylates (PFCs) Emitted from Direct Sources. *Environmental Science & Technology, Vol. 43. 5830-5836.*

Austin, M., Kasturi, B., Barber, M., Kannan, K., MohanKumar, P. and MohanKumar, S. (2003). Neuroendocrine effects of perfluorooctane sulfonate in rats. *Environmental Health Perspectives, Vol. 111, n. 12, 1485-1489.*

Braunbeck, T., Böttcher, M., Hollert, H., Kosmehl, T., Lammer, E., Leist, E., Rudolf, M. and Seitz, N. (2005). Towards an Alternative for the Acute Fish LC50 Test in Chemical Assessment: The Fish Embryo Toxicity Test Goes Multi-species - an Update. *ALTEX, Vol. 22, n. 2, 87-102.*

British Health Protection Agency (2007). Maximum acceptable concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in drinking water. [online] Available at: <http://www.hpa.org.uk/webc/hpawebfile/hpaweb_c/1194947397222> [Accessed 26th of June 2014]

Buck, R., Franklin, J., Berger, U., Conder, J., Counsins, I., de Voogt, P., Jensen, A., Kannan, K., Mabury, S. and van Leeuwen, S. (2011). Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. *Integrated Environmental Assessment and Management, Vol. 7, n. 4, 513-541.*

Butt, C., Muir, D., Stirling, I., Kwan, M. and Mabury, S. (2007). Rapid Response of Arctic Ringed Seals to Changes in Perfluoroalkyl Production. *Environmental Science & Technology, Vol. 41. 42-49.*

Cai, M., Yang, H., Xie, Z., Zhao, Z., Wang, F., Lu, Z., Sturm, R. and Ebinghaus, R. (2012). Per- and polyfluoroalkyl substances in snow, lake, surface runoff water and coastal seawater in Fildes Peninsula, King George Island, Antarctica. *Journal of Hazardous Materials, Vol. 209-210, 335-342.*

Carlsson, G., Örn, S. and Larsson, J. (2009). Effluent from Bulk Drug Production is Toxic to Aquatic Vertebrates. *Environmental Toxicology and Chemistry, Vol. 28, n. 12, 2656–2662.*

Carlsson, A. (2014). *Farliga gifter kan finnas i dricksvattnet.*[online] Available at: <http://www.dn.se/nyheter/sverige/farliga-gifter-kan-finnas-i-dricksvattnet/> [Accessed 26th of March 2014].

Conder, J., Hoke, R., De Wolf, W., Russell, M. and Buck. R. (2008). Are PFCAs Bioaccumulative? A Critical Review and Comparison with Regulatory Criteria and Persistent Lipophilic Compounds. *Environmental Science & Technology, Vol. 42, n. 4, 995-1003.*

Conte, L. and Gambaretto, G. (2003). Electrochemical fluorination: state of the art and future tendencies. *Journal of Fluorine Chemistry, Vol. 125, 139-144.*

Dietz, R., Bossi, R., Rigét, F., Sonne, C. and Born, E. (2008). Increasing Perfluoroalkyl Contaminants in East Greenland Polar Bears (Ursus maritimus): A New Toxic Threat to the Arctic Bears. *Environmental Science & Technology, Vol. 42, n.7, 2701-2707.*

Ellis, D., Martin, J., Mabury, S., Hurley, M., Andersen, M. and Wallington, T. (2003). Atmospheric Lifetime of Fluorotelomer Alcohols. *Environmental Science & Technology, Vol. 37, n.17, 3816-3820.*

Embry, M., Belanger, S., Braunbeck, T., Galay-Burgos, M., Halder, M., Hinton, D., Léonard, M., Lillicrap, A., Norberg-King, T. and Whale, G. (2010). The fish embryo toxicity test as an animal alternative method in hazard and risk assessment and scientific research.*Aquatic Toxicology, Vol. 97, n. 2, 79-87.*

Environment Canada (2013). *Toxic Substances List.* [online] Available at: <http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=0DA2924D-1> [Accessed 19th of March 2014].

