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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Title and subtitle: Exposure to perfluorinated alkyl substances and health effects in pregnant women and their children

Abstract: Per- and polyfluorinated alkyl substances (PFAS) are chemicals that have gained attention during the last years. About 2,100 sites with a point source of high PFAS contamination, so-called hotspots, have been discovered around Europe and it is expected that more sites will emerge in the future. Almost everyone in the world is exposed to PFAS, and the most common sources are food, drinking water, and the indoor environment. PFAS accumulate in the human body and are also transferred from mother to child during pregnancy. Several adverse health conditions are associated with PFAS exposure, for example, impaired immune function, testicular and kidney cancer, increased cholesterol levels, and thyroid disease. The overall aim of this thesis was to use epidemiological methods to investigate the effects of PFAS exposure on complications during pregnancy, and the health and development of children after prenatal PFAS exposure. Studies I, III, and IV were based on the Ronneby population which was exposed for decades to high levels of PFAS in drinking water after contamination from firefighting foam. Study I is a register-based cohort study investigating the association between high exposure to PFAS and gestational hypertension, preeclampsia, and gestational diabetes mellitus. We did not find evidence of an association between high or intermediate exposure to PFAS and gestational hypertension, preeclampsia, or gestational diabetes mellitus. Study II is a case-control study investigating the association between prenatal background-level exposure to PFAS and the risk of overweight at 4 years of age We found no association between prenatal PFAS exposure and overweight at 4 years of age. Study III is a register-based cohort study, investigating the association between high prenatal exposure to PFAS and developmental language disorders in children aged 0-7 years. Girls who had high prenatal PFAS exposure had a 62% higher (HR 1.62, 95% CI 1.12-2.35) risk of being diagnosed with developmental language disorders and a 36% (HR 1.36, 95% CI 1.02-1.80) higher risk of being referred to a speech and language pathologist compared to girls who had prenatal exposure at background levels. Study IV is a register-based cohort study, investigating the association between high prenatal exposure to PFAS and common infectious diseases in children aged 0-7 years. We found an increased risk of 28% (HR 1.28, 95% CI 1.05-1.55) for urinary tract infections and a 9% (HR 1.09, 95% CI 1.00-1.19) increased risk of ear infections in children who had high prenatal PFAS exposure compared to children with prenatal exposure at background levels. In summary, my thesis contributes to new knowledge about high PFAS exposure and its effect on pregnant women and their prenatally exposed children. My studies provide a missing piece of information to the big puzzle of how different PFAS exposure profiles and exposure levels affect human health, by providing estimates of health effects from a population highly exposed to PFAS from drinking water contamination by firefighting foam run-off.

Key words: Per-and polyfluorinated alkyl substances, prenatal exposure, epidemiology, maternal health, child health, child development, register studies.

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Till min familj

Table of Contents

Abstract

Per- and polyfluorinated alkyl substances (PFAS) are chemicals that have gained attention during the last years. There are about 10,000 different PFAS compounds, and due to their unique chemical characteristics, PFAS has been used in a variety of industrial applications and household products since the 1950s. Today, concerns about their toxicity and persistence have led to the regulation and banning of individual PFAS, but new substances have been introduced in their place. New directives will enter into force in 2026 regarding PFAS levels in drinking water in Sweden, and a proposal to ban all PFAS has been authored by several countries. About 2,100 sites with a point source of high PFAS contamination, so-called hotspots, have been discovered around Europe and it is expected that more sites will emerge in the future.

Almost everyone in the world is exposed to PFAS, and the most common sources are food, drinking water, and the indoor environment. PFAS accumulate in the human body and have elimination half-lives of several years as they bind to albumin in the blood and are effectively reabsorbed in the major excretion routes. PFAS are also transferred from mother to child during pregnancy through the placenta. The mechanisms of PFAS toxicity are not clear, but several adverse health conditions are associated with exposure, for example, impaired immune function, testicular and kidney cancer, increased cholesterol levels, and thyroid disease.

The overall aim of this thesis was to use epidemiological methods to investigate the effects of PFAS exposure on complications during pregnancy, and the health and development of children after prenatal PFAS exposure. Studies I, III, and IV were based on the Ronneby population which was exposed for decades to high levels of perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonic acid (PFHxS) in drinking water after firefighting foam had leaked into the groundwater from a military airport. One out of two water treatment plants in Ronneby distributed the contaminated water, providing drinking water to a third of the population.

Study I is a register-based cohort study investigating the association between high exposure to PFAS and gestational hypertension, preeclampsia, and gestational diabetes mellitus. The exposure was assessed using a proxy variable based on residential history and the outcomes were retrieved from the National Medical Birth Register. We considered body mass index (BMI), birth country, educational status, maternal smoking, parity, and maternal age as confounders. We did not find evidence of an association between high or intermediate exposure to PFAS and gestational hypertension, preeclampsia, or gestational diabetes mellitus.

Study II is a case-control study investigating the association between prenatal background-level exposure to PFAS and the risk of overweight at 4 years of age. Serum samples from the Southern Sweden Maternity Cohort biobank were analyzed for PFAS concentrations, and the outcome was retrieved from medical journals from

child health care centers in Malmö. Information on confounders was retrieved from a self-administered questionnaire. We found no association between prenatal PFAS exposure and overweight at 4 years of age.

Study III is a register-based cohort study, investigating the association between high prenatal exposure to PFAS and developmental language disorders in children aged 0-7 years. The exposure was assessed using a proxy based on maternal residential history, which was validated against measured serum concentrations in the same population. The outcome was retrieved from the administrative regional healthcare register. We considered educational status, maternal smoking, parity, and maternal age as confounders. Girls who had high prenatal PFAS exposure were found to have a 62% higher (HR 1.62, 95% CI 1.12-2.35) risk of being diagnosed with developmental language disorders and a 36% (HR 1.36, 95% CI 1.02-1.80) higher risk of being referred to a speech and language pathologist compared to girls who had prenatal exposure at background levels. We found no associations for children with intermediate prenatal PFAS exposure.

Study IV is a register-based cohort study, investigating the association between high prenatal exposure to PFAS and common infectious diseases in children aged 0-7 years. The exposure was assessed by using a proxy based on maternal residential history and the outcome was retrieved from the regional healthcare register. We considered educational status, maternal smoking, parity, and maternal age as confounders. We found an increased risk of 28% (HR 1.28, 95% CI 1.05-1.55) for urinary tract infections and a 9% (HR 1.09, 95% CI 1.00-1.19) increased risk of ear infections in children who had high prenatal PFAS exposure compared to children with prenatal exposure at background levels. We also found an increased risk of 6% (HR 1.06, 95% CI 1.02-1.11) for upper respiratory tract infections among children with intermediate prenatal PFAS exposure, compared to children with prenatal background exposure.

In summary, my thesis contributes to new knowledge about high PFAS exposure and its effect on pregnant women and their prenatally exposed children. This information is important not only for the populations that are affected today but also for emerging hotspot populations. My studies provide a missing piece of information to the big puzzle of how different PFAS exposure profiles and exposure levels affect human health, by providing estimates of health effects from a population highly exposed to primarily PFOS and PFHxS from drinking water contamination by firefighting foam run-off. The results from this thesis are valuable for decision-making regarding new restrictions and phase-outs of PFAS compounds and for public health management in PFAS hotspots.

Populärvetenskaplig sammanfattning

Per- och polyfluorerade alkylsubstanser, förkortat PFAS, är ett samlingsnamn för en grupp mycket stabila kemikalier. PFAS har använts sedan 1950-talet både inom industriella processer och i konsumentprodukter såsom teflonpannor, fritidskläder, och matförpackningar då de har fett- och vattenavvisande egenskaper. Under de senaste åren har dock PFAS-användningen begränsats, och i januari 2026 kommer nya regler införas för hur mycket PFAS som får finnas i dricksvatten i Sverige. Dessa åtgärder speglar de senaste årens ökande oro för vilka effekter dessa substanser kan ha på vår hälsa, men även på vår omgivning då man har sett att de inte bryts ner i naturen.

En undersökning har visat att det finns ungefär 2,100 platser runt om i Europa som har förhöjda nivåer av PFAS i mark eller grundvatten på grund av utsläpp från fabriker eller läckage av släckskum från brandövningsplatser. Dessa platser brukar kallas för ”hotspots”, och en av de som har högst nivåer är Ronneby i södra Sverige. Här upptäcktes 2013 att brandsläckningsskum från en övningsplats på en militär flygplats hade läckt ner till grundvattnet och förorenat dricksvatten till en tredjedel av Ronnebys befolkning under flera årtionden. Upptäckter av liknande föroreningar, om än inte lika omfattande, tror man kommer att bli mer vanliga i framtiden då användning av släckskum har ägt rum i princip på alla militära och civila flygplatser.

Så gott som alla människor i världen har mätbara nivåer av PFAS i blodet, då dessa substanser har spridit sig över hela jordgloben. De huvudsakliga exponeringskällorna för PFAS är via dricksvatten, mat, inomhusluft och damm. Väl i kroppen binder PFAS till proteiner i blodet, och det tar flera år innan nivåerna av PFAS i kroppen minskar. Man vet inte helt hur PFAS beter sig i kroppen och vilka processer som de kan påverka, men man vet att PFAS förs över från mamma till barn under graviditeten. Det har rapporterats om flera hälsoeffekter till följd av PFAS-exponering och några av dessa är påverkan på sköldkörtelhormon och kolesterolnivåer, njur- och testikelcancer samt lägre födelsevikt hos barn.

Det övergripande målet med denna avhandling har varit att undersöka hur exponering för PFAS påverkar hälsan hos gravida mödrar, liksom hälsan hos deras barn efter att de utsatts för PFAS under graviditeten.

Vi undersökte om att vara utsatt för höga nivåer av PFAS under femårsperioden innan förlossningen kunde öka risken för att den gravida kvinnan skulle drabbas av förhöjt blodtryck, havandeskapsförgiftning eller graviditetsdiabetes. Vi hämtade in uppgifter om dessa sjukdomstillstånd från sjukvårdens register, och använde oss av kvinnornas historiska folkbokföringsadresser för att uppskatta hur mycket PFAS de hade utsatts för. Resultatet från denna studie var att vi inte såg samband mellan att vara utsatt för höga nivåer av PFAS och ökat blodtryck, havandeskapsförgiftning eller graviddiabetes.

I en annan studie undersökte vi om att vara utsatt för normala nivåer av PFAS under graviditeten kunde ha ett samband med övervikt hos barnet när det var 4 år. Vi använde sparade blodprover från graviditeten för att mäta mammornas PFAS nivåer, och använde oss av uppgifter insamlade från barnavårdscentraler och frågeformulär för att inhämta uppgifter om barnets längd och vikt. Vi såg inget samband mellan mammans PFAS nivåer under graviditeten och risken för övervikt vid 4 års ålder.

