



Hälsoeffekter av högfluorerade ämnen (PFAS)

En litteraturbaserad riskbedömning för delpopulationer i Uppsala.

The health effects of per- and polyfluoroalkyl substances (PFAS)

A literature-based risk assessment for subpopulations in Uppsala.

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ABSTRACT

Per- and polyfluoroalkyl substances (PFAS) have been widely used in industry since the mid 20th century. They are not degradable in a natural environment and old emissions can make their way into living organisms to bioaccumulate. The Uppsala-Ärna airport used firefighting foam containing PFAS for several years and, as a consequence, PFAS was found to contaminate groundwater wells in Uppsala. This study compiled serum concentrations of three types of PFAS; PFOS, PFOA and PFHxS, from previous studies of three subpopulations in Uppsala in order to make a risk assessment. As a basis for the risk assessment, a literature review was made of the health effects due to PFAS exposure and the causes for the PFAS contamination in Uppsala. The PFAS concentrations found in the subpopulations were plotted over time. While the concentrations of PFOS and PFOA had decreased, PFHxS concentrations had increased prior to the discovery of the water contamination. Further, the concentrations considered to cause increased risks of negative health effects were compared to the concentrations measured in Uppsala. No increased risk could be determined when observing individual PFAS, although all concentrations, especially that of PFHxS, were higher than the European average. However, when combining the effects of PFOS and PFOA, an increased risk of high total cholesterol levels among senior citizens was found. Further effects of the PFAS contamination in Uppsala may be uncovered with new findings and deepened understanding of the subject.

Keywords: PFAS, risk assessment, Uppsala

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INTRODUCTION

The use of per- and polyfluoroalkyl substances (PFAS) is widespread in industry due to their ability to form smooth surfaces that are capable of repelling both water and fat, as well as being able to withstand high temperatures. PFAS can be found in anything from textiles, paper and cleaning detergents to firefighting foam (1). As these substances are very stable and not easily degradable, they remain in the environment long after the emissions occurred. Due to this long half-life, they may reach and contaminate ground water resources several years after their release into the environment. The risks of PFAS-exposure has become a subject of great interest as high levels of contamination are revealed across the globe due to previous and current emissions (2). In Uppsala, several ground water wells were found to be contaminated with PFAS in 2012. The affected wells were shut down for a period of investigation after the discovery, and have since reopening had the water purified with active coal before distribution (3).

Perfluoroalkyl acids (PFAA) is the subgroup of PFAS most frequently detected in organisms and the substances most commonly found in human serum include perfluorooctane sulfonic acid (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonic acid (PFHxS). Although they have been detected in human populations all over the world, the potential health effects related to human exposure remain poorly understood. To aid in this direction, in 2018 the European Food Safety Authority (EFSA) released a report where the health risks of PFOS and PFOA were investigated. The report went on to calculate benchmark doses for the health effects considered statistically significant, based on results from the included studies (4). The same year the Agency of Toxic Substances and Disease Registry (ATSDR) released a toxicological profile for perfluoroalkyls, evaluating the possible health effects of perfluoroalkyl acids (PFAA) (5). In Uppsala, several studies investigating the serum concentrations of PFAS for different subpopulations have been made since the 1990s, allowing for observations over longer periods of time and patterns to be discovered. By comparing the serum levels of PFAS found in Uppsala with the current knowledge of its health effects, it is possible to make a risk assessment for some of the subpopulations in Uppsala.

Purpose

The purpose of this literature-based study is to investigate the potential risks to the Uppsala population due to the local contamination and subsequent exposure of PFAS, focusing on the three most common compounds in human serum: PFOS, PFOA and PFHxS.

In order to do so, this essay aims to:

- Compile the existing literature on the serum levels in the different subpopulations of Uppsala and to compare it to the Swedish national levels caused by background exposure alone.
- Examine what the potential health effects of the measured serum levels are in Uppsala, based on the EFSA-report, the toxicological profile of the ATSDR, as well as other relevant literature.

MATERIAL AND METHOD

In order to make a risk assessment of the PFAS-exposure in Uppsala, a literature review on the health effects of PFAS, the general pathways of exposure and the existing data on local exposure in Uppsala was put together. Information about the main local contamination source and the geographical spread of PFAS was also included. The review is presented in the section “Literature review” and further results and discussion rely heavily on its conclusions.

Method of literature search

All article searches for this essay were made during the period February-March 2020. The articles used as sources for the serum levels in Uppsala were found using the databases PubMed, DiVa and Google Scholar. The search words included “Uppsala” in varying combinations with” PFAS”, “serum”, “concentration”, “health effects”, “PFOS”, “PFOA” and “PFHxS”. References cited in the articles found were also used to find further studies applicable to the subject.

Contaminated water is generally recognised as the cause of the elevated serum levels of PFAS in Uppsala compared to national levels. To find articles on the causes of contamination presented in the literature review, the same search engines and key words were used as for finding the serum levels of the Uppsala population, with the addition of the search word “contaminated water” and the exclusion of the word “health effects”.

The EFSA-study ”Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food”(4), as well as the ATSDR toxicological profile for perfluoroalkyls (5), were used for the major portion of information regarding the health effects of PFAS in the literature review. These reports were chosen as they were both released fairly recently (2018) and both review most of the available literature in the field. Because of this they are able to compare available results, making their conclusions more reliable, as well summarizing the information available in the field. The EFSA-study was the source of the benchmark doses for the effects of PFOS and PFOA, while the ATSDR-report was used for reference values in

the risk assessment of PFHxS. As the Swedish national levels of exposure are on par with the European averages (6), this data was also taken from the EFSA-report.

In order to attain an extensive overview of the PFAS exposure in Uppsala, all Uppsala based studies found from 1990 and onwards were included in the collection of data. A flow chart of the data selection is presented in figure 1. Out of the 200 articles resulting from use of the search words and the databases listed above, the abstracts of 12 articles were screened based on their titles suggesting relevance to this study. One of these 12 articles was excluded as it was a duplicate of another screened study. The remaining 11 articles were read in full to determine the methods used and how the participants in the studies were chosen. In the case of overlapping data, the smaller studies were excluded from further processing in favour of the study with the largest number of participants. Thus, overlapping data would not affect the final results. For studies using the same data, the most recent article was assumed to contain the most updated, and hence the most relevant results, and was therefore used while the older articles were excluded. The resulting articles were evaluated using eight quality parameters in order to ensure sufficient quality before data extraction (see appendix).

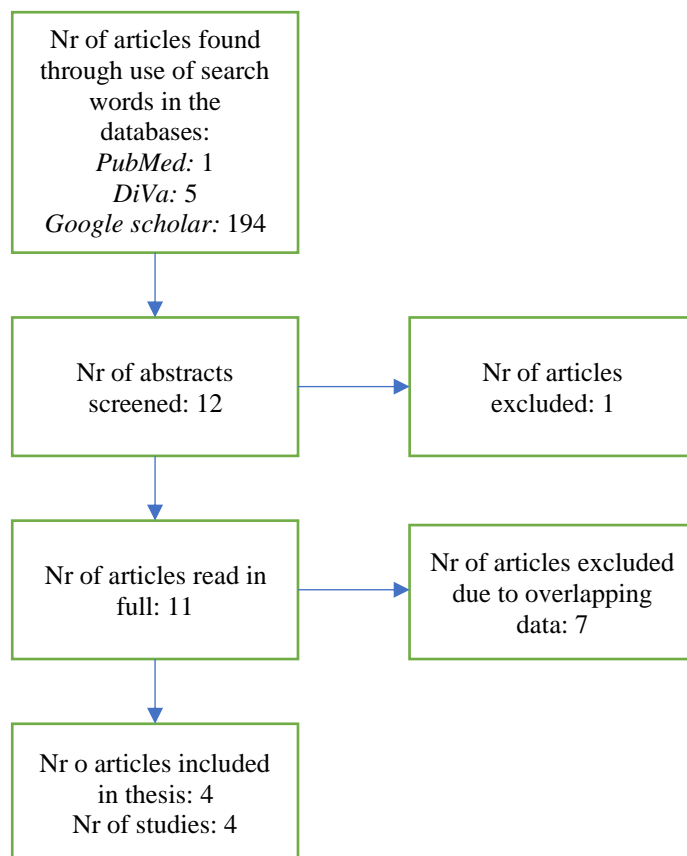


Figure 1: Flow chart of the data selection

Data extraction from literature for risk assessment

Mean values of the serum concentrations in Uppsala, either taken directly from the results of the included studies or calculated from the presented data, were used for the risk assessment. Four local studies, measuring the concentration of PFAS in serum, were used to investigate the PFAS exposure in Uppsala. These were the PIVUS- (7) and POPUP-study (8), a study by Gyllenhammar et al. (9) and a study by Glynn et al. (10). The four articles used for data extraction from these studies are listed in table 1.

The PFAS concentrations investigated were PFOS (L-PFOS), PFOA and PFHxS. All available data from the included studies was plotted to give an overview of the pattern of exposure over time. For comparison with national levels, comparison between the subpopulations studied and risk assessment, mean values from the year 2009 was used. This year was chosen for the data collection, as the most data was available during this time period and since it predates the discovery of the water contamination in 2012. The

concentrations of PFOS, PFOA and PFHxS were also relatively high that year, making the risk assessment more meaningful.

Table 1: The studies included for data extraction of PFAS serum concentrations in the Uppsala population. While the article by Glynn et al. (10) accounts for a smaller study, the articles by Stubleski et al. (7) and Gyllenhammar et al. (2017) (8) are based on larger studies about which several articles have been written. The article by Gyllenhammar et al. (2016) (9) is a continuation of the POPUP study where the children of the mothers that had been part of POPUP were examined.