Environment Canada (2001). *Threats to Sources of Drinking Water and Aquatic Ecosystem Health in Canada.* [online] Available at: <http://www.ec.gc.ca/inre-nwri/default.asp?lang=En&n=235D11EB-1> [Accessed 27th of March 2014].

EU Commission, 2010. *Commission Regulation (EC) No 850/2004* [pdf] Official Journal of the European Union. Available at: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:223:0029:0036:EN:PDF> [Accessed 26th of March 2014].

EU Parliament, 2006. *Directive 2006/122/EC of the European Parliament and of the Council* [pdf] Official Journal of the European Union. Available at: <http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:372:0032:0034:en:PDF> [Accessed 26th of March 2014].

Faithfull, N. and Weers, J. (1998). Perfluorocarbon Compounds. *Vox Sanguinis, Vol. 74, n. 2, 243–248.*

Feige, J., Gelman, L., Michalik, L., Desvergne, B. and Wahli, W. (2006). From molecular action to physiological outputs: Peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. *Progress in Lipid Research, Vol. 45, n. 2, 120-159.*

Giesy, J. and Kannan, K. (2001). Global Distribution of Perfluorooctane Sulfonate in Wildlife. *Environmental Science & Technology, Vol. 35, 1339-1342.*

Giesy, J. and Kannan, K. (2002). Perfluorochemical Surfactants in the Environment. *Environmental Science & Technology, Vol. 36, n. 7, 146A-152A.*

Glynn, A. (2012). Riskvärdering: Perfluorerade alkylsyror (PFAA) i Uppsalas dricksvatten. *National Food Agency, dnr 1192/2012.*

Gump, B., Wu, Q., Dumas, A. and Kannan, K. (2011). Perfluorochemical (PFC) Exposure in Children: Associations with Impaired Response Inhibition. *Environmental Science and Technology, Vol. 45, 8151-8159.*

Hagen, D., Belisle, J., Johnson, J. and Venkateswarlu, P. (1981). Characterization of Fluorinated Metabolites by a Gas Chromatographic - Helium Microwave Plasma Detector - The Biotransformation of 1H,1H,2H,2H-Perfluorodecanol to Perfluorooctanoate. *Analytical Biochemistry, Vol. 118, 336-343.*

Hagenaars, A., Vergauwen, L., De Coen W. and Knapen D. (2011). Structure–activity relationship assessment of four perfluorinated chemicals using a prolonged zebrafish early life stage test. *Chemosphere, Vol. 82, 764-772.*

Hallare, A., Kosmehl, T., Schulze, T., Hollert, H., Köhler, H. and Triebskorn R. (2005). Assessing contamination levels of Laguna Lake sediments (Philippines) using a contact assay with zebrafish (Danio rerio) embryos. *Science of the Total Environment, Vol. 347, 254-271.*

Hangen, E., Klemm, A., Kronawitter, H. and Schubert, A. (2010). Perfluorooctanoate (PFO) in Forest Soils near a Fluoropolymer Manufacturing Facility. *Water, Air, & Soil Pollution, vOL. 212, 491-499.*

Haukås, M., Berger, U., Hop, H., Gulliksen, B. and Gabrielsen, G. (2007). Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web. *Environmental Pollution, Vol. 148, 360-371.*

Hekster, F., de Voogt, P., Pijnenburg, A. and Laane, R. (2002). Perfluoroalkylated substances: Aquatic environmental assessment. *Report RIKZ/2002.043*. July 2002. 99 pp.