I två studier undersökte vi om exponering för höga PFAS nivåer under graviditeten kunde påverka barnets hälsa och utveckling när de var 0–7 år gamla. Vi undersökte barnens språkutveckling som ett mått på hjärnans utveckling och förekomsten av vanliga infektionssjukdomar. Uppgifter om barnens hälsa hämtades från Blekinge hälso- och sjukvårdsregister. I båda studierna använde vi mammans historiska folkbokföringsadresser för att uppskatta vilka PFAS nivåer barnen varit utsatta för under graviditeten. Resultaten visade att flickor som varit utsatta för höga nivåer av PFAS under graviditeten hade 62% högre risk att få en diagnos för en störning i språkutveckling. Vi såg också att barn som blivit utsatta för höga nivåer av PFAS under graviditeten hade 28% respektive 9% högre risk att drabbas av urinvägsinfektioner och öroninfektioner än barn som hade utsatts för låga nivåer av PFAS under graviditeten.

Sammanfattningsvis så har denna avhandling bidragit till att visa hur hög PFAS-exponering påverkar hälsan hos gravida kvinnor och deras barn. Denna information är viktig för de människor som utsätts för höga nivåer av PFAS, men också för sjukvården som behöver kunskapsunderlag om förväntad hälsopåverkan för att kunna ta hand om befolkningen, och för myndigheter som regleringar användningen av PFAS.

List of Papers

Paper II was chronologically the first published paper, and it was published under my former last name, Martinsson.

Paper I

Ebel M, Rylander L, Fletcher T, Jakobsson K, Christel N. 2023. Gestational hypertension, preeclampsia, and gestational diabetes mellitus after high exposure to perfluoroalkyl substances from drinking water in Ronneby, Sweden. Environmental Research 239(Pt 1):117316.

Paper II

Martinsson M, Nielsen C, Björk J, Rylander L, Malmqvist E, Lindh C, Rignell-Hydbom A. 2020. Intrauterine exposure to perfluorinated compounds and overweight at age 4: A case-control study. PLoS ONE 15(3):e0230137.

Paper III

Stübner C, **Ebel M**, Jakobsson K, Gillberg C, Nielsen C, Miniscalco C. 2023. Developmental language disorders in preschool children after high exposure to perfluoroalkyl substances from contaminated drinking water in Ronneby, Sweden. Environmental Epidemiology 7(1):e233.

Paper IV

Ebel M, Blomberg A, Jöud A, Kold Jensen T, Nielsen C. 2023. Common childhood infections after high prenatal exposure to perfluoroalkyl substances from drinking water in Ronneby, Sweden. *In manuscript.*

Author's contribution to the papers

Paper I

I am the first author. I contributed to methodology, investigation, data curation, formal statistical analysis, writing original draft, visualization, article submission, reviewer and journal correspondence.

Paper II

I am the first author. I contributed to formal statistical analysis, visualization, writing original draft, article submission, reviewer and journal correspondence.

Paper III

I am a shared first author. I contributed to study design, methodology, investigation, data curation, formal statistical analysis, writing original draft, and visualization.

Paper IV

I am the first author. I contributed to study design, methodology, investigation, data curation, formal statistical analysis, writing original draft, and visualization.

Abbreviations

AFFF	Aqueous Film-Forming Foams
BMI	Body Mass Index
CI	Confidence Interval
DAG	Directed Acyclic Graph
EFSA	European Food Safety Authority
GDPR	General Data Protection Regulation
HBM4EU	Human Biomonitoring for EU
HR	Hazard Ratio
ICD	International Classification of Diseases
ISO-BMI	Age and sex-adjusted Body Mass Index
LISA	Longitudinal Integrated Database for Health Insurance and Labour Market Studies
OAT	Organic Anion Transporter
PFAS	Per- and polyfluorinated alkyl substances
PFBS	Perfluorobutane sulfonic acid
PFHxS	Perfluorohexane sulfonic acid
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PPAR	Peroxisome Proliferator-Activated Receptors

Introduction

Per- and polyfluorinated alkyl substances

Characteristics and use

PFAS is a collective name for a group of organic compounds that have been produced since the 1950s. PFAS have a carbon chain, where the hydrogen atoms are fully (*per*fluorinated alkyl substances) or partly (*poly*fluorinated alkyl substances) replaced by fluor atoms (1). Different functional groups, e.g., carboxylic acids, sulfonic acids, and alcohols can be connected to the carbon chain (2). The length of the fluorinated carbon chain characterizes PFAS into short and long chained, where long chained are considered substances with chain lengths of six carbons (C6) or more, such as the common PFAS perfluorooctanoic acid (PFOA, C8), perfluorooctane sulfonate (PFOS, C6), and perfluorohexane sulfonic acid (PFHxS, C6). Figure 1 visualizes the molecular structures of PFOA, PFOS, and PFHxS. The length of the chain can influence the substance's behavior in the environment and organisms as it affects the physicochemical properties (3).

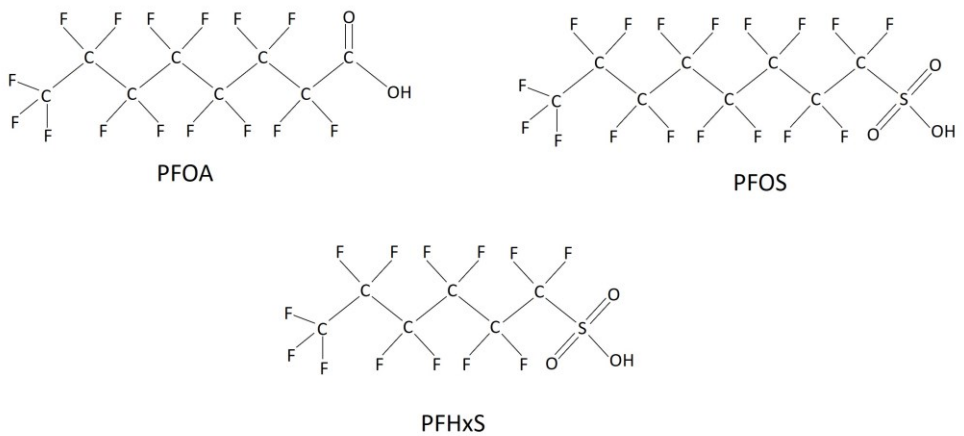

Figure 1. Molecular structures
Molecular structures for perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonic acid (PFHxS).

The functional group of the PFAS molecule is hydrophilic and the carbon-fluor chain is hydrophobic, which makes PFAS prone to form a layer between e.g., water and organic solvents or between fluid and solids (1). The carbon-fluor chain is also oleophobic, which together with the hydrophobic properties makes PFAS effective surfactants and surface protectors (4). The molecule is also very stable due to the strong carbon-fluor bond that gives the molecule its outstanding chemical and thermal stability (5). Because of these unique properties, PFAS are used in a variety of industrial applications and commercial household products. Some of the areas of use are in non-stick coatings, waterproof fabrics, and protective coatings (2) such as food packaging, outdoor clothing, frying pans, cosmetics, and cleaning products (1). PFAS are also used in aqueous film-forming foams (AFFF), i.e., a type of firefighting foam, where PFAS create a film between the foam and the burning liquid (1).

It is now estimated that there are at least 10,000 different PFAS and approximately 4,730 of these have CAS numbers (a unique numeric substance identifier assigned by the Chemical Abstracts Service) (6). In the European Economic Area, around 850,000 tons of PFAS are used every year and the annual emissions are estimated to be approximately 75,000 tons (excluding waste) (7). Through the whole life cycle of PFAS, i.e., during the production, use, and disposal, the substances are released into the environment, with the vast majority ending up in the aquatic environment (8). PFAS are emitted both through point- and diffuse sources. Point sources include manufacturing plants, landfills, use of PFAS-containing products in a certain area, and municipal sewage treatment plants. Atmospheric deposition is an important diffuse source of PFAS, where the original sources are manufacturing plants, landfills, sewage treatment plants, and households (8). PFAS have long-range transport properties and can travel long distances in water and through aerosols. Volatile precursors travel in the atmosphere, reaching remote areas such as Svalbard (9, 10). Due to the strong carbon-fluor bond, which is the strongest bond there is to a carbon atom, PFAS is resistant to degradation (5). Some PFAS do not degrade at all, while some slowly break down into other PFAS compounds. No study has been able to show a complete degradation of PFAS in the environment – hence PFAS is called “a forever chemical” (1).

Regulation and legislation in the European Union

PFOS, PFOA, and PFHxS are regulated under the Stockholm Convention on Persistent Organic Pollutants, a global treaty to protect human health and the environment from chemicals by eliminating or restricting their production and use (11). PFOA has been included in Annex A (eliminate manufacturing and use) since 2019 together with PFHxS since 2022, and PFOS has been included in Annex B (restrict manufacturing and use) since 2009 (12). The Stockholm Convention is implemented in the European Union (EU) by the “POP Regulation” (no.

2019/1021) (13). As the use of PFOA, PFOS, and PFHxS are phased out, they are commonly called “legacy PFAS”.

One reason why only three of many PFAS are regulated is due to the time-consuming process of individual-substance testing of toxic effects. So far, only <1% of all PFAS have been evaluated for toxicity, which illustrates that it is not possible to assess every individual PFAS within a reasonable time frame. If authorities instead would manage all PFAS as a class, rather than as individual substances, it would facilitate this process, and the risk of replacing well-studied hazardous substances with new structurally similar, but unstudied, substances would then decrease (14). In 2023, the Swedish Chemicals Agency together with authorities in Germany, the Netherlands, Norway, and Denmark presented a proposal to regulate PFAS in the EU, stating that all PFAS should be assessed and regulated as one class (7). Further, the proposal stated that all PFAS should be banned, but with use-specific time-limited derogations based on the availability of alternatives to PFAS.

The concentration of PFAS in drinking water is regulated in the EU under the Drinking Water Directive from 2020 (EU 2020/2184) (15). This is a minimum directive, where each member state can introduce stricter legislation. The Swedish Food Agency decided in 2022 to present a regulation that will limit PFAS in drinking water to 4 ng/L for 4 PFAS (PFOS, PFOA, PFHxS, and perfluorononanoic acid (PFNA)) and 100 ng/L for 21 PFAS (4 PFAS and additional PFAS), which will enter into force 1 January 2026 (16). These levels were based on a report authored by the European Food Safety Authority (EFSA) in 2020, which introduced a new safety threshold of 4.4 ng/kg of body weight per week for the sum of PFOS, PFOA, PFHxS, and PFNA (9). The PFAS concentration action limit in drinking water in Sweden has been 90 ng/L for 11 PFAS since 2014 (17). In 2022, the European Commission presented a proposal, which was approved by the European Commission in 2023, to revise the list of pollutants in surface- and groundwater, which needs to be monitored and controlled. This proposal added PFAS to the list for both surface- and groundwater pollutants, which will require the EU member states to monitor them and ensure that the concentration limits are not exceeded (18). Finally, in 2023 new PFAS regulations regarding concentration limits in meat, fish, and eggs were introduced in the EU (EU 2023/915) (19) after the report by EFSA was released (9).