Title	Author	Study	Year of publishing
Perfluorerade organiska ämnen i blod under graviditet och amning	<i>Glynn et al.</i>	-	2009
Temporal trends of poly- and perfluoroalkyl substances (PFASs) in serum from children at 4, 8, and 12 years of age, in Uppsala 2008-2015	<i>Gyllenhammar et al.</i>	Children from POPUP	2016
Changes in serum levels of perfluoroalkyl substances during a 10-year follow-up period in a large population-based cohort	<i>Stubleski et al.</i>	PIVUS	2016
Temporal trends of poly- and perfluoroalkyl substances (PFASs) in pooled serum samples from first-time mothers in Uppsala 1997-2016	<i>Gyllenhammar et al.</i>	POPUP	2017

The PIVUS-study (7) sampled plasma in the general population of Uppsala at ages 70, 75 and 80 between 2001 and 2009. In the study, mean values for three time periods, of three years each, was presented. Therefore, the values used in the risk assessment are in fact mean values for the time period 2006-2009 when samples were taken from senior citizens aged 75 years. In the POPUP-study, samples were taken from first time mothers three weeks after delivery. Gyllenhammar et al. (2017) (8) prepared three pooled serum samples for almost every year between 1996-2009, containing serum from 10 participants each. The mean of the pooled serum samples of 2009 was used for the risk assessment. The study by Gyllenhammar et al. (2016) (9) sampled children aged 4, 8 and 12 years between 2008 and 2012. The mean values of the serum concentrations in the different ages for this period were assumed to be representative for 2009 and were

hence used for the risk assessment. The study by Glynn et al. (10) had no data from this year and was hence excluded from the risk assessment.

Reference values and methods for risk assessment

The benchmark dose (BMD) is defined as the “dose or concentration that produces a predetermined change in the response rate of an adverse effect”, the change being known as the benchmark response (BMR). The BMR is usually a 5% or 10% increase in the frequency of the health effect in question, as compared to a control group. The lower confidence limit of the benchmark dose (BMDL) is often used to calculate human health guidance values, it corresponds to a dose that causes an effect smaller than the BMR and is therefore more conservative. The BMDL is commonly used to estimate guidance values for oral or dermal exposure (11). The BMD and BMDL were presented for PFOS and PFOA in the EFSA report and used for comparison in the results of this thesis. The time weighted average (TWA) is used in the report by the ATSDR and is defined as the average exposure over time (12), in this case of PFHxS. In lack of BMD and BMDL, the TWA was used for comparison in the results of this thesis.

Risk characterization ratio (RCR) is a method that can be used for risk assessment. It is calculated by dividing the actual exposure with tolerable exposure levels. An RCR value over one indicates that the exposure is higher than the tolerable level, and hence that there is a potential risk (13). The combined effect of several substances on a specific endpoint can be calculated by adding up the RCRs for that endpoint. If the sum is higher than one, an increased risk is indicated (14). To examine the combined effect for shared endpoints, RCRs were calculated for PFOS and PFOA using the BMDL values as tolerable exposure levels. The RCRs were then added to give a total RCR for each endpoint.

Ethical considerations

Since this study is based on already published material where personal data is not included, there is no need for further anonymization of data. The fact that the PFAS-exposure in Uppsala is a sensitive subject to those affected by the contaminated drinking water, could be an ethical issue in this study. Results indicating an increased risk of disease could cause distress for some of these individuals if the information reached them. However, all the information in this study comes from already published sources, a review of this information should therefore not worsen the situation.

LITERATURE REVIEW

Structure and characteristics of PFAS

There is a vast amount of different substances classified as per- and polyfluoroalkyl substances (PFAS). The most common subgroup of PFAS detected in living organisms is perfluoroalkyl acids (PFAA). These compounds are constituted by a hydrophobic fluorinated alkylic chain, of varying length, ending with a hydrophilic functional group (2). PFAA can in turn be divided in perfluoroalkyl sulfonic acids (PFSA) as well as perfluoroalkyl carboxylic acids (PFCA) depending on the hydrophilic group (1). In table 1 some of the most common PFAAs are listed according to sub-group and carbon chain length.

Table 2: Abbreviations and carbon length of some perfluoroalkyl acids. All data in the table is retrieved from the article by Gyllenhammar et al. (9).

Substance	Abbreviation	Number of carbons in fluorinated chain
Perfluoroalkyl sulfonic acids (PFSA)		
Perfluorobutane sulfonic acid	PFBS	4
Perfluorohexane sulfonic acid	PFHxS	6
Perfluorooctane sulfonic acid	PFOS	8
Perfluoroalkyl carboxylic acids (PFCA)		
Perfluorohexanoic acid	PFHxA	5
Perfluoroheptanoic acid	PFHpA	6
Perfluorooctanoic acid	PFOA	7
Perfluorononanoic acid	PFNA	8
Perfluorodecanoic acid	PFDA	9
Perfluoroundecanoic acid	PFUnDA	10
Perfluorododecanoic acid	PFDoDA	11
Perfluorotridecanoic acid	PFTriDA	12
Perfluorotetradecanoic acid	PFTeDA	13
Perfluoropentadecanoic acid	PFPeDA	14

PFAS, being extremely stable compounds as a result of their strong carbon-fluoride bonds, are very resistant to degradation by thermal, chemical and biological means (15). This resistance causes them to be long lived in the environment. PFAA are regarded as stable end products and do not degrade further in natural conditions. The degradation of polyfluorinated precursors will also generate PFAA (16). The stability of PFAA renders them bioaccumulative in the environment, with a bioconcentration factor (BCF) proportional to the length of the carbon chain. They also have a tendency for biomagnification going up the food chain (2). The three PFAA compounds found in the highest concentrations in human serum are typically PFOS, PFOA and PFHxS (4). For the reasons listed above, the usage of PFOS and its precursors has been banned within the EU since 2008, PFOA has also been banned from 2020 onwards (1).

PFAS in humans

The serum levels of PFAS are determined by how much is absorbed, how much is excreted and what happens to the compounds in between. This is covered in the following section. As PFAA is the subgroup of PFAS mainly found in living organisms, this will be the main focus of this thesis.

Absorption

PFAA can be absorbed through oral as well as respiratory and dermal exposure. The oral fractional absorption, as measured in rodents, can vary between ~50% for PFHxS, to >95% for PFOA, PFNA, PFBA, PFDeA, PFUA and PFDoA. The mechanisms for absorption have, however, not been fully explained as of yet. The chronic exposure of PFOS and PFOA is largely made up of contaminated food and drinking water among the European and North American population (5). Contaminated drinking water, followed by food and dust, is currently the greatest source of PFHxS-exposure in the Swedish population (3). Suggestions have been made that food intake is the major source of exposure for long chain PFAA (>7 C), while water contamination is the major source of short chain PFAA (<7 C) (17).

Distribution

The highest levels of PFAA can be found in the liver, although there is significant distribution throughout the whole body (5). Other areas of high concentrations are the kidneys and the blood stream. PFAA binds to several proteins in the blood, albumin being one of them. PFOS has a higher affinity than PFOA regarding the binding of human serum albumin, contributing to its longer half-life in humans. The mechanisms for how PFAA passes from the blood to the liver are not yet fully clarified but may involve organic anion transporters (5). Regarding the transport of PFAA into the kidney cells, some is known about PFOA. The substance seems to be transported into the tubular epithelial cells through organic anion transporters and, once through, binds to intracellular proteins. Furthermore, PFAA can pass through the placenta to the foetus in pregnant women. There is also evidence of it passing to the child through intake of breastmilk during nursing (5).

Metabolism

To our current knowledge, PFAA is not metabolized nor does it undergo chemical reactions in the human body (5).

Elimination

Elimination of PFAA occurs primarily through the passing of urine, and to a lesser extent, faeces. There is substantial biliary excretion, but due to reabsorption not much is actually eliminated that way. In fertile women, some is also eliminated through menstruation blood and breastmilk. The specific exposure route (e.g. oral, intraperitoneal, intravenous) is not likely to affect the elimination rate of PFAA substantially.

The elimination rate of PFAA varies greatly depending on the chemical species properties. In humans, the half-life of PFBA is 72-81h, while the half-lives of PFOS, PFOA and PFHxS are 3.1-7.4, 2.1-8.5 and 4.7-15.5 years respectively. More generally, the perfluoroalkyl sulfonates have a longer half-life than the carboxylates. Longer carbon-chains, as well as increased branching, are also factors related to slower elimination rates (5).

Health effects of PFAA

The following effects are based on the main conclusions from the EFSA- and ATSDR-reports.

Fertility and pregnancy

One of the outcomes of PFOS- and PFOA- exposure considered statistically significant by EFSA was the causality with reduced birth weight. The findings were relatively consistent, but the glomerular filtration rate (GFR) may be a confounding factor (5) . A causal relationship has been indicated between a decreased GFR and a reduction in birth weight (18). The clinical relevance also comes into questions as, despite indications of reduced birth weight, no connection to low birth weight (<2500g) was observed. The mechanisms behind the reduction in birth weight may involve an inverse relation between insulin-like growth factor 1 (IGF-1) and PFOS levels, as observed in studies on humans, causing a reduction in growth rate. In rodents, reduced body weight and an upregulation of uncoupling protein 1 (UCP-1) was observed. UCP-1 has been “associated with energy expenditure and regulation of food consumption”, possibly explaining the reduction in body weight (4). No associations have been found between low birth weight and PFHxS-exposure (5).