Higgins, C. and Luthy, R. (2006). Sorption of Perfluorinated Surfactants on Sediments. *Environmental Science and Technology, Vol. 40, 7251-7256.*

Hoffman, K., Webster, T., Weisskopf, M., Weinberg, J. and Vieira, V. (2010). Exposure to Polyfluoroalkyl Chemicals and Attention Deficit/Hyperactivity Disorder in U.S. Children 12–15 Years of Age. *Environmental Health Perspectives, Vol. 118, n. 12, 1762-1767.*

Houde, M., Martin, J., Letcher, R., Solomon, K. and Muir, D. (2006). Biological monitoring of polyfluoroalkyl substances: A review. *Environmental Science & Technology, Vol. 40, n. 11, 3463-3473.*

Hu, W., Jones, P., Upham, B., Trosko, J., Lau, C. and Giesy, J. (2002). Inhibition of gap junctional intercellular communication by perfluorinated compounds in rat liver and dolphin kidney epithelial cell lines in vitro and Sprague-Dawley rats in vivo. *Toxicological Science, Vol. 68, n. 2, 429-436.*

Hurley, M., Sulbaek Andersen, M., Wallington, T., Ellis, D., Martin, J. and Mabury, S. (2004). Atmospheric Chemistry of Perfluorinated Carboxylic Acids: Reaction with OH Radicals and Atmospheric Lifetimes. *The Journal of Physical Chemistry, Vol. 108, 615-620.*

Ishibashi, H., Kim, E. and Iwata, H. (2011). Transactivation Potencies of the Baikal Seal (Pusa sibirica) Peroxisome Proliferator-Activated Receptor r by Perfluoroalkyl Carboxylates and Sulfonates: Estimation of PFOA Induction Equivalency Factors. *Environmental Science & Technology, Vol. 45, 3123-3130.*

Jensen, A. and Leffers, H. (2008). Emerging endocrine disrupters: perfluoroalkylated substances. *International journal of andrology, Vol. 31, n. 2, 161-169.*

Kallenborn, R., Berger, U. and Järnberg, U. (2004). Perfluorinated alkylated substances (PFAS) in the Nordic environment. Norden. AIP 2004:552. Jan 2004. 112 pp.

Kannan, K., Koistinen, J., Beckmen, K., Evans, T., Gorzelany, J., Hansen, K., Jones, P., Helle, E., Nyman, M. and Giesy, J. (2001). Accumulation of Perfluorooctane Sulfonate in Marine Mammals. *Environmental Science & Technology, Vol.35, n. 8, 1593-1598.*

Keller, J., Kannan, K., Taniyasu, S., Yamashita, N., Day, R., Arendt, M., Segars, A. and Kucklick, J. (2005). Perfluorinated compounds in the plasma of loggerhead and Kemp's ridley sea turtles from the southeastern coast of the United States. *Environmental Science & Technology, Vol. 39, n. 23, 9101-9108.*

Kennedy, G., Butenhoff, J., Olsen, G., O'Connor, J., Seacat, A., Perkins, R., Biegel, L., Murphy S. and Farrar D. (2004). The Toxicology of Perfluorooctanoate. *Critical Reviews in Toxicology, Vol. 34, n. 4, 351–384.*

Key, B., Howell, R. and Criddle, C. (1997). Fluorinated Organics in the Biosphere. *Environmental Science & Technology, Vol.31, n. 9, 2445-2454.*

Kärrman, A., Elgh-Dalgren, K., Lafossas, C. and Møskeland, T. (2011). Environmental levels and distribution of structural isomers of perfluoroalkyl acids after aqueous fire-fighting foam (AFFF) contamination. *Environmental Chemistry, Vol. 8, n. 4, 372-380.*

Lammer, E., Carr, G., Wendler, K., Rawlings, J., Belanger, S. and Braunbeck T. (2009). Is the fish embryo toxicity test (FET) with the zebrafish (Danio rerio) a potential alternative for the fish acute toxicity test? *Comparative Biochemistry and Physiology, Vol. 149, n. 2, 196-209.*

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

Liew, L., Ritz, B., von Ehrenstein, O., Bech, B., Aagaard Nohr, E., Fei, C., Bossi, R., Henriksen, T., Bonefeld-Jørgensen, E. and Olsen, J. (2014). *Prenatal Exposure to Perfluorinated Chemicals, Attention-deficit/hyperactivity disorder and Autism.* [online] Available at: <https://wce.confex.com/wce/2014/webprogram/Paper3692.html> [Accessed 23rd of June 2014]