Environmental PFAS contamination

A network of journalists from several European countries has gathered data to build a map of the PFAS contamination in Europe, called “The Forever Pollution Project” (20). The results show that there are more than 17,000 sites that are contaminated all over Europe, including 20 PFAS manufacturing facilities. More than 2,100 sites can be considered “hotspots”, i.e., areas with PFAS contamination levels that are considered to be hazardous to the health of exposed people.

On a global scale, PFAS hotspots that have been discovered and researched include the Ohio River Valley in the USA (21), the Veneto region in Italy (22), Korsør in Denmark (23), and Ronneby in Sweden (24). The contamination from C8 and Veneto is from industrial emissions, while in Korsør and Ronneby it originates from firefighting foam use. The difference in contamination sources is reflected in the PFAS exposure profile – generally, the areas that are contaminated by industrial facilities have high levels of PFOA, while the areas with AFFF contamination have high levels of PFOS and PFHxS. The serum levels in the populations from the different hotspots are visualized in Figure 2. The serum levels in the populations affected by these contamination hotspots can have up to a hundredfold the levels of background exposed populations (24).

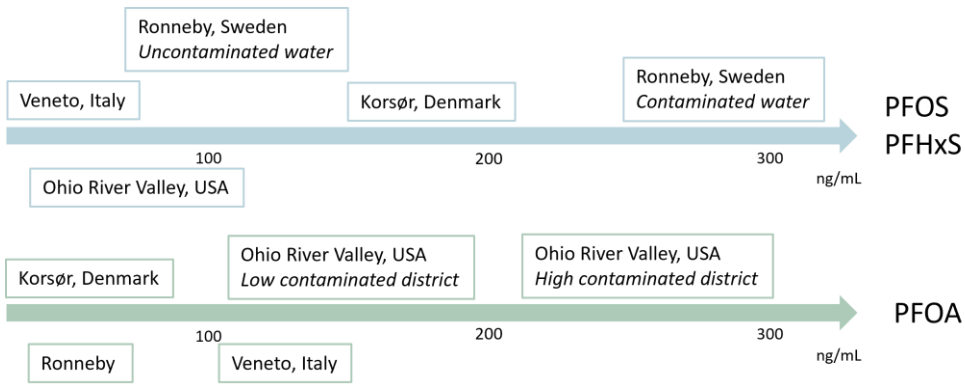

Figure 2. Overview of contamination at hotspots.
PFAS levels in serum samples from the populations of the hotspot areas Ronneby in Sweden, Veneto in Italy, Ohio River Valley in the USA, and Korsør in Denmark. The picture is adapted from an overview in a research report from The Ronneby Research Program (25).

Human exposure to PFAS and health effects

Human exposure routes

PFAS, especially PFOS, PFOA, PFHxS, and PFNA, are measurable in most human populations from Europe (26-31), the USA (32, 33), Australia (34), and China (35). This gives reason to believe that almost everyone in the world is exposed to PFAS.

The common routes for PFAS exposure in background-exposed adult populations are through food, drinking water, indoor air, and dust (9, 36). Contamination of food can come from two processes – bioaccumulation in aquatic and terrestrial food chains, and transfer from materials that are in contact with food such as non-stick cookware (9) or packaging (1). An investigation of Swedish drinking water in 2021

showed that water treatment plants, that served drinking water to 5.8 million people, had detectable levels of PFAS. Notable is that not all municipalities test their drinking water, and some municipalities do not test all their water treatment plants (37). The exposure from the indoor environment is probably dominated by exposure from house dust (38) from historical and current uses of PFAS in electronics (flame retardants), construction products (additives in paint and coatings), household products (floor polish and cleaning agents), etc. (39). During the lifetime of these products, they abrade and shed material components, which can end up as house dust. There can also be volatilization from consumer products into the air (40).

Toddlers are especially exposed to dust via hand-to-mouth contact or by putting items into their mouths (40), and it is estimated that dust is equally responsible as food for PFAS exposure in 2-year-old children (41). Children also have a larger consumption of both food and drinking water and also breathe more air per kg body weight, than adults (40). All of these pathways may increase their exposure to PFAS. Infants are also exposed to PFAS through breastfeeding as PFAS is transferred from maternal serum into the milk (42).

Time trends

Human biomonitoring is used to assess exposure to environmental chemicals in populations. In Europe, Human Biomonitoring for EU (HBM4EU) was an EU-funded project that ended in 2022, working to coordinate and advance biomonitoring with 30 countries participating (43). HBM4EU had PFAS on its priority-substances list, highlighting the need for a better overview of PFAS in Europe.

PFAS has been monitored in European populations over the last decades to create an understanding of the time trends (27-30, 44, 45). These studies all see an increase in serum levels of PFOA and PFOS up until around the year 2000, and then a decrease. This might be explained by the voluntary phase-out of PFOA and PFOS by the manufacturer 3M in 2002 (46), and the inclusion of these substances into the Stockholm Convention. Other substances such as PFHxS and PFNA do not show a strong time trend in the studies, but there is a tendency towards a decline. This indicates that restrictions and voluntary phase-out of PFAS have had an impact on PFAS serum levels in several populations in Europe.

As PFOS and PFOA started to be phased out, manufacturers replaced these compounds with new short-chained alternatives, for example, hexafluoropropylene oxide dimer acid which has the tradename GenX, and perfluorobutane sulfonic acid (PFBS) (47). All new substances that are used might not be included in laboratory analyses yet, which means that only a few of the thousands of existing PFAS are measured in time trend studies, and it is not possible to conclude if a decrease of one PFAS means an increase in another (44).

The serum levels in the Swedish population are reported in two different study populations in recent studies within the National Environmental Monitoring Program by the Swedish Environmental Protection Agency. One study with serum levels from men and women in the last year of upper secondary school in Scania in 2017 showed the levels (μg/L); PFOS 2.54, PFOA 1.08, PFHxS 0.29, and PFNA 0.38 (48). The other study presented similar levels (measured in ng/g, which can be assumed to be approximately as μg/L (49)); PFOS 3.2, PFOA 1.0, PFHxS 1.8, and PFNA 0.4, sampled 2017-2019 in primiparous women 3 weeks after delivery in Uppsala (50). The higher level of PFHxS in women from Uppsala might be because of earlier drinking water contamination (51).

These PFAS levels from Sweden are comparable to a sample of women aged 16-49 from the general population in the USA 2017-2018 (52). However, compared to 16–30-year-olds in background exposed populations in Australia 2016-2017, the Swedish levels are somewhat lower (34).

Toxicokinetics

Due to the hydrophobic carbon-fluor chain, PFAS does not accumulate in lipids (53) but has an increased affinity for proteins (54). A study showed that PFAS could be measurable in biopsies from the human liver, brain, lung, kidney, and bone in different compositions of substance and concentration (55). PFAS can also be found in the non-cellular blood fraction, where it binds to albumin with high affinity, which is why it is primarily measured in serum or plasma in humans (56). Over 90% of PFAS in blood is bound to albumin, and the rest is unbound (57). The number of PFAS molecules binding to each albumin has been reported to be between 1 and 50.

PFAS are eliminated from the human body by urine and feces (58), and for women, menstruation, pregnancy, and breastfeeding are also contributing to eliminating the body burden (42, 59, 60). Continuous exposure to PFAS causes bioaccumulation, as the excretion is slowed down by the binding to albumin, enterohepatic circulation (reabsorption from bile back into blood and the liver), and active reabsorption of PFAS in the kidneys (61). The reabsorption in the kidneys is due to transport proteins that actively reabsorb PFAS from pre-urine into the proximal tubule cells in the kidneys, and thereafter reuptake into the blood (62). One transporter important for reabsorption in humans is the organic anion transporter (OAT) 4, but other transporter proteins such as OAT1 and OAT3 may also be of importance (61).

The half-life of a substance means the time required for the substance to be eliminated to half of the measured level, which can be investigated with repeated measurements over time in a study population. There might be differences in half-lives between species, for example, for PFAS between rats and humans. The reason for rats having a much shorter half-life of PFAS compared to humans is due to not having OAT4 expressed in their kidneys (63).

The half-lives for eight-carbon PFAS are longer compared to four-carbon PFAS, and longer for sulfonates compared to carboxylates (62). The PFAS with the shortest reported serum half-life is 25.8 days for PFBS (64), while for PFOS it is 3.4 years, for PFOA 2.7 years, for PFHxS 5.3 years (58), and PFNA 3.5 years (65). Differences in half-lives between PFAS may be explained by, for example, how well it is transported by OAT4 (61), or binding affinity to albumin (66).

There can be variability in half-lives between individuals, and also between the sexes (58), which can be accounted for by the extra elimination routes for women (menstruation, childbirth, and breastfeeding) (67). An example from the highly PFAS-exposed population in Ronneby (24) showed the importance of these elimination routes, as the sex difference between PFAS levels was the highest at the ages 20-50 years, with women having lower levels. Between the ages of 51-60 years, the difference between men and women decreased. Some individual variations in half-lives cannot be explained by these variables but are instead possibly arising from physiological factors (58).

The long half-lives of PFAS can be especially troublesome for populations exposed to hotspot PFAS contamination. For example, children who were exposed to high PFOS and PFHxS levels from drinking water in Ronneby, Sweden, are not expected to have the same levels as the background population until they are 60-70 years old (58). The consequence is that children born to highly exposed mothers will have PFAS transferred to them from the mother during pregnancy and breastfeeding, affecting a second generation as well.

Effects on the human body

PFAS is an endocrine disruptor, which according to the definition of the World Health Organization (WHO) means "…an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or sub(populations)" (68). The mechanism of action of PFAS is still unclear, but there are two major potential molecular mechanisms through which PFAS might act on the endocrine system: impact on steroidogenesis (the process where cholesterol is converted to steroid hormones) and interaction with nuclear receptors (69).

PFAS might affect the steroidogenesis in itself, or act by disturbing the cholesterol homeostasis, which is the precursor to all steroid hormones. A review by Mokra et al. (69) reported that the steroidogenesis was disturbed by PFAS both through alteration in the expression of genes involved in sex-hormone synthesis, and by lower levels of an important steroid hormone precursor, used for the synthesis of androgens and estrogen. Further, it was reported that disturbances in liver cell function and alteration through peroxisome proliferator-activated receptors

(PPARs) could be critical for cholesterol homeostasis, which could affect steroidogenesis (69).

The stimulation of the sex hormones testosterone, dihydrotestosterone, and estradiol is regulated via the nuclear receptor superfamily, a large group of proteins important for several cell events, which functions as ligand-activated transcription factors (70), mainly the androgen receptor and the estrogen receptor. Also, the thyroid hormone receptor is a member of the superfamily. PFAS has been reported to interact with both the estrogen- and thyroid hormone receptors and disrupt the function of estrogen and thyroid hormones. Several PFAS have an affinity to the estrogen receptor and androgen receptor and have been reported to inhibit the binding of testosterone to the androgen receptor. PFAS are also thought to disturb the thyroid feedback loop, possibly resulting in alterations in the levels of thyroid hormones and the thyroid hormone homeostasis (69). The involvement of sex hormones in the PFAS route of action would explain differences in health effects between the sexes.