According to the EFSA, there is insufficient evidence for intrauterine PFOS/PFOA-exposure causing an increase in the prevalence of miscarriage, stillbirths, birth defects or infertility (4). The epidemiological studies included in the ATSDR-report suggest impaired fertility as an effect of PFOS/PFOA- exposure. Impaired fertility is in this case defined as a longer period of time before pregnancy or infertility. There were similar findings for PFHxS, although the results were inconsistent between different studies. PFHxS did not seem to be associated with an increased risk for miscarriage (19) or preterm birth (20). A correlation between PFOS, PFOA, PFHxS and sperm quality was observed epidemiologically, although the severity of the alterations in the sperm was hard to assess (5).

EFSA determined that there was not sufficient evidence of an increased risk of pregnancy hypertension as a result of PFOS/PFOA-exposure (4). The ATSDR, on the other hand, holds forth that “the strongest methodological study” of those examining highly PFOA-exposed communities, showed an increased risk of pre-eclampsia. There are, however, mixed results looking at other studies. Two epidemiological community studies pointed out PFOS as a contributor to pre-eclampsia, a disorder occurring in pregnancy involving high blood pressure and protein in the urine (21). PFHxS did not seem to affect the risk of pre-eclampsia according to general population studies (5).

Development

Through epidemiological studies of intrauterine exposure of PFOS and PFOA, there is insufficient support to establish causality with changes in neurodevelopment, growth in infancy and childhood, puberty onset or semen quality (4). Neither has it been possible to link intrauterine exposure of PFHxS to changes in body weight of children at 20 months post-partum, the body mass index (BMI) of 2-8 year olds or an increase in the risk of childhood obesity/overweight (5).

Animal studies on rodents indicate delays the mammary gland development due to intrauterine exposure of PFOS and PFOA (4). Exposure to PFHxS did not affect food consumption nor body weight (5).

Neurotoxicity

Epidemiologically, there is insufficient support of a causality between PFOS- or PFOA exposure and cognitive, neurobehavioural or neuropsychiatric effects in both children and adults (4). ATSDR found that several studies showed a connection between a decreased risk of attention deficit hyperactivity disorder (ADHD) and exposure to PFOS, PFOA and PFHxS. Two studies also found a decrease in the risk of memory loss after exposure to the same (5).

In animal studies, widespread effects on gene expression, affecting the production of proteins involved in brain signal transmission, were observed due to PFOS and PFOA exposure. The most common effect in behaviour linked to PFOS-exposure was a

decrease in spontaneous activity, while PFOA seemed to cause an increase in the spontaneous activity (4). No morphological alterations were observed due to PFOS or PFOA, and no changes in neurological functions were found due to PFHxS. Conflicting with the results of the two epidemiological studies, one study found that PFOS seemed to cause impaired learning and memory in animals (5).

Immunology

The immunological connection to PFOS-exposure, specifically on serum antibody response after vaccination, was found to be statistically significant by EFSA. Less conclusive, but similar effects were seen for PFOA. The most vulnerable subgroup to the immunological effect is children, an increased risk of infection also being observed as an effect of intrauterine PFOS/PFOA-exposure in epidemiological studies (4). The ATSDR agrees with the connection between PFOS/PFOA and decreased antibody response following vaccination, adding that PFHxS may have a similar effect. However, they deemed the evidence for a decreased resistance to disease due to PFOS-, PFOA- or PFHxS-exposure insufficient (5).

Animal studies support the notion of the immune system being a sensitive target for PFOS and PFOA, while the only available study for PFHxS on the subject did not find such results (5).

A hypothesis of the mechanism underlying this effect, is that PFOS and PFOA interact with peroxisome proliferator-activated receptor (PPARs) in the cells of the immune system, modulating gene regulation as well as affecting apoptosis and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) transcription. Lymphoid cells exposed to PFOS or PFOA display differences in cytokine profiles, indicating slight differences in the underlying mechanisms for the two chemical species (4).

Further, ATSDR connects PFOA-exposure to an increased risk of asthma (5). The evidence for such a link is, however, deemed as insufficient by EFSA (4). The evidence for PFOS and PFHxS having the same effect is too conflicting, or comprised of too few studies, for conclusions to be made according by the ATSDR (5). The EFSA also deems

the evidence for an increased risk of allergies due to PFOS- or PFOA-exposure, insufficient (4). PFHxS has been suggested to increase the risk of food allergies (22), as well as sensitivity to allergens, such as plants (23), dust mites and pets, in adolescents (5).

Endocrine effects

EFSA deemed that there was insufficient support for a connection between changes in the menstrual cycle, endometriosis, the timing of puberty or menopause, semen quality, sex hormones or thyroid function, and exposure to PFOS and PFOA. Neither was the milk production of breastfeeding significantly affected (4). The ATSDR agreed with EFSA, stating that the available studies on reproductive hormones and PFOS/PFOA were too few and inconsistent for any conclusions to be drawn. No connection between PFHxS and reproductive hormones has been observed (5). Further, they pointed out that there are indications of early onset menopause being triggered by PFOS-, PFOA- and PFHxS-exposure. The connection might, however, be due to reverse causation as the lack of regular menstruation may lead to less PFAA to be eliminated, thus increasing serum levels (5).

The ATSDR also noted that multiple epidemiological studies on both exposed communities as well as general populations, indicate a connection between PFOA in serum and an increased incidence of thyroid disease (5). Such a connection was however more elusive with regard to PFOS. However, as there is conflicting evidence regarding the effect of PFOS and PFOA on thyroid hormones, no conclusions can be drawn as of now. For PFHxS there are very few studies available, and no consistent findings on its effect on thyroid hormone levels (5).

In animal studies, alterations of thyroid hormone levels due to PFOS- or PFOA-exposure have been observed. For PFHxS, a connection to histopathological alterations in the thymus has been observed, most likely a secondary effect caused by hepatocellular hypertrophy (5).

Metabolism

EFSA concludes that there is strong support for a causality between PFOS- or PFOA-exposure and increased levels of cholesterol in serum, and the ATSDR shares this opinion(4)(5). There were not sufficient evidence for PFHxS having an effect on serum lipid levels (5). EFSA also found causality for PFOA-exposure and increased serum levels of the liver enzyme alanine transferase (ALT) (4). The ATSDR, on the other hand, considers there to be sufficient evidence for causality between alterations in ALT-levels and PFOS, PFOA and PFHxS in serum (5).

EFSA considers the evidence for a connection between PFOA-exposure and liver disease, insufficient (4). ATSDR found that increased serum levels of liver enzymes and decreased levels of bilirubin might indicate liver damage as a consequence of exposure to PFOS, PFOA and PFHxS. However, they could not find an increased risk of liver disease connected to PFOS- or PFOA- exposure, looking at epidemiological studies (5).

Neither obesity and metabolic syndrome were sufficiently proven as causal to exposure of PFOS or PFOA (4). There was insufficient support for the connection between PFOS or PFOA and ulcerative colitis (5). EFSA found that there was not sufficient evidence of an increased risk of diabetes due to exposure of PFOS or PFOA (4). The ATSDR adds that PFHxS-exposure does not seem to increase the risk of diabetes either (5).

Animal studies strongly suggest that the liver is very sensitive to exposure of PFOS, PFOA and PFHxS. Both in rodent and monkey studies, a strong connection has been made between increased liver weight and administration of PFOS and PFOA. Repeated dose administration also induced peroxisomal β -oxidation in rodents (4). Histological signs of liver damage could be observed in rodents. Gastrointestinally, however, no histological alterations were found after exposure to PFOS, PFOA or PFHxS. Some indications of gastrointestinal irritation were observed after gavage administration of PFOA (5).

The mechanisms behind the observed increase in serum alkaline phosphatase (ALP)-levels, due to PFOS- or PFOA-exposure in humans, are presently unclear. The liver

toxicity observed in rodents is regarded as partly dependent on PFOS and PFOA acting as ligands to peroxisome proliferator-activated receptor alpha (PPAR α) in the cell nucleus (5)(4). Experiments on PPAR- α -null mice suggest that other mechanisms are also involved (5). However, the effects on lipid metabolism are very different between rodents and humans, the cholesterol levels in rodents decreasing contrary to the effect in humans. Altogether there is no evidence that the findings in rodents have any human relevance, PPAR α likely having a different function in human lipid metabolism (4).

Kidney

A few studies have connected PFOA to kidney disease, but the findings are inconsistent. There are links between PFOS- and PFAS-exposure and decreased GFR and increased levels of uric acid in serum. This may however be due to inverse causality, as a decreased renal function inhibits the elimination of PFAA (5). The EFSA-report agrees with these conclusions (4). The studies on the effect of PFHxS on renal function are too few and inconsistent to draw any conclusions (5).

Carcinogenicity

According to EFSA, there is insufficient support through epidemiological studies for causality between exposure of PFOS/PFOA and carcinogenicity in humans. This goes for both for occupational exposure and general exposure (4). ATSDR references epidemiological studies of highly exposed groups, both occupational and community exposure, associating PFOA-exposure with an increased risk for kidney- and testicular cancer (5). No other consistent evidence has been found for other types of cancer being linked to PFOA. PFOS was connected to bladder cancer in one epidemiological study, but the findings have not been supported by later studies. There is, so far, no evidence for an increased breast cancer incidence due to PFHxS-exposure. Neither did PFHxS increase the risk for prostate cancer, unless the participants already had strong heredity for the condition (5).

In animal studies, no “direct genotoxic mode of action” was observed. Some evidence of PFOS and PFOA inducing oxidative stress and liver tumours has been observed in rodents. This, as well as mechanistic studies, implies that PFOS can act as a tumour

promoter (4). In one study on rats, PFOA was observed to cause Leydig cell tumours, possibly caused by the hormonal imbalances induced by the exposure. The findings were, however, considered inconsistent with regard to hyperplasia in the pancreas and tumours in the mammary gland and liver (4).