Loewen, M., Halldorsson, T., Wang, F. and Tomy, G. (2005). Fluorotelomer Carboxylic Acids and PFOS in Rainwater from an Urban Center in Canada. *Environmental Science & Technology, Vol. 39, 2944-2951.*

Martin, J., Mabury, S., Solomon, K. and Muir, D. (2003). Bioconcentraion and Tissue Distribution of Perfluorinated Acids in Rainbow Trout (Oncorhynchus Mykiss). *Environmental Toxicology and Chemistry, Vol. 22, n. 1, 196-204.*

Min, J-Y. Lee, K-J., Park, J-B. and Min, K-B. (2012). Perfluorooctanoic acid exposure is associated with elevated homocysteine and hypertension in US adults. *Occupational & Environmental Medicine, Vol. 69, Issue 9, 658-662.*

Nagel, R. (2002). DarT: The Embryo Test with the Zebrafish Danio rerio - a General Model in Ecotoxicology and Toxicology. *ALTEX, Vol. 19, n. 1, 38-48.*

Naile, J., Khim, J., Wang, T., Chen, C., Luo, W., Kwon, B., Park, J., Koh, C., Jones, P., Lu, Y and Giesy, J. (2010). Perfluorinated compounds in water, sediment, soil and biota from estuarine and coastal areas of Korea. *Environmental Pollution, Vol. 158, 1237–1244.*

NICNAS (2013). *Regulatory Actions.* [online] Available at: <http://www.nicnas.gov.au/regulation-and-compliance/nicnas-handbook/handbook-appendixes/data-requirements-for-notification-of-new-chemical-substances-containing-a-perfluorinated-carbon-chain/regulatory-actions> [Accessed 27th of March 2014]

OECD (2007). *Report of an OECD Workshop on Perfluorocarboxylic Acids (PFCAs) and Precursors.* [online] Available at:

<http://search.oecd.org/officialdocuments/displaydocumentpdf/?doclanguage=en&cote=env/jm/mono(2007)11> [Accessed 31st of March 2014]

OECD (2013). *OECD Guidelines for the Testing of Chemicals; Fish Embryo Acute Toxicity (FET) Test.* [online] Available at: <http://www.oecd-ilibrary.org/docserver/download/9713161e.pdf?expires=1403789921&id=id&accname=guest&checksum=CD81D3DD3B22B86E2C48D1EDB219AB7E> [Accessed 26th of June 2014]

Pabon, M. and Corpart, J. (2002). Fluorinated surfactants: synthesis, properties, effluent treatment. *Journal of Fluorine Chemistry, Vol. 114, 149-156.*

Petersen, G. and Kristensen, P. (1998). Bioaccumulation of Lipophilic Substances in Fish Early Life Stages. *Environmental Toxicology and Chemistry, Vol.17, n. 7, 1385-1395.*

Plassmann, M. and Berger, U. (2013). Perfluoroalkyl carboxylic acids with up to 22 carbon atoms in snow and soil samples from a ski area. *Chemosphere, Vol. 91, 832-837.*

Plassmann, M., Denninger, A. and Berger, U. (2011a). Environmental occurrence and fate of semifluorinated n-alkanes in snow and soil samples from a ski area. *Chemosphere, Vol. 85, 1458-1463.*

Plassmann, M., Meyer, T., Lei, Y., Wania, F., McLachlan, M. and Berger, U. (2011b). Laboratory Studies on the Fate of Perfluoroalkyl Carboxylates and Sulfonates during Snowmelt. *Environmental Science and Technology, Vol. 45, 6872-6878.*

Prevedouros, K., Cousins, I., Buck, R. and Korzeniowski, S. (2006). Sources, Fate and Transport of Perfluorocarboxylates. *Environmental Science & Technology, Vol. 40, n. 1, 32-44.*

Pyati, U., Look, T. and Hammerschmidt, M. (2007). Zebrafish as a powerful vertebrate model system for in vivo studies of cell death. *Seminars in Cancer Biology, Vol. 17, 154-165.*