Other members of the nuclear receptor superfamily are PPARs. There are three isoforms of the PPAR, α, β/δ, and γ, but PPARα is generally reported to be the targeted isoform for PFAS activation (71). Several PFAS have been shown to activate PPARα (72) which is involved in lipid metabolism, cellular differentiation and growth (73), and inflammatory responses (74). PPARs have been described as interacting with both the innate and the acquired immune system, and EFSA concluded in their latest report that they could be involved in PFAS immunotoxicity, but a detailed understanding of its involvement is lacking (9). Also, all three isoforms are expressed in the placenta, and dysregulation of PPARα and PPARγ has been reported to be involved in the pathophysiology of both gestational diabetes mellitus and preeclampsia (71). There is strong evidence for PPARs as targets for metabolic disease, as they regulate cellular energy and lipid homeostasis (75). Finally, both PPARα and PPARγ are involved in several important mechanisms in both the developing and the adult brain (76, 77).

The dose-response curve of PFAS is not known, but there is a hypothesis that it might be non-monotonic, which is common for endocrine disruptors, and have the shape of a U, or an inverted U (78, 79). A U-shaped dose-response curve suggests that the effect of PFAS on human health at intermediate exposure levels may be different from the effects at low or high levels.

Depending on exposure characteristics such as magnitude, duration, route of exposure, and individual factors such as age, sex, health, and genetic predisposition, PFAS have the potential to produce a wide range of adverse health effects (80). According to the EFSA report from 2020, there is evidence for PFAS to have an effect on birth weight, vaccine response, clinical infections, cholesterol, and liver damage (9). There are also studies showing associations between PFAS and several additional health outcomes, as reported by the European Environment Agency (81).

Here, thyroid disease, kidney cancer, and testicular cancer are considered to be associated with PFAS exposure with high certainty, while breast cancer, ulcerative colitis, increased time to pregnancy, increased risk for miscarriage, and gestational hypertension and preeclampsia are considered to be associated to PFAS exposure with lower certainty. The European Environment Agency also reported that prenatal exposure to PFAS might be associated with delayed mammary gland development, low sperm count and mobility, obesity, and early puberty onset in the child (81). Figure 3 provides a visual summary of the health effects associated with PFAS exposure.

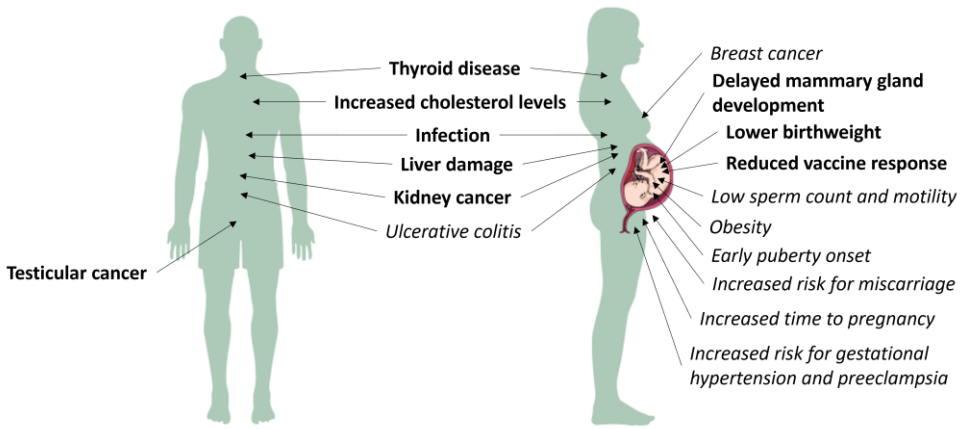

Figure 3. Health effects from PFAS exposure
Health effects from PFAS exposure in men and women, and in children after prenatal exposure. Health effects in bold have more consistent evidence, while those in italics have less evidence. The figure was inspired by a picture from the European Environment Agency (81), and additional information was retrieved from a report from the European Food Safety Authority (9).

There are several different health outcomes reported to be associated with high exposure to PFAS in different hotspot populations. Compared to settings with background exposure, the PFAS profile is often unique in each hotspot, and the results may therefore not be translatable to other hotspot populations. Research from Ohio River Valley, USA, with high levels of PFOA, has reported associations between high exposure and elevated cholesterol and increased risk of ulcerative colitis, thyroid disease, testicular cancer, kidney cancer, and gestational hypertension (82). In Veneto, Italy, where exposure was also dominated by PFOA, research has reported associations between high exposure and elevated blood lipids (83), an increased risk of behavioral problems in children (84), and newborns being small for gestational age (85). A Veneto study on mortality found statistically significant associations with several health outcomes, for example, kidney and breast cancer, diabetes, and myocardial infarction (86). Health outcomes that have been reported to have an association with the PFAS contamination in Ronneby,

Sweden, with an exposure profile dominated by PFOS and PFHxS, are elevated cholesterol, reduced breastfeeding duration, and increased risk of diabetes mellitus type II, kidney cancer, testicular cancer, fractures associated with osteoporosis and polycystic ovary syndrome (25).

In the EFSA report from 2020, the tolerable weekly intake (TWI) was established for 4 PFAS (PFOS, PFOA, PFHxS, and PFNA) to 4.4 ng/kg body weight to describe safe levels of intake. This implies that this is the maximum amount of 4 PFAS that can be ingested in food, every week for a whole lifetime, to avoid adverse health effects. The main critical health effect when determining the TWI was considered to be decreased antibody response to childhood vaccinations. This differed from the previous report from EFSA in 2018 when increased cholesterol was considered the main critical health effect (87). EFSA also concluded that more studies on the effects of PFAS exposure on the immune system should be conducted, both regarding response to vaccines, but also regarding other immune outcomes, such as the risk for infections (9).

In the report from several European countries that supports the proposal for restriction of PFAS in Europe, it is stated that if the contamination of PFAS is not minimized, it will increase until effects are inevitable, and by that time, the exposure will be irreversible (7). This statement highlights the fear that today's background levels might not be associated with health effects, but continued use of PFAS may eventually lead to elevated levels with consequences for human health.

Prenatal PFAS exposure

PFAS exposure is thought to have the most negative effects during pregnancy, infancy, childhood, and adolescence due to the dynamic developmental processes taking place (40). The *in-utero* environment can affect both the antenatal and future health of a child and exposure to endocrine disruptors during sensitive developmental time windows has the potential to irreversibly alter the risk of future morbidity among children (88). It is a well-known hypothesis that the prenatal environment decides the health of the child, commonly known as the developmental origins of health and disease (DOHaD) (89).

PFAS is transferred from mother to fetus during pregnancy via the placenta. Positive correlations have been reported between maternal and fetal serum PFAS concentrations for long-chained PFAS (90), but the efficacy of transfer might vary by PFAS compound. The transfer efficacy likely depends on the perfluorocarbon chain length, isomers, and functional groups (91). It can also be concluded from previous studies that it might be that the transfer efficacy has a U-shaped relationship with fluoroalkyl chain lengths, with short and long chains having high transfer efficacy, while those in between have lower (90, 91). It is also reported that branched isomers might cross the placenta to a greater extent than corresponding

linear isomers (92). When it comes to functional groups, PFAS with a carboxyl group seems to have a higher transfer efficacy than PFAS with a sulfonyl group (90, 91). It is assumed that only the non-protein bound PFAS fraction can be transferred over the placenta (93). Therefore, the binding affinity to plasma proteins is one of the hypothesized determinants for transfer efficacy, where lower affinity would mean a higher fraction of free PFAS available for transfer (92).

The ratio between maternal and cord serum levels of PFAS can be used to calculate and compare the transfer efficacy. Generally, the results in previous studies have been below 1, meaning that there is a higher concentration of PFAS in the mothers' serum (40). It is not clear which mechanisms are responsible for the placental transfer of PFAS, but it might, based on previous research on drug transfer in the placenta, involve both passive transport and active transport with transporter proteins. It is suggested that OAT4 receptors are involved in PFAS placental transport (94). The binding of PFAS to transporter proteins might either facilitate the transmission or restrict it (91).

The fetus can be directly affected by PFAS during pregnancy as PFAS may disturb the balance in many hormonal processes required for normal growth and development (95). The fetus may also be affected indirectly because insults to the mother's health may adversely affect the uterine environment (96). This can be the case if the mother develops e.g., preeclampsia, an outcome for which the literature suggests an association with PFAS exposure (97), that can have adverse effects on the child from a young age and through the life course (98).

The placenta is thought to be a possible target for PFAS, as it shares features with other known target organs, and is involved in the etiology of pregnancy disorders that have been associated with PFAS exposure (99). Fetal growth and development depend on proper placental function, and dysfunction in the placenta can be associated with poor health outcomes. PFAS might interact with several molecular targets in the placenta, but there is strong evidence that PPARs are involved. In the placenta, PPARs regulate trophoblast differentiation and function, which PFAS might interfere with and result in diseases like preeclampsia and gestational diabetes (71).

PFAS epidemiology

The definition of epidemiology has changed over time but can be summarized as "the study of distribution and determinants of disease and health in the population" (100). Further, environmental epidemiology studies the effect on human health of environmental factors, such as physical, biological, or chemical exposures (101).

To assess health risks from environmental contamination, toxicological research gives valuable insight into the toxicokinetics, i.e., absorption, distribution, metabolism, and excretion of a toxicant, and toxicodynamics, i.e., the interaction of a toxicant with a biological target and the effect. However, differences across species and non-representative exposure levels might pose challenges for the translation of results from experimental studies to humans. Environmental epidemiology helps with these challenges by collecting data from events occurring in defined populations. Toxicological and epidemiological research are both needed for human health risk assessment, to successfully create interventions to protect health (102).

To be able to investigate the health effects of PFAS exposure in humans, epidemiological studies are used – both in background-exposed populations and highly exposed populations. The results from these observational studies, which may include large populations, can be used to identify target areas for future research aiming to determine causation.

Study designs

Epidemiological studies are categorized into observational- and experimental study designs. In observational studies, the researcher observes the cohort and does not intervene, while in experimental studies the researcher intervenes and can decide which participants will receive treatment or intervention and which will be untreated. To decide on which study design to apply depends on several factors, where the most important are the research question and resources, such as time and funding (103). If the research question involves studying a rare disease, a case-control study might be the right choice of design, while if the research question regards cause and effect, a cohort study is a fitting design as exposure and outcome are measured in chronological order.