Mechanistically, PFOS and PFOA are tumour promoters in rodent liver, acting through PPAR α and mediating carcinogenic activity in its ligands. However, this does not seem to be the case in humans. The Leydig cell carcinomas in rats seem to be caused by a reduction in serum testosterone levels, triggering a release of luteotrophic hormone that induces growth and tumour development in the testis (4). This type of tumour does, however, rarely occur in humans (1-4% of all testicular tumours in humans) (24). Neither does the hyperplasia of the pancreas seem to be a concern in humans, making the mode of action in rodents inapplicable to humans and therefore irrelevant for this essay (4).

However, the current findings have made the US environmental Protection Agency (EPA) conclude that “there is suggestive evidence that PFOA and PFOS may cause cancer” in humans in 2016 (25)(5). Further, the International Agency for Research on Cancer (IARC) concluded that PFOA is possibly carcinogenic to humans (group 2B)” in 2017 (26)(5).

Cardiovascular effects

There is insufficient support for a causal relationship between PFOS/PFOA-exposure and an increased risk for cardiovascular disease (4). Neither did the ATSDR find cause to believe that it has a connection to hypertension (5). The limited epidemiological studies of PFHxS did not observe an increased risk of coronary heart disease (27).

Hematological

Some animal studies have observed haematological changes due to high levels of exposure of PFOS, PFOA and PFHxS. The effects included an increase in prothrombin time, decreased haemoglobin concentration and a decreased count of erythrocytes in male rats. Female rats were not significantly affected (5).

Musculoskeletal

While several epidemiological studies found a link between PFOA-exposure and osteoarthritis in women, especially in young participants (<55 years old), no connection was found for men (5). The results for PFOS were more inconsistent. Due to the lack of mechanistic data and understanding the ATSDR found it hard to draw conclusions on the matter (5). Furthermore, osteoarthritis is affected by several factors, some of which also affected by PFAA-exposure (e.g. high levels of uric acid in serum) (5). EFSA also concluded that there is insufficient support for the connection to rheumatoid arthritis, osteoarthritis or bone mineral density (4).

PFHxS was found to be associated with an increased risk of osteoarthritis in one general population study. An inverse relation between serum levels and osteoarthritis as well as total femur bone density in women was found epidemiologically. However, no connection to the mineral density of the femur neck nor the lumbar spine bone was observed (28).

Risk assessment EFSA – PFOS and PFOA

The health effects most strongly linked to PFOS and PFOA exposure, according to EFSA, were increased serum cholesterol (PFOA and PFOS) and decreased antibody response in children (PFOS). Outcomes less supported, but still considered significant, included ALT levels higher than the reference range (PFOA) and decreases in birth weight (PFOA and PFOS) (4).

As the toxicological mechanisms of PFOS and PFOA are poorly understood, EFSA refrained from calculating a health-based guidance value (HBGV). But based on a selection of the studies included in the report, benchmark doses for increased risk were calculated and presented (4). Summaries of the findings are presented in table 3 and 4.

Table 3: Summary of EFSA's BMD analysis (mean values) for PFOS [ng/ml]. BMD = Benchmark dose; BMDL₅ = benchmark dose for a 5% increase (based mainly on random samplings, the calculations are based on 1-3 studies per parameter. In the case of several values per parameter being available, a mean value is presented). Total cholesterol as calculated for 50-year olds. Birth weight calculated with the serum concentration of the mother.

Human response variable	BMD₅ (ng/mL)	BMDL₅ (ng/mL)
<i>Total cholesterol</i>	29.67	22.67
<i>Vaccination response for children</i>	11.6	10.5
<i>Birth weight</i>	36	21

*Table 4: Summary of EFSA's BMD analysis (mean values) for PFOA [ng/ml]. BMD: Benchmark dose; BMDL₅: benchmark dose for a 5% increase (based mainly on random samplings, the calculations are based on 1-3 studies per parameter. In the case of several values per parameter being available, a mean value is presented). Total cholesterol and alanine transferase calculated for 50-year olds. Birth weight calculated with the serum concentration of the mother. *BMD₃ and BMDL₃ is presented for alanine transferase.*

Human response variable	BMD₅ (ng/mL)	BMDL₅ (ng/mL)
<i>Total cholesterol</i>	12.2	9.3
<i>Alanine transferase*</i>	80	21
<i>Birth weight</i>	9.45	7.3

Risk assessment ATSDR – PFHxS

The ATSDR deemed one of the most sensitive endpoints for PFHxS to be developmental toxicity. A provisional intermediate-duration oral minimal-risk level (MRL) for the developmental toxicity of PFHxS was therefore calculated. The MRL was based on follicular damage in thyroid cells, an example of developmental toxicity, in male mice force-fed with PFHxS. The calculation of MRL was based on the time weighted average (TWA) serum concentration observed to give an effect in the mice, which was 73.22µg/ml (5). In lack of better estimates of a benchmark dose, this serum concentration will be used for further risk evaluations.

PFAS-contamination in Uppsala

The usage of firefighting foam containing PFAS was very common in Sweden between 1985 and 2003, for the purpose of extinguishing airplane-related fires. This has made the release of PFAS from airports into the environment a subject of much attention as the widespread pollution, particularly of groundwater wells, and its consequences have come into light (1). One such airport, run by the national defence, is located in Ärna close to Uppsala. (3)

A PFAS-contamination of groundwater wells in Uppsala was discovered in 2012, the previous usage of aqueous fire-fighting foam at Uppsala-Ärna airport likely being a major contamination source. A study by Gyllenhammar et al. (3) aimed to investigate the distribution of PFAA from the contamination source and its subsequent effect on serum levels of PFAA in humans, comparing the water levels of PFAA with serum samples from pregnant women in the time period 1996-2011. In a pilot study from 2012 made by A. Glynn (29), nine drinking water samples from taps at different locations in Uppsala were collected, and the levels of PFAA were analysed. The results showed contamination in some areas with PFHxS, PFOS, PFBS and PFOA in order of decreasing concentrations and Uppsala Vatten och Avfall AB quickly took measures to reduce human exposure (3).

In Uppsala, the public drinking water is extracted from a ground water aquifer flowing from north to south under the city. In total, there are five production well fields where extraction takes place, as well as a total of 29 production wells located within these fields. After extraction, the water is taken for calcium removal at one of the two central water treatment plants. Gyllenhammar et al. took samples from 21 of these production wells in the period July 2012 to February 2014 (3). The spreading of PFAS in the drinking water was then simulated based on the findings. Since the water distribution network changed on two occasions since 1996, a simulation was made for each scenario. The resulting time periods were then 1996-September 2004, October 2004-January 2007 and February 2007-July 2012. The study found that there was a large variation of the level of contamination in the aquifer, with the highest levels measured in the north-western part of Uppsala and decreasing levels further east and south (3). This indicates that the PFAA is dispersed and diluted as the contaminated water from

the aquifer closest to the source enters the larger aquifer below Uppsala. For visual representation of the spread in the area, the reader is referred to figure 2 of the study by Gyllenhammar et al. (3).

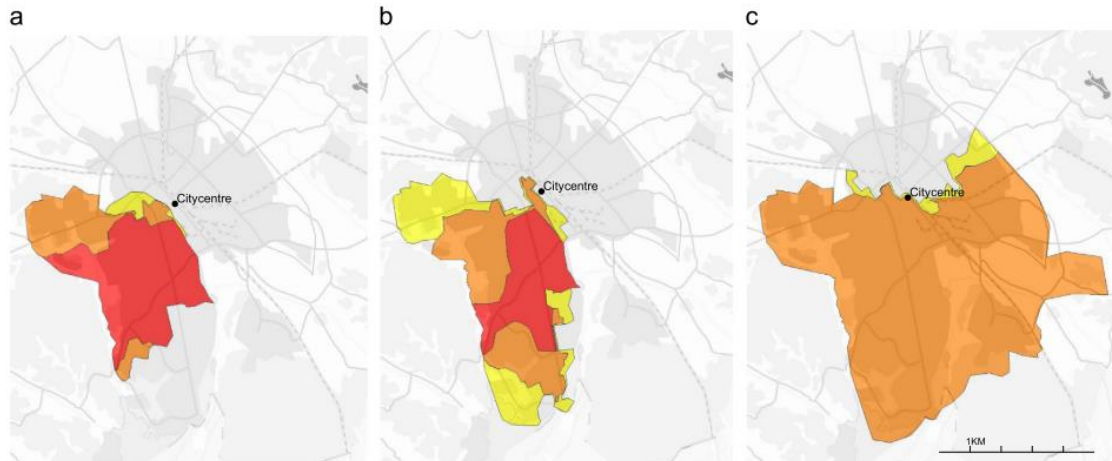


Figure 2: The spread of PFAA in the Uppsala area in a) 1996-2004, b) 2004-2007 and c) 2007-2012. No colour signifies no PFAA contamination of the drinking water, yellow colour signifies that the proportion of the water supply contaminated with PFAA was less than 10%, orange 10-89% and red $\geq 90\%$. The figure is taken from the study by Gyllenhammar et al. 2015 (3).

The most abundant PFAA were found to be PFHxS, PFOS, PFBS and PFHxA, in order of decreasing concentration, which was similar to the findings of the pilot study. This pattern of PFAA concentrations is similar to findings in groundwater nearby firefighting training sites, suggesting the contamination source to be aqueous film forming firefighting foam (AFFF). Hence, the airport at Uppsala-Ärna is a likely point source (3).