Renner, R. (2006). The long and the short of perfl uorinated replacements. *Environmental Science & Technology, Vol. 40, n. 1, 12-13.*

Schiavone, A., Corsolini, S., Kannan, K., Tao, L., Trivelpiece, W., Torres, D. and Focardi, S. (2009). Perfluorinated contaminants in fur seal pups and penguin eggs from South Shetland, Antarctica. *Science of the Total Environment, Vol. 407, 3899-3904.*

Schröder, H. (2003). Determination of fluorinated surfactants and their metabolites in sewage sludge samples by liquid chromatography with mass spectrometry and tandem mass spectrometry after pressurised liquid extraction and separation on fluorine-modified reversed-phase sorbents. *Journal of Chromatography A, Vol. 1020, 131-151.*

Schröder, H. and Meesters, R. (2005). Stability of fluorinated surfactants in advanced oxidation processes - A follow up of degradation products using flow injection-mass spectrometry, liquid chromatography-mass spectrometry and liquid chromatography-multiple stage mass spectrometry. *Journal of Chromatography, Vol. 1082, 110-119.*

Schuetze, A., Heberer, T., Effkemann, S. and Juergensen, S. (2010). Occurrence and assessment of perfluorinated chemicals in wild fish from Northern Germany. *Chemosphere, Vol. 78, n. 6, 647–652.*

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., (2003). [book] *Environmental Organic Chemistry*. John Wiley & Sons, Inc., Hoboken, New Jersey.

Scott, B., Spencer, C., Mabury, S. and Muir, D. (2006). Poly and Perfluorinated Carboxylates in North American Precipitation. *Environmental Science & Technology, Vol. 40, n. 23, 7167-7174.*

Shankar, A., Xiao, J. and Ducatman, A. (2011). Perfluoroalkyl Chemicals and Chronic Kidney Disease in US Adults. *American Journal of Epidemiology, Vol. 174, n. 8, 893-900.*

Sinclair, E. and Kannan, K. (2006). Mass Loading and Fate of Perfluoroalkyl Surfactants in Wastewater Treatment Plants. *Environmental Science & Technology, Vol. 40, n. 5, 1408-1414.*

Stein, C. and Savitz, D. (2011). Serum Perfluorinated Compound Concentration and Attention Deficit/Hyperactivity Disorder in Children 5–18 Years of Age. *Environmental Health Perspective, Vol. 119, 1466-1471*.

Stemmler, I. and Lammel, G. (2010). Pathways of PFOA to the Arctic: variabilities and contributions of oceanic currents and atmospheric transport and chemistry sources. *Atmospheric Chemistry and Physics, Vol. 10, 9965-9980.*

Stock, N., Lau, F., Ellis, D., Martin, J., Muir, D. and Mabury, S. (2004). Polyfluorinated telomer alcohols and sulfonamides in the North American troposphere. *Environmental Science & Technology, Vol. 38, n. 4, 991-996.*

Stockholm Convention (2009). *Governments unite to step-up reduction on global DDT reliance and add nine new chemicals under international treaty.* [online] Available at: <http://chm.pops.int/Convention/Pressrelease/COP4Geneva8May2009/tabid/542/language/en-US/Default.aspx> [Accessed 25th of March 2014]

Swedish Environmental Protection Agency (2008). Förslag till gränsvärden för särskilda förorenande ämnen. [online] Available at: <http://www.naturvardsverket.se/Documents/publikationer/620-5799-2.pdf> [Accessed 26th of June 2014]

Tan, B., Wang, T., Wang, P., Luo, W., Lu, Y., Romesh, K. and Giesy, J. (2014). Perfluoroalkyl substances in soils around the Nepali Koshi River: levels, distribution, and mass balance. *Environmental Science and Pollution Research. [Epub ahead of print]*.