Research ethics is an important factor to consider when designing a study. It is not possible to expose a population to high levels of PFAS to investigate the effects. Instead, observational studies must be used. Sometimes the event of a hotspot is called a "natural experiment", meaning that the situation simulates an experimental setting. For example, in the Ronneby drinking water PFAS contamination case (24), one part of the population received contaminated drinking water in their homes while one part received uncontaminated drinking water – which resulted in a setting where the effects of PFAS exposure could be explored if an experiment would have been conducted, but in an observational study design.

This thesis focuses on observational studies, and the designs that are used in this thesis will be presented below.

Cohort study design

Cohort studies measure the occurrence of disease within one or more cohorts with well-defined exposure assessments and are the original epidemiological studies. A cohort can be defined as "any designated group of individuals who are followed or traced over time" (104). This study design can involve comparing the rate of an outcome between exposed and unexposed individuals. The exposure is the factor whose effect is investigated, which might be e.g., a specific disease or, as in this thesis, PFAS exposure. In cohort studies, the exposure is known before the outcome status. An important requirement for being in a cohort is that cohort members must be at risk for the outcome, which is why a cohort can be described as a "population at risk". Cohort studies start with participants that do not have the outcome in question, and follow them over time, to observe who develops the outcome. Establishing a cohort often takes many years, as the outcome under study might be a disease that takes a long time to develop or that is rare. It is therefore convenient to use already existing population-based registers to follow cohorts. Using population-based registers in research also increases the sample size, and hence the statistical power of the analysis.

Case-control study design

In contrast to cohort studies, case-control studies start by identifying individuals that have the outcome (i.e., the cases), which is then known before their exposure status is assessed. This is a design that fits well when investigating rare outcomes.

The cases and the controls should come from the same underlying population and should be chosen independently of exposure status. Exposure status is then compared between cases and controls. The cases and controls should be as alike as possible to ensure that the difference between them depends solely on their exposure status and not on any other factors. Cases and controls may be matched on characteristics such as sex and age to handle variables that might confound the association between the exposure and the outcome. However, a drawback is that the effect of the matching variables cannot be investigated.

The design of a case-control study is, compared to a cohort study, more time and cost-effective, especially if the outcome of interest takes a long time to develop or is rare, and requires a smaller sample size.

Systematic error

Epidemiological studies may contain systematic or random errors. Systematic error is also called bias and can arise from the way subjects have been selected for a study, the way study variables are measured, or from factors that may confound the association between exposure and outcome. Random error is what is left after the

systematic error has been eliminated and can be defined as variability in data that cannot be explained.

A systematic error continues to be of the same magnitude regardless of how large the study sample is, while random error decreases with a larger study sample to the point that there is almost only systematic error left in a sufficiently large study (104).

The most common systematic errors in epidemiological studies are outlined below.

Selection bias

Selection bias may arise from the procedures that are used to select study participants, and from factors that may affect willingness to participate. When there is selection bias, the association between exposure and outcome is different in the participants in the study, compared to the ones who do not participate (104).

Selection bias can affect both the internal and the external validity of a study. Internal validity is a measure of how well results in a study reflect the true value in the study population while external validity is a measure of how generalizable the results from the study are to another setting. If there is no reason to believe that the associations shown in a study would be different in another population, the external validity is high, and the results are generalizable.

Information bias

This type of systematic error arises from the inaccuracy of measuring variables in a study. If inaccurate information leads to a study participant being categorized in the wrong way, it is called misclassification bias. If the probability of misclassification is the same for all participants in the study, regardless of e.g., exposure and outcome status, the bias is non-differential. If the risk of misclassification depends on exposure or outcome status, the bias is differential (105). In most cases, nondifferential misclassification results in a bias towards the null, which would underestimate a result. Differential misclassification, on the other hand, may depending on the circumstances either underestimate or overestimate the results. Another type of information bias is recall bias, where the accuracy of the reported information differs depending on outcome status.

Confounding

A definition of confounding is “confusion of effects”, which means that the effect of the exposure on the outcome is confused with the effect of another variable. A confounder must be associated with both the outcome and the exposure but cannot be an effect of the exposure (104). Confounding can be separated into measured confounding, for which data is available and adjustment therefore is possible, and unmeasured confounding which is the confounding from unmeasured variables.

Confounding can be accounted for in the study design by randomization, restriction, or matching. Randomization can be used in experimental studies, where participants are randomly assigned either to the treatment group or to the control group. The confounding factors are then evenly distributed over the two groups, and their effects are minimized. Restriction can be used in both experimental and observational studies, where the study population is chosen based on the confounding factor, e.g., if age is a confounder, the study can be performed with only persons within a certain age interval. Matching is used in case-control studies, where persons with the outcome are matched with persons who do not have the outcome based on confounder status. There are two kinds of matching in case-control studies: individual and frequency matching. The individual matching involves matching one or more controls to each case, to be as similar as possible on the confounding variables at an individual level. On the other hand, frequency matching involves the cases and controls on a group level and aims to have a balance in the matched confounding variables between the groups.

Confounding can be handled in the analytical part of the study, by adjustment of one or several confounders in the statistical model, or by stratification. Stratifying means that different groups are created, in which the confounding factor does not vary, e.g., if sex is a confounder, the study population is divided into men and women, and a separate analysis is performed in each group. The disadvantage of stratification is that the effect estimates from the different strata cannot be compared.

Directed acyclic diagrams (DAG) can be used as a tool for visualizing causal pathways and for identifying relationships between variables with the exposure and the outcome, which determines how it should be handled in the statistical analysis. These diagrams are called directed, as arrows show the direction by which a variable causes another, and acyclic because there are no cycles as a variable cannot cause itself (106). An arrow between two variables suggests an association, and a direction, while the absence of an arrow suggests no association. *Confounders* are variables that are associated with both the exposure and the outcome. When a confounder is adjusted for in the statistical model, the effect of that factor is "taken away" from the association between the exposure and the outcome, and the association is not biased by the confounding factor. A *collider* is also a variable associated with both the exposure and the outcome, but that is a cause of these. A collider should not be adjusted for in statistical models, as that might introduce bias from "backdoor paths", which is a non-causal path that opens up when the collider is conditioned on, causing spurious associations. A *mediator* is a variable on the causal pathway between exposure and outcome and should not be adjusted for in statistical models if the aim is to measure the total effect of exposure on the outcome. Unmeasured confounding, i.e., variables where data is missing or where data cannot be collected, can be represented by a latent variable in a DAG. Figure 4 shows an overview of different variables in a DAG.

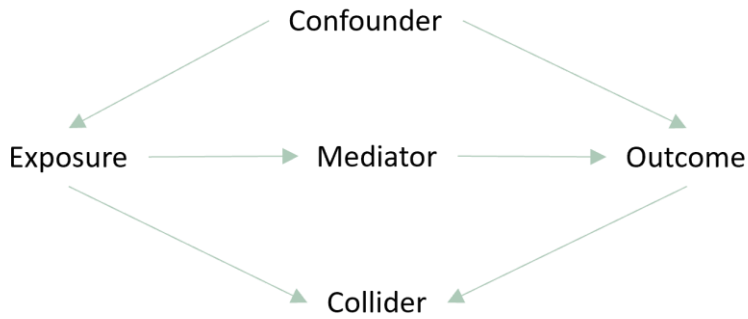

Figure 4. Directed Acyclic Graph (DAG)
Using the DAG as an analytical tool, confounders, mediators, and colliders can be identified.

Effect modification

Effect modification can be explained as an association that varies over different strata, for example, if an association between an exposure and an outcome varies between men and women. Effect modification can be addressed using two methods: stratifying the data or introducing an interaction term in the statistical model. By stratifying the data, the two strata are analyzed separately. Stratification is a simple way to handle effect modification, but it assumes that all variables that are included in the modeling vary by the strata, not only the variable that is an effect modifier. The major drawback of this method is that the estimates from the two strata cannot be compared.

If effect modification is handled in the statistical model, a multiplicative factor is added between the exposure and the effect modifier. Then, all variables except the effect modifier are allowed to have the same effect over the strata where the effect modification is. This method results in one statistical model, which not only increases the power but also makes the estimates comparable.

Aim

Overall aim

The overall aim of this thesis was to use epidemiological methods to investigate the effect of PFAS exposure on maternal health and child health and development.

Specific aims

- To investigate if high pre-pregnancy exposure to PFAS is associated with gestational hypertension, preeclampsia, and gestational diabetes mellitus.
- To investigate if prenatal exposure to PFAS is associated with child overweight at 4 years of age.
- To investigate if high prenatal exposure to PFAS is associated with developmental language disorders in children up to 7 years of age.
- To investigate if high prenatal exposure to PFAS is associated with common infectious diseases among children up to 7 years of age.

Methods

Study design

Study I

A cohort design was applied to investigate the association between pre-pregnancy PFAS exposure, assessed using residential address history as a proxy, and pregnancy complications using national register data on antenatal health.

Study II

A case-control design was used to study the association between prenatal exposure to PFAS, measured in maternal serum in early pregnancy, and overweight in 4-year-old children who had visited child health care centers in Malmö, and whose parents had answered a self-administered questionnaire.

Study III & IV

A cohort study was applied to investigate the association between prenatal PFAS exposure, assessed using residential address history as a proxy, and the outcomes, using data from regional health care registers on primary care visits.

Study participants

The Ronneby population

Studies I, III, and IV investigated health effects in Ronneby, with a population highly exposed to mainly PFOS and PFHxS from contaminated municipal drinking water. The contamination was discovered in December 2013, when the routine water quality monitoring for the first time included PFAS. The contamination came from one of the two water treatment plants, which immediately was closed. At the time, the contaminated water treatment plant provided 1/3 of the population of 28,000 individuals with drinking water. The levels in drinking water were measured at 8,000 ng/L PFOS and 1,700 ng/L PFHxS (24), which can be compared to the Swedish action limit at the time for the sum of 11 PFAS, which was 90 ng/mL (17). The source of the contamination was a military airfield where recruits had training

with firefighting foam containing PFAS. It is still unclear when the use of AFFF with PFAS started in Ronneby, but it is assumed to be in the mid-1980s according to military purchase records.

After the end of exposure to the contaminated water, the exposed population's geometric means of serum concentrations for PFHxS, PFOS, and PFOA were 114, 135, and 6.8 ng/mL, respectively (24). Compared to the levels of a background-exposed population in the same county (i.e., Blekinge), the exposed population had 135 times higher levels of PFHxS, 35 times higher levels of PFOS, and 4.5 times higher levels of PFOA. The biomonitoring also made it clear that there was a need for an external reference group, as the part of the Ronneby population who had not received contaminated water also had elevated PFAS concentrations.

Study I

Women who had a residential address in Blekinge County sometime between 1990-2013, followed by childbirth between 1995-2013 were included. The study included 18,626 women and 31,433 pregnancies.

Study II

Children who participated in the routine 4-year health screening at child health care centers in Malmö between 2003-2008 and whose parents answered a questionnaire. The study included 9,009 children.

Study III

All children born between 1998-2013 who had a residential address in Blekinge County for at least one year from birth to age 7, with mothers who had a residential address within Blekinge for at least one year before childbirth. The study included 15,895 children.