Despite difficulty predicting how the previous emissions of PFAA will affect the drinking water in the future, the study concludes that it is likely that higher concentrations of long-chained PFAA, especially PFOA, PFHxS and PFOS, could reach the production wells of southern Uppsala in the future. As these wells account for 50% of the city's water production, this would pose a serious threat to public health (3).

Studies of serum-PFAS in Uppsala

At the time of writing, the serum levels of PFAS had been studied in four different Uppsala cohorts. The POPUP- and PIVUS-studies, which had the largest number of participants, as well as a study on the children of the mothers in the PIVUS-study (Gyllenhammar et al. 2016 (9)), and an early study where the levels in pregnant and breastfeeding women was examined (Glynn et al. 2009 (10)). Below follows an overview of the methods used in the latest studies (used as data in this essay) based on the four cohorts studied:

PIVUS – Stableski et al. 2016

Study participants from the general population in Uppsala were invited to leave plasma samples at ages 70, 75 and 80. The sampling took place in 2001-2004, 2006-2009 and 2011-2014 respectively. A total of 579 participants were part of all three collections. (7)

POPUP - Gyllenhammar et al. 2017

First time mothers from the general population were recruited for the donation of a blood sample 3 weeks after delivery. The study went on from 1996 to 2016 and three pooled serum samples, containing serum from 10 participants, was prepared for almost every year of the study period. (8)

Gyllenhammar et al. 2016

First time mothers from the POPUP-study were recruited at random for a follow-up study on the mothers and children. The invitation was received during or shortly after pregnancy, and the study went on between 2008 and 2012. The mothers answered questions regarding lifestyle factors and dietary habits, as well as the health of herself and her child. In addition, a blood sample of the child was collected. The ages of the children were 4 (N=78), 8-9 (N=59) and 12 years (N=121). (9)

Glynn et al. 2009

Blood samples from 19 women were collected between 1996 and 1999, during early and late pregnancy. Samples were also taken at 3 weeks and 3 months post-partum. (10)

RESULTS AND DISCUSSION

A number of effects are indicated by the EFSA- and ATSDR-reports, some of which can be considered more statistically supported than others. The outcomes considered to have the strongest support by EFSA were increased serum cholesterol (PFOA and PFOS) and decreased antibody response in children (PFOS). Outcomes less supported, but still considered significant, included ALT levels higher than the reference range (PFOA) and decreases in birth weight (PFOA and PFOS) (4). The ATSDR found developmental toxicity to be one of the most sensitive endpoints for PFHxS (5).

Average serum concentrations 2009

The average serum concentrations of the chosen Uppsala-based studies in 2009 are presented in table 5. Some of the serum concentrations were originally presented in ng/ml, this was however considered equivalent to ng/g since blood serum has roughly the same density as water. The PFOS concentration was approximately three times higher for the 75-year-olds than for the first-time mothers, and 4.7 times higher than for the children. The difference was smaller between children and first-time mothers, but the average concentration of the mothers was higher than that of the children. The concentration of PFOA was the highest for the senior citizens. The difference between children and first-time mothers was small, but one possible explanation for the lower concentration of the mothers is breastfeeding. The breastfeeding may reduce the mothers serum concentration, while being a source of exposure to the baby, as PFAS passes to the child through the milk (5). The PFHxS concentration was the highest for the senior citizens, followed by first time mothers, and was the lowest for children. The differences were quite small, particularly when comparing children and mothers, hence they do not necessarily imply a statistically tenable difference. The concentrations were the highest among senior citizens for PFOS, PFOA and PFHxS. This could possibly be explained by a general decrease in the glomerular filtration rate, and menopause among the women, in the senior population (5). Further, older age may mean a longer period of exposure compared with the children and first-time mothers, potentially contributing to the higher concentration in the senior citizens. It should be noted that the PIVUS study concluded that some PFAS actually decreased with increasing age. However, the decrease in PFOS, PFOA and PFHxS concentrations was recorded in 2011-2014 (7),

during which time the water contamination in Uppsala was remedied (3). This means that the decrease was not necessarily due to older age but rather could have been due to decreased exposure.

PFAA	Gyllenhammar et al. 2016 Mean of all included ages 4-12yr [ng/g]	Stubleski et al. 2016 75-year-old senior citizens [ng/g]	Gyllenhammar et al. 2017 1st time mothers, 3 weeks after delivery [ng/g]
<i>PFOS</i>	3.47	16.4	5.36
<i>PFOA</i>	2.34	4.5	2.13
<i>PFHxS</i>	4.87	6.9	5.18

Table 5: The average serum concentrations of PFAA [ng/g] found in three different studies in the year 2009. The values for Gyllenhammar et al. 2016 (9) and Stubleski et al. 2016 (7) are mean values for the time period 2008-2015 and 2006-2009 respectively, due to lack of individual data for each year. The value for Gyllenhammar et al. 2017 (3) is the mean value of all pooled samples from 2009.

Temporal trends of serum concentrations

The temporal trends of PFAA for the different subpopulations are displayed in figure 3-5. When observing the serum concentrations of the senior citizens and the first-time mothers, the levels have decreased between the initial and the final measurements.

The decrease among the first-time mothers seems to have started before the discovery of the contaminated water in 2012 and the subsequent purification of the water, indicating that pathways other than water contamination may have played a more important role in the exposure (3). This pattern fits well with PFOS and related substances being phased out of production for the leading manufacturer in 2000 (6), and the Stockholm convention aimed at decreasing the emission of persistent organic pollutants entering into force in 2004 (30). There seems to be a slight peak of concentration around 2007 in both the subpopulation of senior citizens and first-time mothers. The reasons for this peak could be many, one possibly being a fluctuation in the levels of water contamination. There was a change in the pattern of water distribution around this time, causing the contamination to be more even between different areas in the city (see figure 2). It is, however, hard to say what effect this would have on the average serum levels as no consideration has been taken to the place of living and working (i.e. sites of exposure) in the processing of data in this essay. The serum concentrations among first-

time mothers have continued to decrease further past 2012, although at what seems to be a slightly slower rate.

The serum concentrations of PFHxS continuously increased for all subpopulations since the initial measurements. There was a sharp decrease for first time mothers in 2012, although the concentrations seemed to increase slightly in the following years. As the only available data for senior citizens was a mean value for the years 2011-2014, it's hard to draw any conclusions on specific patterns around 2012. Due to the lack of more recent data it is hard to draw further conclusions on the development of the PFHxS concentrations in serum. There are suggestions that food intake is the major exposure pathway for PFAA with longer chains (≥ 7), while water contamination is the major pathway for shorter chain PFAA (< 7 C) (17). This fits with the data as the serum levels of PFOS and PFOA (chain length of 8C and 7C respectively) decreased before the water contamination was stopped. The serum concentrations PFHxS (chain length of 6C) continued to increase for both senior citizens and first-time mothers and fell in 2012. The pattern after 2012 is harder to interpret.

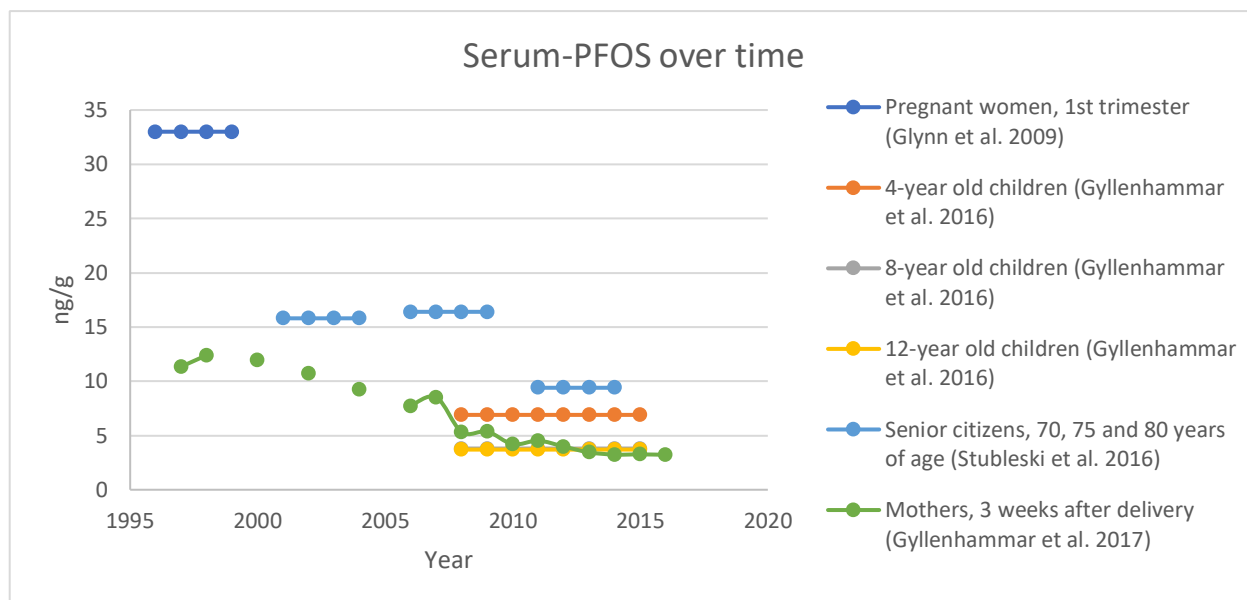


Figure 3: The PFOS concentrations in serum [ng/g] over time for different subpopulations in Uppsala. The concentrations for 8- and 12-year old children are almost identical, therefore only the curve for 12-year olds is visible.