Taniyasu, S., Kannan, K., So, M., Gulkowska, A., Sinclair, E., Okazawa, T. and Yamashita, N. (2005). Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota. *Journal of Chromatography A, Vol. 1093, 89-97.*

Ulhaq, M., Carlsson, G., Örn, S. and Norrgren, L. (2013). Comparison of developmental toxicity of seven perfluoroalkyl acids to zebrafish embryos. *Environmental Toxicology and Pharmacology, Vol. 36, 423-426.*

US EPA (2000). *EPA and 3M announce phase out of PFOS.* [online] Available at: <http://yosemite.epa.gov/opa/admpress.nsf/d0cf6618525a9efb85257359003fb69d/33aa946e6cb11f35852568e1005246b4!OpenDocument&Highlight=2,risk> [Accessed 19th of March 2014].

US EPA (2002). *Perfluoroalkyl Sulfonates; Significant New Use Rule.* [online] Available at: <http://www.fluoridealert.org/wp-content/pesticides/pfos.epa.final.rule.dec.2002.pdf> [Accessed 19th of March 2014]

US EPA (2007). *Perfluoroalkyl Sulfonates; Significant New Use Rule.* [online] Available at: <http://www.epa.gov/fedrgstr/EPA-TOX/2007/October/Day-09/t19828.htm > [Accessed 19th of March 2014]

US EPA (2014). Emerging Contaminants - Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFOA). [online] Available at: <http://www2.epa.gov/sites/production/files/2014-04/documents/factsheet_contaminant_pfos_pfoa_march2014.pdf> [Accessed 26th of June 2014]

Wang, M., Chen, J., Lin, K., Chen, Y., Hu, W., Tanguay, R., Huang, C. and Dong, Q. (2011). Chronic zebrafish PFOS exposure alters sex ratio and maternal related effects in F1 offspring. *Environmental Toxicology and Chemistry, Vol. 30, n. 9, 2073-2080.*

Wedekind, C., von Siebenthal, B. and Gingold, R. (2007). The weaker points of fish acute toxicity tests and how tests on embryos can solve some issues. *Environmental Pollution, Vol. 148, 385-389.*

Yamashita, N., Kannan, K., Taniyasu, S., Horii, Y., Petrick, G. and Gamo, T. (2005). A global survey of perfluorinated acids in oceans. *Marine Pollution Bulletin, Vol. 51, 658-668.*

Young, C., Furdui, V., Franklin, J., Koerner, R., Muir, D. and Mabury, S. (2007). Perfluorinated acids in Arctic snow: new evidence for atmospheric formation. *Environmental Science & Technology, Vol. 41, n. 10, 3455-3461.*

Yue, R. (2008). *Additional information on production and use of PFOS.* [online] Available at: <http://chm.pops.int/Portals/0/Repository/addinfo_2008/UNEP-POPS-POPRC-SUB-F08-PFOS-ADIN-CHI.English.pdf> [Accessed 8th of April 2014]

Appendix

Table A1. Descriptions of the locations for sampling of snow on the 3rd of March. at the start of Vasaloppet

Sample	Latitude	Longitude	Description
A1	N61°6′37″	E13°17′36″	Top 3-5 cm of a track in front of starting area
A2	N61°6′37″	E13°17′36″	Top 3-5 cm of a track adjacent to the above
A3	N61°6′37″	E13°17′36″	Top 3-5 cm of a track adjacent to the above
A4	N61°7′4″	E13°17′25″	Top 3-5 cm of a track next to lake were there are fewer tracks
A5-1	N61°7′4″	E13°17′25″	Top 3-5 cm of a track adjacent to the above
A5-2	N61°7′4″	E13°17′25″	The layer below 5-1 down to 8-10 cm under original surface
A5-3	N61°7′4″	E13°17′25″	The layer below 5-2 stretching down another 5 cm to 13-15 cm
A6	N61°7′4″	E13°17′25″	Top 3-5 cm of track adjacent to 5-1
A7	N61°7′15″	E13°17′37″	Top 3-5 cm of snow cover about halfway up the first climb
A8	N61°7′15″	E13°17′37″	Top 3-5 cm of snow cover closely above 7
A9	N61°7′15″	E13°17′37″	Top 3-5 cm of snow cover closely above 8
A10	N61°6′57″	E13°17′4″	Top 5-10 cm of untouched snow next to reference lake
A11	N61°6′57″	E13°17′4″	Top 5-10 cm of untouched snow close to 10
A12	N61°6′57″	E13°17′4″	Top 5-10 cm of untouched snow close to 11