Study IV

All children born between 2003 and 2013, with mothers who had lived in Blekinge County for at least one year within the five years before childbirth. The study included 17,401 children.

Data sources

National Total Population Register

We used the National Total Population Register to identify the study population in Studies I, III, and IV. The National Total Population Register is an excerpt from the

Population Register administered by the Swedish Tax Agency and contains information about personal identification number, sex, marital status, residential address, births, deaths, etc. The register covers all individuals who are registered as Swedish residents and is held by Statistics Sweden. For Study IV, the Multigeneration Register, which is part of the National Total Population Register, was used to link mothers to their children.

Longitudinal Integrated Database for Health Insurance and Labour Market Studies

Variables from the Longitudinal Integrated Database for Health Insurance and Labour Market Studies (LISA) register were used in Studies III and IV to control for confounding. LISA is held by Statistics Sweden and contains annual information about education, employment, income, sick benefits, etc. The register covers annual data on all individuals that are aged 16 and older (from 2010, 15 years and older), born from 1990 and forward, who had a residential address in Sweden on the 31st of December each year. In Study I, data from the register over Education of the Population, which is one of the registers that contribute to LISA, was used to control for confounding by socioeconomic variables.

National Medical Birth Register

We used the National Medical Birth Register in Study I to assess outcome variables, and in Studies I, III, and IV to retrieve data on potential confounders. The National Medical Birth Register contains information about pregnancies, births, and newborn children, and includes variables like smoking in pregnancy, parity, clinical International Classification of Diseases (ICD) (107) diagnoses for mother and child, sex, weight, and length of the child, etc. The register covers pregnancies that ended in childbirth in Sweden since 1973, including live births and stillbirths from 22+0 gestational weeks. It is held by the National Board of Health and Welfare. Healthcare providers are obliged by law to report antenatal, obstetric, and neonatal data to the National Medical Birth Register (108).

Blekinge Healthcare Register

The Blekinge Healthcare Register covers all consultations in primary and specialized health care in Blekinge County. Information about primary and secondary causes is registered as ICD-10 diagnoses. The register also holds information regarding the type of consultation, i.e., physical visit or by telephone.

The coverage of healthcare consultations in this register is assumed to be high, as it is necessary to record into the register for financial reimbursement purposes for the healthcare provider.

Nationally, healthcare registers only include specialized care and Blekinge is one of a few regions in Sweden that hold a healthcare register that includes primary care.

The Southern Sweden Maternity Cohort

The Southern Sweden Maternity Cohort includes Scania women from 1986 and forward who were routinely screened for rubella immunity in gestational week 14 and who had a stored serum sample in Region Skåne's Biobank. Women could opt out of having the sample biobanked for use in future research.

Questionnaire in Study II

The questionnaire in Study II was administered to parents at the routine 4-year health screening at child healthcare centers in Malmö between 2003-2008. It was self-administered and included 32 questions, in Swedish, regarding the health of the child, the family situation, socioeconomic status, etc.

Ronneby Water Distribution Documents

The municipal water company provided information on annual water distribution to individual households between 1980-2013. With this information, it was known which residential addresses that were provided with water from the contaminated water treatment plant during which years. This information could then be used together with the individual residential addresses to assess the participants' exposure status.

Exposure assessment

Chemical analyses

In Study II, PFOS, PFOA, PFHxS, and PFNA were analyzed in biobanked maternal serum. The analyses were performed at the Division of Occupational and Environmental Medicine at Lund University, using liquid chromatography-tandem-mass-spectrometry (LC/MS/MS) (109).

The laboratory regularly participates in the inter-laboratory program the German External Quality Assessment Scheme (G-EQUAS) for analyses of PFOA and PFOS, and it has also participated and been approved in the ICI/EQUAS quality exercises within HBM4EU for PFOS, PFOA, PFHxS and PFNA.

Proxy

In population-based Studies I, III, and IV, it was not feasible to collect serum samples. Instead, we used a proxy for maternal exposure based on residential history. The five years before childbirth was used as the exposure window of interest, which would capture maternal exposure within 1.5 half-lives of PFOS and PFHxS (58). Exposure status was categorized in a hierarchy as:

1. High: Women who had a residential address within Ronneby municipality, with contaminated water, for at least one year during the five years before childbirth.
2. Intermediate: Women who had a residential address within Ronneby municipality, with uncontaminated water, for at least one year during the five years before childbirth.
3. Background: Women who had a residential address in Blekinge County at the year of childbirth, and who had not lived in Ronneby municipality within five years before childbirth.

This categorization was applied to each pregnancy.

Validation of proxy

Before using the proxy variable of exposure in our studies, it was validated against measured serum concentrations in the biomonitoring cohort from Ronneby and Karlshamn, a nearby municipality with background PFAS exposure. We included all women of reproductive age and categorized their exposure based on their residential addresses five years before the sampling. We then compared the measured PFAS concentrations in the exposure categories, visualized in Figure 5. The serum concentrations were higher in the highly exposed group with medians of PFOS 169 ng/mL, PFHxS 129 ng/mL, and PFOA 9 ng/mL compared to the intermediate exposed group with medians of PFOS 48 ng/mL, PFHxS 40 ng/mL and PFOA 3 ng/mL, and the background exposed group with medians of PFOS 4 ng/mL, PFHxS 0.8 ng/mL and PFOA 2 ng/mL.

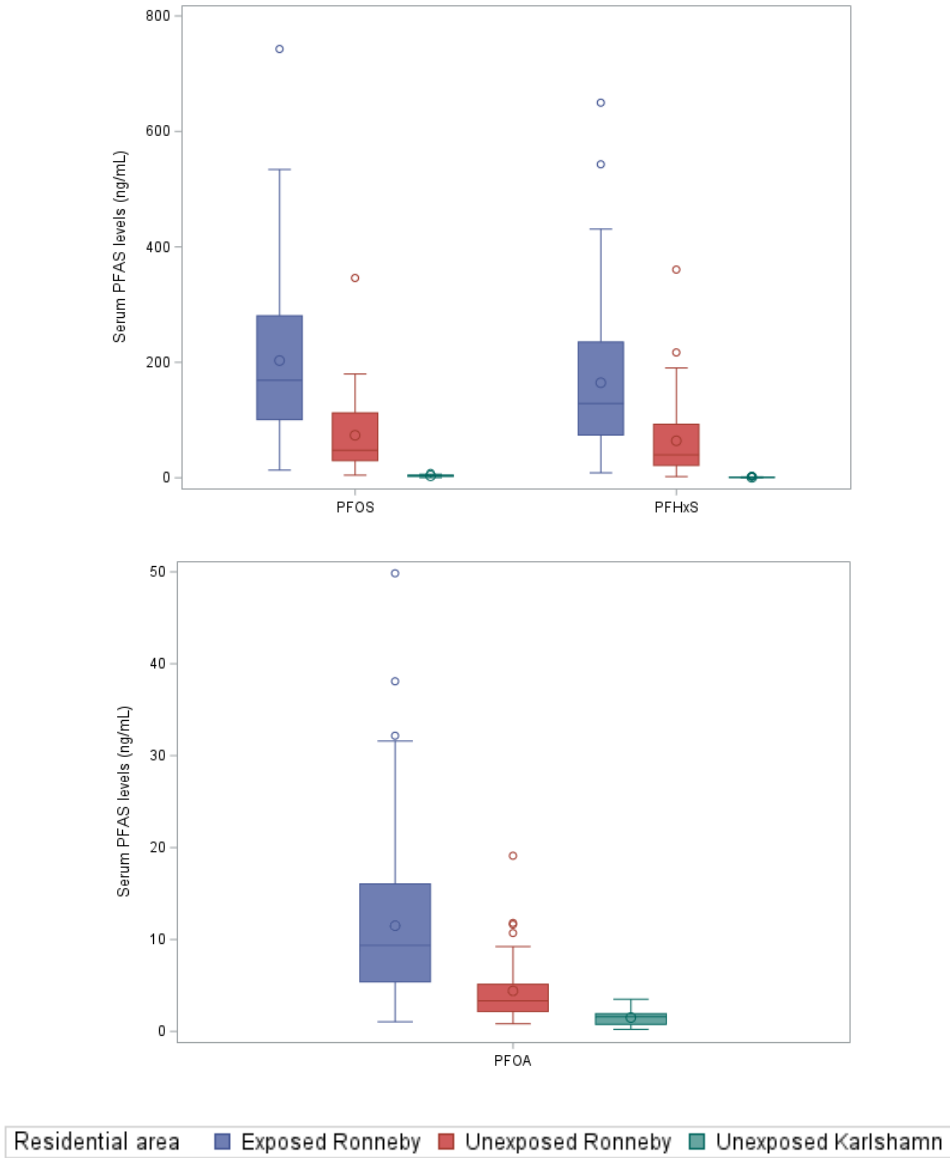

Figure 5. Validation of the proxy variable against measured serum concentrations
Study participants in the Ronneby biomonitoring cohort were categorized based on their historical residential address, and their serum concentrations were visualized in boxplots. The boxplots were previously published by Stübner et al. (110).

Outcome definitions

Study I

The health outcomes that were investigated in this study were gestational hypertension, preeclampsia, and gestational diabetes mellitus. The outcomes were defined according to ICD-9 or ICD-10 diagnoses. As hypertension is a part of the diagnosis of preeclampsia, we chose to analyze these two outcomes together to avoid misclassification, and we performed a sensitivity analysis to investigate this assumption. Women with pre-pregnancy diagnosed hypertension or diabetes mellitus type I or II were excluded from the analysis for gestational hypertension/preeclampsia and gestational diabetes mellitus respectively, as they were not considered at risk of the outcomes.

Study II

The health outcome investigated in this study was overweight at 4 years of age. The cases were defined by age and sex-adjusted body mass index (ISO-BMI) $\geq$18 kg/m^2, and the controls by an ISO-BMI$\leq$17 kg/m^2. To create a contrast between cases and controls, children with an ISO-BMI in between these thresholds were excluded.

Study III

In this study, developmental language disorder was investigated. We used two outcome definitions, with two different professions from different levels of care who defined the outcome: child health care nurses from primary care in outcome 1 and speech and language pathologists from specialized care in outcome 2.

1. Referral to a speech and language pathologist after routine screening at child health care centers: followed by an assessment by a speech and language pathologist.
2. Clinical diagnosis of developmental language disorder, defined as children with an ICD-10 diagnosis within speech, language, or communication set by a speech and language pathologist on at least two occasions.

The clinical diagnosis outcome was also further categorized into subtypes, i.e., expressive language disorder and mixed receptive language disorder.

Study IV

The health outcomes that were investigated in this study were common infectious diseases. The diagnoses were defined based on ICD-10 codes from primary care visits and were categorized into infections in the eyes or ears, upper respiratory tract infections, lower respiratory tract infections, and urinary tract infections. We also included all these diagnoses in a joint analysis of the overall risk of infection.