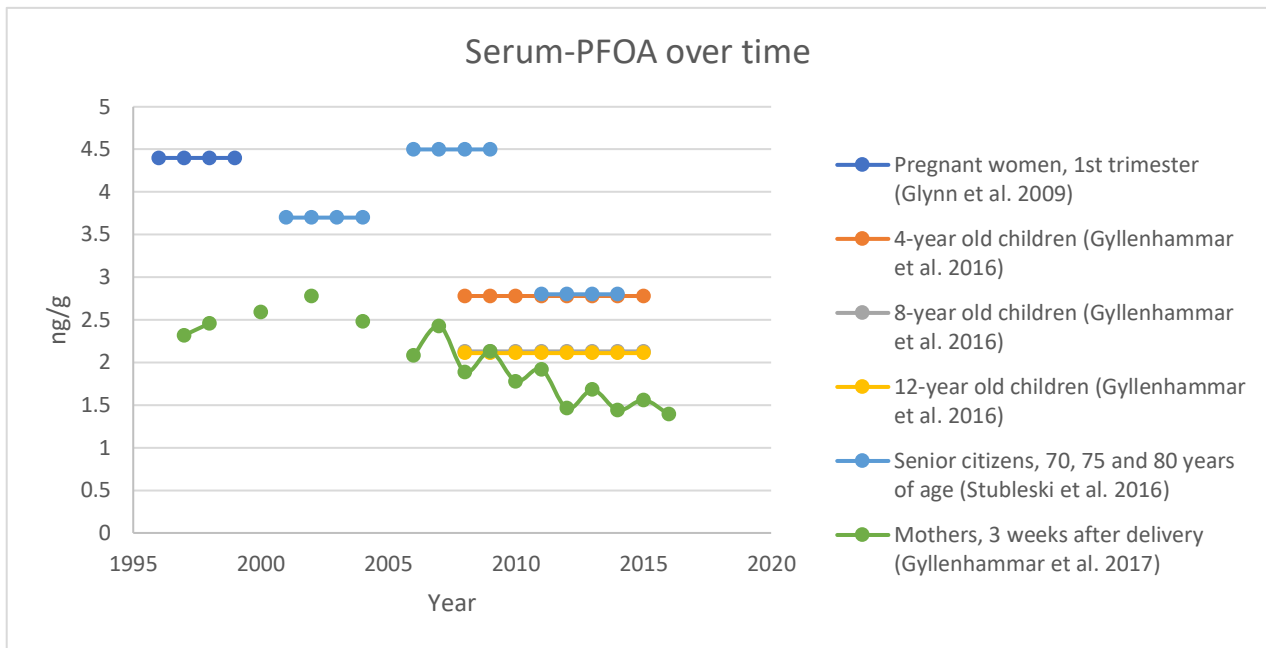


Figure 4: The PFOA concentrations in serum [ng/g] over time for different subpopulations in Uppsala. The concentrations for 8- and 12-year old children are almost identical, therefore only the curve for 12-year olds is visible.

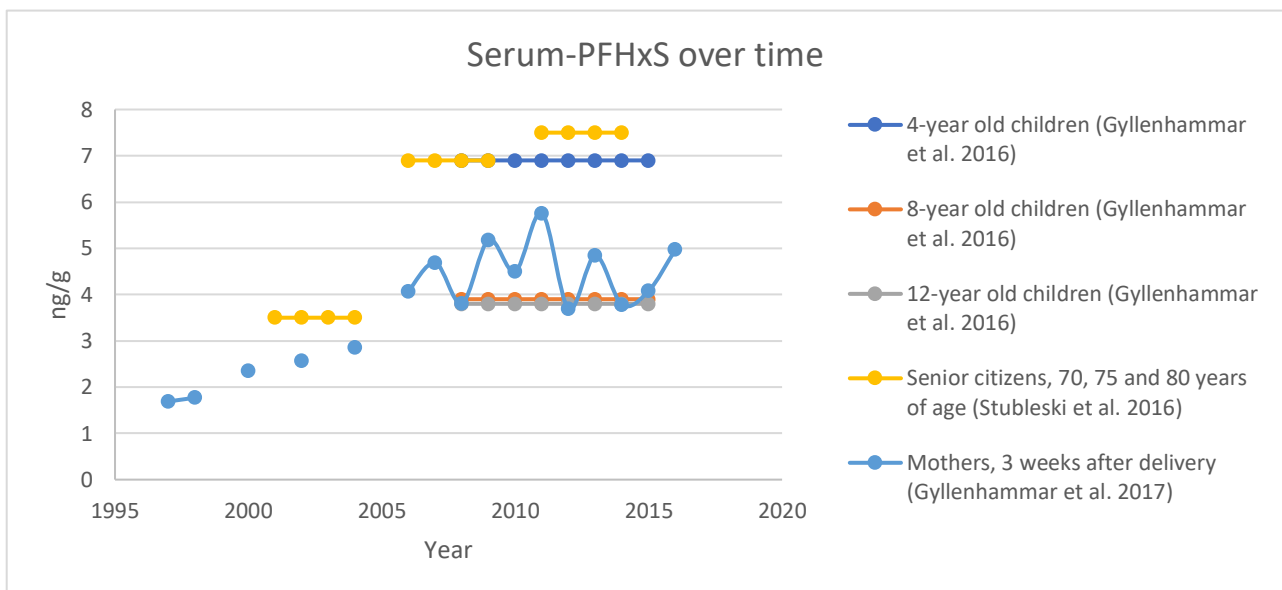


Figure 5: The PFHxS concentrations in serum [ng/g] over time for different subpopulations in Uppsala.

Serum concentrations of Uppsala-subpopulations compared to literature

The mean values for the different subpopulations in Uppsala are compared with the lowest BMDLs for the three most common PFAA in figure 6-8. The unit ng/mL in which the BMDLs were presented (see literature review) is considered equivalent to ng/g since blood serum has roughly the same density as water. As the Swedish national levels of PFAS are similar to the European average, mean values from the EFSA-report (2007-2015) are used as a comparison for the local serum concentrations of PFOS and PFOA (4)(6). The serum concentrations of PFOA were below the BMDL of 9.3ng/g for all subpopulations in 2009, although they were slightly above the European average. Hence, no increased risk due to the PFOA exposure can be expected. All subpopulations except the senior citizens had serum PFOS concentrations lower than the European averages and were below the BMDL of 10.5 ng/g. The senior citizens had an average serum concentration of 16,4ng/g, the high levels possibly due to the reasons listed earlier in this section. The exceeding of the BMDL would mean that there is 5% increase in the risk for an impaired vaccination response in children, but as this risk is not applicable to adults it cannot be considered relevant. The risk of a lower birth weight, with a BMDL of 21 ng/g, cannot be considered relevant for this subpopulation either. One could argue that the impaired response to vaccination is a symptom of a weakened immune system and as such would cause increased risks in senior citizens as well. However, the EFSA deemed the evidence for an increased risk of disease insufficient (5). The BMDL for a 5% increase of the risk of getting high cholesterol levels was 22,67ng/g, which the serum levels in senior citizens did not exceed. It can therefore be concluded that to our current knowledge, no increased risk can be expected.

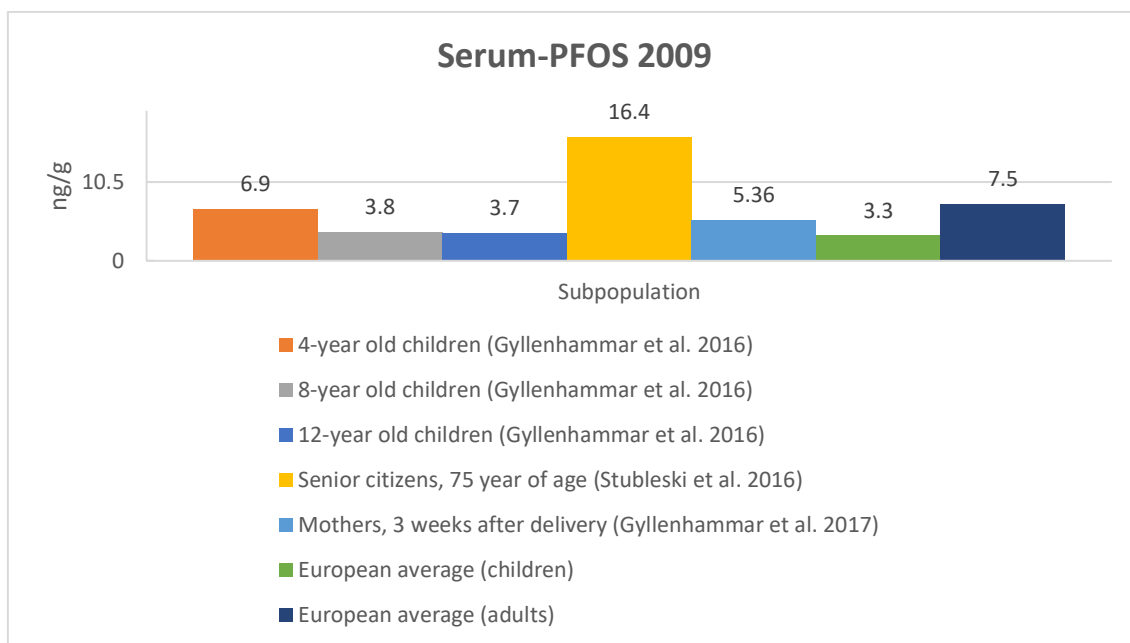


Figure 6: The serum concentrations of PFOS [ng/g] for different subpopulations in 2009. The lowest BMDL provided by EFSA was 11.6 for an alteration in the vaccination response in children. This concentration is only reached for senior citizens. The European average is taken from the EFSA-report (4).