Table A2. Descriptions of the locations for sampling of surface water on the 29th of April at the start of Vasaloppet

Sample	Latitude	Longitude	Description
B1	N61°6′57″	E13°17′5″	About three meters out from the western shore close to the southern end of the reference lake
B2	N61°7′0″	E13°17′1″	About three meters out from the western shore at the middle of the lake of the reference lake
B3	N61°7′3″	E13°16′59″	About three meters out from the western shore at the northern end of the lake of the reference lake
B4	N61°6′53″	E13°17′30″	About three meters out from the western shore at the northern end of three lake of the contaminated lake
B5	N61°6′59″	E13°17′26″	About three meters out from the western shore at the northern end of the lake of the contaminated lake
B6	N61°7′5″	E13°17′25″	About three meters out from the western shore at the northern end of the lake of the contaminated lake
B7	N61°7′8″	E13°17′25″	From the middle of the water pool in the depression
B8	N61°5′24″	E13°18′53″	From the river about two meters out from the western shore just north of the bridge south of the starting area
B9	N61°6′34″	E13°17′35″	From the river about two meters out from the eastern shore next to the starting area
B10	N61°8′54″	E13°16′20″	From the river about two meters out from the eastern shore just south of the bridge north of the starting area

Table A3. Descriptions of the locations for sampling of soil and sediment on the 29th of April at the start of Vasaloppet

Sample	Latitude	Longitude	Moisture (%)	OM (%)	Description
C1	N61°6′58″	E13°17′5″	42	18	Top 3 cm of sediment from about three meters out from the western shore close to the southern end of the reference lake
C2	N61°6′58″	E13°17′5″	25	5	Close to C1
C3	N61°7′0″	E13°17′2″	50	11	Top 3 cm of sediment from about three meters out from the western shore at the middle of the lake of the reference lake
C4	N61°7′0″	E13°17′2″	67	22	Close to C3
C5	N61°7′0″	E13°17′2″	34	14	The 3 cm thick layer under C4
C6	N61°7′0″	E13°17′2″	65	20	The 3 cm thick layer under C5
C7	N61°7′3″	E13°17′0″	69	23	Top 3 cm of sediment from about three meters out from the western shore at the northern end of the lake of the reference lake
C8	N61°7′3″	E13°17′0″	25	22	Close to C7
C9	N61°7′5″	E13°17′26″	29	24	Top 3 cm of sediment from about three meters out from the western shore at the northern end of three lake of the contaminated lake
C10	N61°7′8″	E13°17′25″	38	1	Top 3 cm of sediment from the middle of a depression in the filed north of the potentially contaminated lake
D1	N61°6′37″	E13°17′36″	82	28	Top 3 cm of soil from just in front of the starting area
D2	N61°7′15″	E13°17′37″	79	32	Top 3 cm of soil about halfway up the first climb
D3	N61°7′16″	E13°17′35″	75	22	Top 3 cm of soil out in the forest next to D2

Table A4. The pairs of sediment and water samples for which K_d and K_{oc} were calculated

Sediment sample	Water sample
C10	B7
C9	Average of B4, B5 and B6
Average of C7 and C8	B3
Average of C3 and C4	B2
Average of C1 and C2	B1

Table A5. Which endpoints showing in which group at respective screening time for the test of water samples B1, B6 and B7

Time/ sample	Coag.	Move.	Circ.	Blood pool	Edema	Sidelaying	Unhatched	Dead	Crooked spine
24h B1	0	0							
24h B6	0	3							
24h B7	1	1							
24h TW	1	1							
48h B1				1					
48h B6				2	2				
48h B7	1		1	1					
48h TW	1				1				
144h B1								1	
144h B6					1			1	
144h B7	1				1				1
144h TW	2					2	6		