Statistical analysis

Study I

In this study, we investigated the association between high prenatal exposure to PFAS, using the proxy measure of exposure, and gestational hypertension, preeclampsia, and gestational diabetes mellitus. The confounders were identified *a priori* and included body mass index (BMI) in early pregnancy, birth country, educational status, maternal smoking status in early pregnancy, parity, and maternal age at childbirth.

Patterns of missingness in the confounder variables were investigated and considered to be missing at random. Then, a multiple imputation was performed, including all confounder variables, exposure, and outcome in the imputation model. The fully conditional specification-imputation algorithm was used to enable both categorical and continuous variables in the imputation model. The variables that contained missing data were smoking and educational level, which were modeled using logistic regression, and maternal BMI, which was modeled using linear regression. For each model, 20 imputed datasets were generated.

The association between the exposure and the outcome was modeled by logistic regression with a logit link function, adjusting for all confounding variables. Effect modification by child sex was investigated by introducing an interaction term between the exposure and child sex in the model.

Women with more than one childbirth in the data introduced clusters as their pregnancies were considered correlated. Generally estimated equations were used, together with quasi-likelihood under the independence model criterion-values to choose the exchangeable correlation structure, which assumes the same correlation for all pairs of measurements from the same mother.

Study II

This study investigated the association between prenatal exposure to PFAS, measured in biobanked serum samples, and overweight in children at 4 years of age. A two-step strategy based on risk scores was used, as overweight is a multifactorial disease, and matching cases and controls based on risk strata can decrease the risk of unmeasured confounding. The methodological approach in detail is described elsewhere (111). First, the risk of being overweight was modeled with logistic regression, including confounding variables from the questionnaire: maternal smoking during pregnancy, birth weight, economic strain, being a tenant, maternal obesity, and paternal obesity. Then, the risk model was used to categorize each child with a complete set of confounding variables into one of three risk strata: low (0-5%), intermediate (6-13 %), and high (≥14%) risk for being overweight. The cut-offs were set using a data-driven approach to create contrast between the low and

intermediate strata. All cases were matched with two controls from the same risk strata and were also matched on sex.

Logistic regression with a logit link function was used to model the association between overweight and quartiles of PFOS, PFOA, PFHxS, and PFNA. Analyses were performed both with all PFAS in one model, and also with one model for each PFAS. The modeling was applied both on the full dataset, adjusting for risk strata, the difference from the strata-specific mean, and sex, and stratified on risk strata, adjusting for the difference from the strata-specific mean and sex. Effect modification by risk strata was investigated in the full dataset models.

Study III

In this study, we investigated the association between high prenatal exposure to PFAS, measured with a proxy and categorized into exposure categories, and developmental language disorders. Cox proportional hazards regression was used to estimate hazard ratios (HR) with 95% confidence intervals (CI) between children with high, intermediate, or background prenatal exposure and one of the two outcomes: referral to a speech and language pathologist, and a clinical diagnosis of developmental language disorder. The *a priori* chosen confounders that were adjusted for in the model were parity, maternal age, maternal educational attainment, and maternal smoking in early pregnancy.

The proportion of participants that had missing data in one or more of the confounders was 6%, and therefore we were running the model on a complete case dataset. We used calendar year as the underlying time variable, and children were censored at the outcome, at age 7, or at the end of the study period.

Women with several children in the dataset introduced clusters of correlated observations, that were accounted for by using the robust sandwich covariance estimate. Effect modification by sex was investigated by an interaction term between exposure and sex. We also investigated effect modification by time period with an *ad hoc* cutoff in the year 2005, as we hypothesized that exposure levels would have increased over time, reaching the highest levels towards the end of the contamination.

We checked the proportional hazards assumption by including time-varying covariates in the model and evaluating if they were statistically significant ($p < 0.05$). Parity did not fulfill the assumption and was therefore stratified. In addition, the proportional hazards assumption was not fulfilled in the subgroup analysis, and we therefore stratified parity and maternal age in the model for expressive language disorder, and maternal education in the model for mixed-receptive language disorder.

Study IV

This study investigated the association between high prenatal PFAS exposure, measured by our proxy variable, and common childhood infectious diseases. We used Cox proportional hazards regression with the Andersen and Gill extension for recurrent events to estimate HR. Person-years at risk were included from birth, or for those that were not born in Blekinge County, from the first year with a registered address within Blekinge County. Censoring occurred on the 31st of December in the year the child turned 7 years old, when the child moved out from Blekinge County, in the case of death, or at the end of the follow-up period in 2020. All models were adjusted for maternal smoking status, parity, maternal educational status the year of birth, and maternal age at childbirth.

The analyses were performed on complete cases as the percentage of missingness was low.

We used a washout period of 14 days between events to avoid counting the same infection multiple times. Effect modification by sex was investigated by including an interaction term between exposure and sex in the model for overall infections.

Ethical considerations

All studies included in this thesis have been approved by the Regional Ethics Committee in Lund (Studies I and II) and the Swedish Ethical Review Authority (Studies III and IV). Studies I, III, and IV were register-based studies, where participants had the opportunity to opt out. Study II also had an opt-out opportunity to be part of the study, and participants provided informed consent for using the serum samples for research purposes.

Healthcare data is sensitive personal information. All data used in this thesis was stored and managed according to the General Data Protection Regulation (GDPR) (EU 2016/679). All data in the register-based studies were pseudonymized before we received it, and the code keys are stored at Statistics Sweden. The data in Study II was pseudonymized before the study was conducted. All data was stored at Lund University's secure servers: first at servers that required login, and after GDPR entered into force, at a server with two-step authentication.

To use opt-out in register-based studies instead of gathering informed consent is needed as informed consent would be an impossible task in many cases, reaching out to hundreds of thousands, or sometimes millions, of study participants. If informed consent was required, the register studies that are performed today would not be possible, considering the time and cost the researcher would have to invest. Instead, opt-out is used, where it is possible for each individual included in a study to decline participation. The information about studies, and how to opt-out, was

previously advertised in newspapers, but different platforms for this purpose are now used, for example, Lund University Population Research Platform. An ethical question is if all participants have an opt-out possibility in practice, as not all are reached by the study information and therefore are not aware of their participation. For the research, it is preferred when no one leaves the cohort as it increases the risk of selection bias, and then opting out is a good method, but for the individual who wants to have control over their health data, it is not. The usefulness of the research must then be weighed against the risk of not all participants being aware that their healthcare data is used in research.

Another ethical issue arises when research is performed in a population that has been highly exposed to contamination, as in the case of the Ronneby population. This population has been subject to extensive research during the years since the contamination was discovered, which might have its drawbacks for those that are affected. To repeatedly be invited to participate in different research projects, or to receive information about study results might cause psychological stress as it is a reminder of the contamination. However, it is needed to research the population, as the exposure levels are unique, and no one knows how they affect health. Investigation of the health effects of exposure is necessary to inform decisions on potential interventions needed to protect the health of those who are exposed.

One aspect is also the communication between the researchers and the affected population, as the results need to be communicated. The way this is done is important, as this information should be accessible and understandable for everyone in the population. Our studies from Ronneby have not included analysis of individual samples, but other studies have reported individual serum PFAS levels back to the participants as a way of giving back. The results from studies I and III have been included in a report (25) over the results from the ten years of research that have passed since the discovery of the contamination, which has been published on the Ronneby PFAS Research Program's blog, which is a means to communicate results to the affected population.

Main results

Study I

The results from this study showed no association between high or intermediate exposure to PFAS during the five years before childbirth and the risk of gestational hypertension, preeclampsia, or gestational diabetes mellitus.

Study II

PFHxS exposure had an overall association with child overweight in the multi-pollutant model that included all exposures. Some odds ratios for PFOS, PFHxS, and PFNA were associated with the outcome in specific quartiles, but no consistent pattern was found.

Study III

There was a 23% (HR 1.23, 95% CI (1.03-1.47) increased risk for referral to a speech and language pathologist among children with high prenatal PFAS exposure compared to children with prenatal background PFAS exposure. We found no association between the outcomes and intermediate levels of prenatal PFAS exposure. When investigating possible effect modification by sex, only the results for girls had confidence intervals that did not include 1. Girls with high prenatal PFAS exposure had a 36% (HR 1.36, 95% CI 1.02-1.80) increased risk for referral to a speech and language pathologist and a 62% (HR 1.62, 95% CI 1.12-2.35) increased risk for being diagnosed with a developmental language disorder compared to girls that had prenatal background exposure to PFAS.

Study IV

The result from this study showed no association between the overall risk of infection and high or intermediate prenatal exposure to PFAS. We found a 28% (HR 1.28, 95% CI 1.05-1.55) increased risk of urinary infections and a 9% (HR 1.09, 95% CI 1.00-1.19) increased risk for ear infections in the children with high prenatal PFAS exposure compared to the children with prenatal background exposure. We also found a 6% (HR 1.06, 95% CI 1.02-1.11) increased risk of upper respiratory tract infections and a 12% (HR 0.88, 95% CI 0.81-0.96) lower risk of eye infections for children with intermediate prenatal PFAS exposure compared to children with prenatal background exposure to PFAS.

Discussion

In this thesis, I show that high prenatal exposure to PFAS is associated with increased risks of developmental language disorders among girls up to 7 years of age, and common infections among children up to 7 years of age. I also show that high pre-pregnancy exposure did not increase the risk for gestational hypertension, preeclampsia, and gestational diabetes mellitus. Further, I show that prenatal exposure background levels of PFAS are not associated with an increased risk of overweight in children at four years of age.

Previous studies

Most of the research on health effects from PFAS exposure has been carried out in background levels of exposure, but with the studies in this thesis, I can increase the knowledge about health effects in high exposed populations. The number of populations that have been exposed to high levels of PFAS from a point source such as an industry or AFFF run-off is constantly increasing, which creates a need to understand the consequences of these contaminations. Based on our findings, neurodevelopment and immunotoxicity might be sensitive outcomes after high prenatal PFAS exposure. The results are in line with results from background-exposed populations, which also show an association with language development (112) and immunotoxicity (113-116).

Previous studies investigating gestational hypertension, preeclampsia, and gestational diabetes mellitus have found associations between these outcomes and prenatal PFAS background exposure (117-121), which I did not see in our high exposed population. A possible reason why there are differences in results might be because the effect occurs at low exposure levels. In our study, due to the use of a proxy for exposure assessment, all our low-exposed women were categorized into one group, and we could therefore not distinguish women with very low levels of PFAS from those that have low levels. We might therefore have been unable to detect an effect.

Even if we used a method to investigate overweight as a multifactorial disorder, we did not find an association with prenatal background exposure to PFAS. Some previous studies investigating the association in background-exposed populations have found associations (122, 123), while others have not (124). It may be that the

exposure contrasts in our study, especially for PFHxS and PFNA, were too small to be able to detect a difference between the PFAS quartiles.