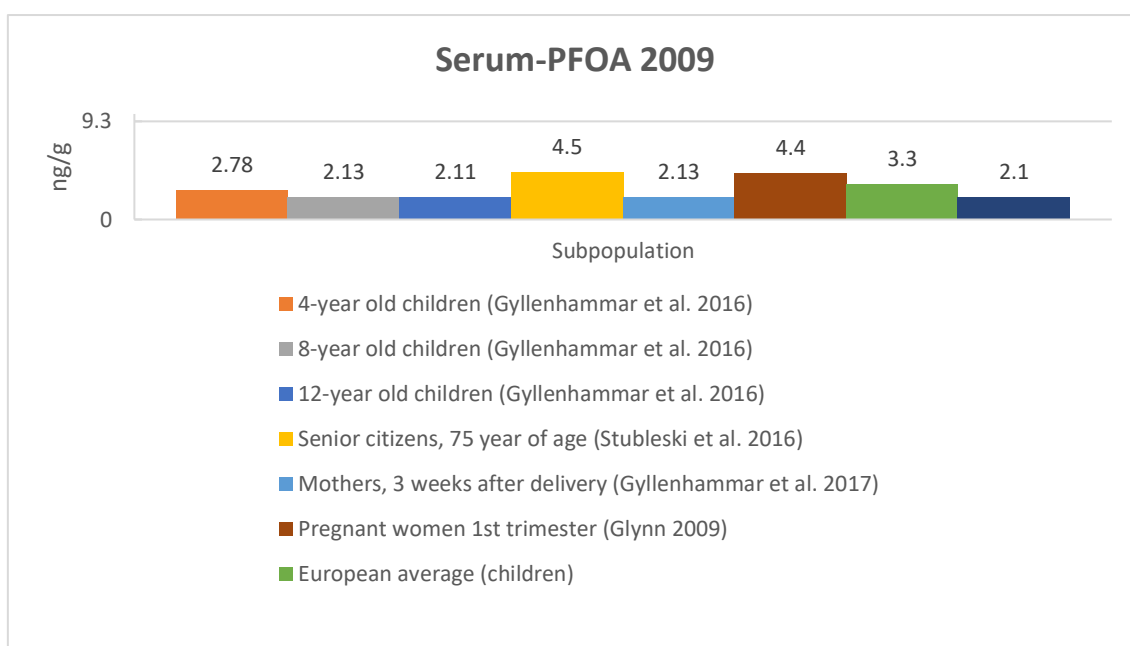


Figure 7: The serum concentrations of PFOA [ng/g] for different subpopulations in 2009. The lowest BMDL provided by EFSA was 5.8 for an increase of total cholesterol. This concentration is reached by none of the subpopulations. The European average is taken from the EFSA-report (4).

For the PFHxS-concentrations in serum, the levels for all subpopulations were at least six times higher than the European average. Most likely due to the water contamination which at the time had not been discovered (3). However, the TWA serum concentration found to cause developmental toxicity in rodents, according to the ATSDR report, was more than 10³ times higher than the highest values in the European and Swedish population presented in figure 6. These data therefore indicate no increased risk to the Uppsala population due to PFHxS exposure.

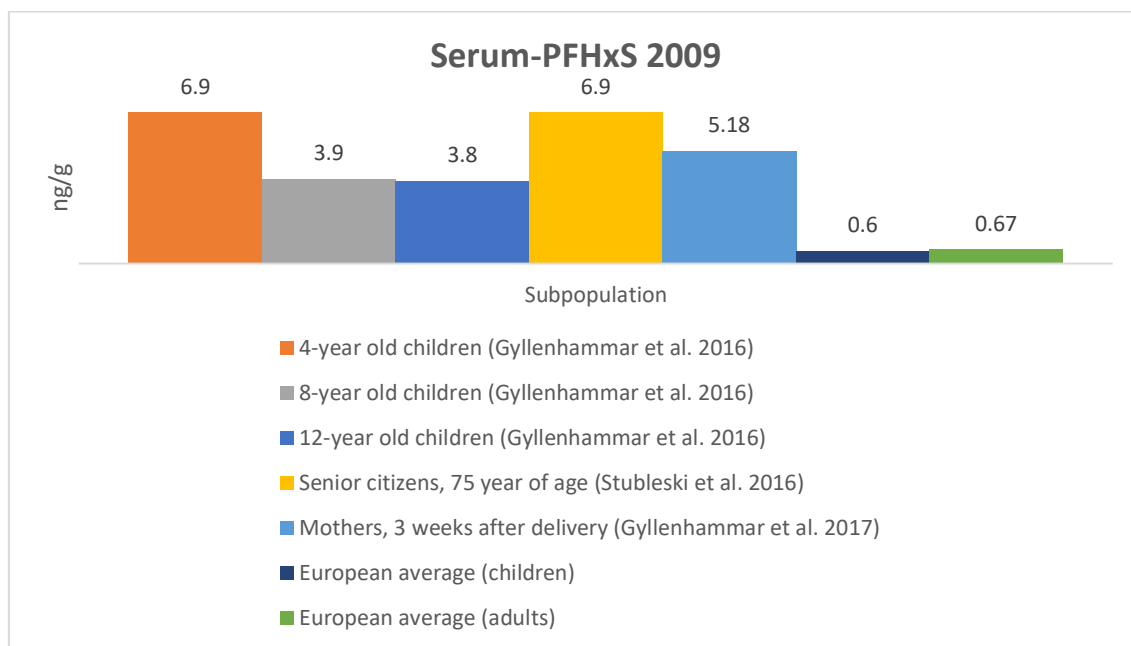


Figure 8: The serum concentrations of PFHxS [ng/g] for different subpopulations in 2009. The time weighted average (TWA) serum concentration of PFHxS, observed to cause developmental toxicity in rodents, was 73.22 µg/ml. This value is more than 10³ times greater than the concentrations found in the subpopulations of Uppsala. The European average is taken from the drafted scientific opinion by EFSA (31).

Combined risk assessment

To examine the effect of combined exposure, total risk characterization ratios (RCR) were calculated for the endpoints shared by PFOS and PFOA. All total RCRs, but that for high total cholesterol levels in the senior citizens, were below one, indicating no increased risk of the endpoint despite co-exposure. The total RCR for high total cholesterol levels in the senior citizens was, however, higher than 1 which implies that there was an increased risk due to the combined exposure of PFOS and PFOA (13).

Figure 9: The total risk characterization ratio for the endpoints total cholesterol and low birth weight due to PFOS- and PFOA-exposure in Uppsala 2009. As the risk of low birth weight is only applicable for young women, it was not calculated for senior citizens.

Endpoint	Total RCR senior citizens	Total RCR first-time mothers	Total RCR children (mean of all included ages)
<i>High total cholesterol</i>	1.21	0.47	0.40
<i>Low birth weight</i>	-	0.55	-

Strengths and limitations

Firstly, it should be noted that the results from this study are only applicable for the specific subpopulations studied. The number of participants in the studies, particularly for first time mothers, is not very large. Therefore, outliers or participants who were otherwise not representative of the subpopulation as a whole, will have a greater impact on the results than if the number of participants had been larger. This could potentially skew the results. Assuming that the results are representative for the studied subpopulations, the results may indicate a pattern for the whole population, but no firm conclusions can be drawn. This since the concentrations may have been drastically different for other subpopulations during the year of measurement, which is not unconceivable judging by the high PFOS-concentrations in senior citizens compared to the other subpopulations.

Further, this study used mean values to attain a general risk assessment. This does, however, not take into account the increased risk to individuals with higher serum concentrations of PFAS than the mean. In order to examine the risk for these

individuals, a risk assessment could be made with the highest measured concentrations from each subpopulation i.e. the maximum concentrations. This would likely result in a higher risk than the one found in this study, where mean concentrations were used.

It should also be noted that the TWA concentration, used for the risk assessment of the PFHxS serum concentrations, are not comparable to the BMDL values. This as a BMDL corresponds to a concentration below which there should be no increased risk for any subpopulation, including more sensitive individuals, making the tolerable concentration much lower. The TWA, on the other hand, corresponds to a concentration where an effect was actually observed. Further, this is a value obtained from animal studies and the effect and the concentrations at which they occur in humans could be different. However, no BMDL values were found for PFHxS, making the TWA concentration the only available option. It can be concluded that there are no statistically founded increases of risk due to the PFHxS exposure, but it should be noted that the obtained reference value leaves much to be desired.

As no estimate of the emissions of PFAS from Uppsala-Ärna airport was found in literature, and the travel time of PFAS through the soil into the ground water is hard to predict (3), it is also difficult to estimate the exposure of the Uppsala population since the emissions began. For this reason, the risk assessment in the present study was based on mean concentrations in 2009, instead of exposure over time. This approach is not perfect, particularly as some of the included values are mean values for several years around 2009. However, the uncertainties of a more sophisticated calculation cannot be considered much more reliable than the method chosen due to the many uncertainties involved. In addition, the available literature examining the health effects of PFAS exposure are largely based on studies executed over a short period of time and the available longitudinal studies are few. As a consequence, there is little information about the long-term effects of exposure. Hence the differences in effect due to temporarily high levels as opposed to lower concentrations over longer time frames, are still uncertain.

The EFSA- and ATSDR-reports try to examine the effects of the different PFAA separately, although this is often difficult due to combined exposure in epidemiological studies. This makes both study results and their application in risk assessment unreliable. A study draft on the risks due to combined exposure has been made by the EFSA but had not been reviewed or published at the time of writing, its results are therefore not included in this study. The European average serum concentration of PFHxS was however taken from this unpublished study as no other source was found and the value was considered paramount for discussion of the data. To attempt examining the combined effects of PFOS and PFOA on shared endpoints, the sum of their RCR-values for each endpoint was calculated. It should be noted that this does not take into account any potential synergy between the substances, which is defined as an increase in effect that is larger than the sum of the individual effects (32). However, it provides a more realistic assessment than the individual comparisons to BMDL-values alone.

In the EFSA report, an increased risk of cardiovascular disease was not considered a significant effect of PFAS exposure. It is however noteworthy that an increased risk of high cholesterol, which was considered sufficiently supported, is considered to increase the risk of cardiovascular disease in turn.

The focus on PFOS, PFOA and PFHxS in this study was based on the high concentrations both in serum and water samples before purification of the wells in Uppsala. While other PFAA were present in quite high levels in the water samples, e.g. PFBS, they were not as high in the serum samples and hence excluded from further analysis. The lesser concentration in serum may possibly be due to short chain length (5).

The conclusions drawn from the results are limited to information available at the time of writing. This was in turn dependent on the available research of the health effects due to PFAS exposure at the time of inclusion in the EFSA- and ATSDR-reports. It would be interesting to repeat a similar study as this one, once more studies have been conducted and, hopefully, deepened our understanding of this subject.

Potential developments of this study could be to study geographical differences, focus on gender specific differences or include more PFAA. Another option could be not to limit the study to preexisting data, but to take new measurements in different subpopulations over time. This would allow inclusion of more subpopulations. This would however have to be a very large project in order to include enough participants. Further, there would be no access to historic data at the time of the highest observed PFAS concentrations which could be problematic if historic concentrations turn out to be of significance.

Conclusion

In conclusion, mean serum concentrations of the examined PFAA, PFHxS in particular, were higher in the Uppsala population than the European, and by extension the Swedish, average. Despite this, based on the statistically established effects by the EFSA- and ATSDR-reports, no increases in health risks due to exposure to individual PFAS were found at the serum levels observed the Uppsala population in 2009. When combining the potential effects of PFOS and PFOA, an increased risk for high total cholesterol levels among the senior citizens was found. Future discoveries may, however, lead to a deepened understanding regarding the risks associated with PFAS exposure and hence different outcomes of risk assessments such as this one.

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APPENDIX

Overview of the studies included in the collection of data for risk assessment

Authors	Time of data collection	Participants <i>Subpopulation: nr (female, male)</i>	Purpose	Method	Results	Quality¹
Stubleski et al. (2016) (7)	2001-2004 2006-2009 2011-2014	Senior citizens at 70, 75 and 80 y.o (PIVUS): 579 ²	To describe the longitudinal trends of eight PFAS concentrations in the PIVUS cohort, ages 70-80, where age and location was the same for all individuals. The sex-specific changes in levels of PFASs over time was also examined.	Collection and analysis of serum samples at 70, 75 and 80 years of age.	A decrease in concentrations of PFOSA and PFOS was observed while the remaining PFAS tested for increased between ages 70-75. All PFAS decreased between ages 75-80. PFHxS, PFUnDA, PFNA and PFDA increased during the entire period of measurement, while PFOSA, PFHpA, PFOS and PFOA decreased. PFHxS increased more among women and PFHpA decreased more among men.	High (7/8) ³

¹ The quality parameters are explained in detail below.

² The proportion of women and men was not clearly presented.

³ Weaknesses of the study are not discussed, although most likely acceptable.

Gyllenhammar et al. (2017) (8)	1997-2016	First time mothers (POPUP): ~ 480 (3 pooled samples from 9-10 women each included year)	To present the serum levels of PFAS among first time mothers 1997-2016.	Collection of serum samples 3 weeks after delivery in first time mothers. Around 30 samples per year was divided into 3 representative samples (9-10 samples per representative sample) which were then analysed.	Levels of PFOS and its precursors decreased due to a drop in production 2002. Levels of PFOA decreases, although at a slower rate than PFOS due to it not being completely phased out of production. PFHxS increased until 2011, after which no trend was observable due to the water contamination being resolved.	High (8/8)
Gyllenhammar et al. (2016) (9)	2008-2015	Children 4 y.o: 57 (21, 36) 8 y.o: 55 (21, 34)	To examine the change in serum concentrations of PFAS among 4-, 8- and 12 year old children	Collection and analysis of serum samples.	PFOS, PFHpA, PFOA and PFDA decreased over time while PFHxS and PFUnDA increased. The concentration of some substances varied depending on where the child lived. PFHpA decreased among children	High (8/8) ⁴

⁴ Unclear which children were followed over time (multiple samples) and which were sampled only once.

		12 y.o: 119 (56, 63)	in Uppsala between 2008-2015.		who had not received contaminated water. The decrease of PFOS was faster for children who had not received contaminated water and the increase in PFHxS levels was faster for children who had received contaminated water. Overall, the exposure to PFAS through drinking water affects the serum concentrations of PFAS among children.	
Glynn et al. (2009) (10)	1996-1999	First time mothers and their children: 19	To evaluate if a single blood sample during pregnancy or the period of breastfeeding can accurately depict the exposure to PFOS, PFOA and PFNA.	Collection and analysis of blood from the umbilical chord as well as serum samples from the mother at early- and late pregnancy, 3 weeks and 3 months after pregnancy.	The correlation between all samples was good, indicating that one sampling during or after pregnancy is enough to estimate the exposure of PFOS, PFOA and, most likely PFnA, in both mother and child.	Medium (6/8) ⁵

⁵ The participants were few and no ethical approval was mentioned in the report.

Quality parameters – adaptation of questions for quality assessment from Friberg (2012) (33).

1. Clear purpose and research question(s)?
2. Appropriate method design for answering the research question(s)?
3. Selection of participants: Relevant inclusion and exclusion criteria for the participants to be considered a relatively uniform subpopulation.
4. Sufficient number of participants included for reliable results?
5. Ethical review and approval?
6. Sufficient quality of measurements?
7. Appropriate analysis of data?
8. Are the weaknesses of the study discussed and acceptable?

Low quality: <3

Medium quality: 4-6

High quality: >6

Serum concentrations of PFAA over time in different subpopulations

Table 1: Serum concentrations of PFOS

Tidsöversikt PFOS	Pregnant women, 1st trimester (Glynn et al. 2009 (10))	4-year old children (Gyllenhammar et al. 2016 (9))	8-year old children (Gyllenhammar et al. 2016 (9))	12-year old children (Gyllenhammar et al. 2016 (9))	Senior citizens, 70, 75 and 80 years of age (Stubleski et al. 2016 (7))	Mothers, 3 weeks after delivery (Gyllenhammar et al. 2017 (8))
1996	33					
1997	33					11.35
1998	33					12.4
1999	33					
2000						11.97
2001					15.8	
2002					15.8	10.73
2003					15.8	
2004					15.8	9.22
2005						
2006					16.4	7.73
2007					16.4	8.53
2008		6.9	3.8	3.7	16.4	5.31
2009		6.9	3.8	3.7	16.4	5.36
2010		6.9	3.8	3.7		4.2
2011		6.9	3.8	3.7	9.4	4.52
2012		6.9	3.8	3.7	9.4	3.98
2013		6.9	3.8	3.7	9.4	3.44
2014		6.9	3.8	3.7	9.4	3.24
2015		6.9	3.8	3.7		3.26
2016						3.22

Table 2: Serum concentrations of PFOA

Tidsöversikt PFOA	Pregnant women, 1st trimester (Glynn et al. 2009 (10))	4-year old children (Gyllenhammar et al. 2016 (9))	8-year old children (Gyllenhammar et al. 2016 (9))	12-year old children (Gyllenhammar et al. 2016 (9))	Senior citizens, 70, 75 and 80 years of age (Stubleski et al. 2016 2016 (7))	Mothers, 3 weeks after delivery (Gyllenhammar et al. 2017 (8))
1996	4.4					
1997	4.4					2.32
1998	4.4					2.46
1999	4.4					
2000						2.59
2001					3.7	
2002					3.7	2.78
2003					3.7	
2004					3.7	2.48
2005						
2006					4.5	2.08
2007					4.5	2.43
2008		2.78	2.13	2.11	4.5	1.89
2009		2.78	2.13	2.11	4.5	2.13
2010		2.78	2.13	2.11		1.78
2011		2.78	2.13	2.11	2.8	1.92
2012		2.78	2.13	2.11	2.8	1.46
2013					2.8	1.68
2014					2.8	1.44
2015						1.56
2016						1.39

Table 3: Serum concentrations of PFHxS

Tidsöversikt PFHxS	4-year old children (Gyllenhammar et al. 2016 (9))	8-year old children (Gyllenhammar et al. 2016 (9))	12-year old children (Gyllenhammar et al. 2016 (9))	Senior citizens, 70, 75 and 80 years of age (Stubleski et al. 2016 2016 (7))	Mothers, 3 weeks after delivery (Gyllenhammar et al. 2017 (8))
1996					
1997					1.69
1998					1.77
1999					
2000					2.36
2001				3.5	
2002				3.5	2.57
2003				3.5	
2004				3.5	2.86
2005					
2006				6.9	4.07
2007				6.9	4.69
2008	6.9	3.9	3.8	6.9	3.81
2009	6.9	3.9	3.8	6.9	5.18
2010	6.9	3.9	3.8		4.5
2011	6.9	3.9	3.8	7.5	5.76
2012	6.9	3.9	3.8	7.5	3.69
2013	6.9	3.9	3.8	7.5	4.84
2014	6.9	3.9	3.8	7.5	3.78
2015	6.9	3.9	3.8		4.08
2016					4.97