In general, associations between PFAS and different health outcomes often differ between studies – where one study finds an association, another does not. There are large differences in study design between PFAS studies that might explain part of those differences. An association could be investigated in the background or hotspot population, and the exposure could be measured in biological samples or by using a proxy. Information about the outcome could be collected by self-assessed measures or retrieved from register data. Further, based on the study design, the studies could have different confounding, selection bias, and information bias, possibly affecting the results. Also, the exposure windows can be different between the studies, measuring PFAS in early pregnancy or during childhood or adult life, which might affect how PFAS is associated with different health outcomes. Taken all these factors together, it is difficult to compare results.

PFAS toxicity

A non-monotonic dose-response curve of PFAS would have implications, as it suggests that the effect in a background-exposed population cannot be extrapolated into populations with higher exposure levels. Therefore, it is of great importance to research PFAS effects in populations exposed to different PFAS levels – from background to high exposure. Understanding the health effects of intermediate exposure levels might be of interest in the future, as emerging hotspots often have exposure levels that fall within this range. Studies I, III, and IV were designed to assess exposure by using a proxy and investigation of crude dose-response relationships. Results from Study III and IV show suggestions for a monotone dose-response relationship, with a higher risk in the high exposed category. The result for gestational diabetes mellitus in Study I showed a tendency toward lower risk of the outcome in the intermediate exposed women compared to the background and high-exposed women, which would be expected if PFAS was acting with a dose-response curve of U-shape. However, studies with measured serum levels including the whole spectrum of exposures are needed to make conclusions regarding PFAS dose-response curve.

The studies in this thesis cannot develop the understanding of possible mechanisms of PFAS effects further, but with epidemiological studies in combination with experimental studies, research can provide new insights. However, mechanisms acting through both steroid hormones and PPARs could, in theory, be likely candidates for PFAS toxicity in relation to pregnancy complications and child health and development. In previous studies, steroid hormones have been shown to have a role in the development of the fetal brain (125) and immune system (126), they play a part in metabolism (127), and are secreted by the placenta (128). PPARs are associated with all the outcomes in this thesis, as previous studies have reported

involvement in the pathophysiology of gestational diabetes mellitus and preeclampsia (71), association with the metabolic state, regulation of several immune cells (129), and involvement in early brain development (76).

Implications of the research

The results from this thesis can be used for risk assessment in hotspots and to make decisions regarding public health management. As most studies that are used for risk assessment are performed in background-exposed populations, our results contribute valuable information regarding high exposure. The results from my studies cannot alone lead to a change in guidelines in health care in hotspot populations but they emphasize that it is important that children's health is monitored. The outcomes that we observed increased risk of, in association with high prenatal PFAS exposure, are monitored by the child health care centers up until school age. The child health care centers can, if it is needed, refer the child to specialized health care such as speech therapy. The results from these studies therefore emphasize the importance of children being enrolled in the standard health care monitoring programs at child health care centers.

Methodological discussion

A great strength of this thesis is that I applied a population-based cohort approach in three out of four studies. When using the whole population as the study cohort the risk of selection bias is minimized. It also allowed me to have the statistical power to investigate rare outcomes such as preeclampsia. In the cohort studies, we retrieved healthcare data from administrative registers, and no data was self-assessed which minimized the risk of recall bias. We also performed a case-control study with a methodology designed to investigate a multifactorial disease as overweight.

Exposure and outcome assessment

For Studies I, III, and IV, a proxy was used for the PFAS exposure. Using a proxy instead of serum measurements has its advantages but also its drawbacks. When a proxy is used for exposure assessment, potential confounding arising from individual variation in pregnancy-related physiological changes, such as increased glomerular filtration rate and plasma volume expansion, is avoided (130). Increasing plasma volume dilutes the PFAS concentrations in serum, while increased glomerular filtration rate might increase excretion, which may confound the association between exposure and outcome if these pregnancy-related

physiological changes also are associated with the outcome. The changes in these factors are individual and gradual as the pregnancy progresses (131). Another major advantage of using a proxy instead of measurements is the cost – using data on e.g., residential history is cheaper than analyzing serum samples for PFAS, and also more time effective. It is then possible to include a larger population sample.

The major concern with using a proxy is the risk of exposure misclassification. To assess the risk of exposure misclassification, we validated the proxy variable against measured serum levels of PFAS. The results from the validation showed that the proxy method with categorization into high, intermediate, and background exposure reflected the serum levels of PFAS well on a group level. There is no reason to believe that any misclassification would be differential in Studies I, III, and IV, as the risk would be the same for all participants irrespective of outcome status. Non-differential misclassification is more likely to lead to an underestimation of the effect than an overestimation (132), which implies that our estimates may be lower than the true effect.

As in all register-based research, only those women and children who visit the health care system are present in the data. This raises a concern for potential selection bias. However, care is equally accessible for all residents in Sweden, as healthcare is publicly funded, and all healthcare is free for children. This ensures that all children have the same access to health care in the case of infectious disease, even though healthcare-seeking behavior between the parents may vary. For Study I, the outcome was retrieved from the National Medical Birth Register, which holds maternal diagnoses from pregnancy and childbirth. The National Medical Birth Register is a population-based register, and all pregnancies that lead to childbirth in Sweden are included, which minimizes the risk of selective reporting.

Studies II and III utilize data from routine screening of child health and development at child health care centers, that covers 99% of all children in their standard health-monitoring program (133). Within this program, nurses are taking measurements of the children regularly from birth (data used in Study II) and are also performing screening tests e.g., speech and language development, and can make referrals to speech therapists (data used in Study III). By using data from the child health care centers, we are likely to find close to all of the children with the outcome.

In Study IV, we used data from primary care, which is the first step to seeking care in non-urgent cases and is therefore suitable for investigating common infectious diseases. As the study population is from one county and the health care is free, it should be equally accessible to all children. In this study, children with infections of brief, mild symptoms can probably not be found in the data, as the parents will not seek health care in those cases. Also, cases with very severe and urgent symptoms will probably not be included as those children will most likely seek specialized care at the emergency unit.

A great strength of this thesis is that all the studied outcomes are clinically relevant. For example, in the case of preeclampsia, we have studied the clinical diagnoses of preeclampsia rather than using physiological biomarkers of preeclampsia, such as blood pressure in pregnancy.

An important aspect to discuss is also if the awareness of the exposure might have influenced the tendency of paying attention to certain symptoms, both among parents and health care professionals. The risk of this would probably be very low in the background exposed cohort in Study II, as the levels of PFAS in the pregnant women were not reported to the participants in the study.

When it comes to the highly exposed population of Ronneby, included in Studies I, III, and IV, it might, in theory, be different. The exposure was discovered in December 2013, and the population was then informed about the high PFAS levels in the drinking water. Study I was performed on women who had a pregnancy sometime between the years 1995-2013, which was before discovery. Studies III and IV include children who were born up until the discovery of the exposure but were followed until they were seven years old, i.e., as long as the year 2020. In Study III, we were in contact with speech and language pathologists in Blekinge County and they were not aware of the possible association between PFAS exposure and developmental language disorders. I therefore consider the possibility of a child being diagnosed differently depending on exposure status to be small. When it comes to Study IV, there might be a possibility that parents would have another health-seeking behavior after knowing about the exposure and seek care more often. If only individuals who knew that they had been exposed to the contaminated water would seek health care more often when the child experienced symptoms of common infectious diseases, that would bias our results upwards. However, it is unlikely that some diagnoses would be more often registered by healthcare professionals than others after the exposure was discovered.

Bias

To handle possible confounding, I adjusted for several variables in all studies. In Studies I, III, and IV, I knew from earlier studies of the same population that the area that received contaminated water had a lower socioeconomic status than the other areas in Ronneby and the background exposed population of Blekinge. It was therefore of the greatest importance to adjust for socioeconomic factors. However, the estimates only change marginally between crude and adjusted analyses in our studies. This indicates that our confounding variables did not affect the associations between the exposure and the outcome much. In Study II, confounding was handled in another way, where several confounding variables were included in creating a risk model, which then was used to define the cases and controls. Cases and controls were chosen from the same risk strata, and as a consequence, they were (more) alike regarding the confounding variables.

Gestational hypertension is part of the preeclampsia diagnosis, and in Study I, we therefore decided to analyze these diagnoses together as one outcome to minimize the risk of misclassification bias. Gestational hypertension/preeclampsia and gestational diabetes mellitus are considered severe diagnoses that are confirmed via tests and would not easily be missed or misdiagnosed by a physician. Also, it has been concluded in a study that the validity of these diagnoses in the National Medical Birth Register, where the outcomes are retrieved, is good (108).

In Study II, measures to calculate BMI, weight, and length were retrieved from child health care center medical journals. These measurements are standard procedures performed by trained nurses, and there is no reason to believe that any measurement error would differ systematically between exposure groups and hence introduce systematic error. In Study III, we used two different outcomes. The first outcome was to capture those who were referred by child health care center nurses to a speech and language pathologist. The second outcome was stricter and required at least two visits to a speech and language pathologist with ICD-coded diagnoses within speech, language, or communication, to be considered as having the outcome. The requirement of at least two visits with a predefined diagnostic code was applied to improve the validity of the outcome assessment.

Finally, in Study IV, the main outcome was overall infection and we used primary care health records. We also categorized common infectious diseases into subgroups of infections in eyes, ears, upper- and lower respiratory infections, and urinary tract infections. Some diagnoses may be less prone to misclassification than others – those that are more severe and require antibiotics or other pharmaceuticals are probably more valid than less severe infections.

Generalizability

Concerning the generalizability of my results, I will distinguish Studies I, III, and IV from Study II. Studies I, III, and IV are based on the highly exposed Ronneby population with especially high levels of PFOS and PFHxS. The results from these studies are, based on the high exposure and specific PFAS profile not generalizable to background-exposed populations. It could also be that my results are not generalizable to hotspots with another profile i.e., where the contamination has another source. When it comes to Study II, the study population consisted of children from parents born in Sweden (due to the reason that the questionnaire was in Swedish) that was living in Malmö, which is a multicultural city. However, we have no reason to believe that the association between the exposure and outcome would be any different in any other background-exposed population, and therefore those results are generalizable to other background-exposed populations within the approximate same exposure levels.

Future research

PFAS research has increased over the last decade, from 10 publications containing the word “PFAS” published on PubMed in the year 2010 to 1,109 publications so far in the year 2023 (134). This increase can mirror the growing concern over the effects of PFAS on the environment and human health. The current attention to PFAS is partly due to the proposal to heavily restrict these substances in the future, and new restrictions are coming next year in Sweden to limit the levels in drinking water which will force water treatment plants to act. Also, the growing number of hotspots that are discovered all over the world is gaining media attention, and not much is known about its possible health effects. In summary, research needs to continue to investigate PFAS as there is a growing concern about them in society, and unfortunately, regardless of how regulations will be in the future, there will be a large amount of PFAS in the environment that we will be having around for many years to come.

As of today, the mechanisms of PFAS in the human body are not clear. Future research should utilize research from hotspots, background exposed populations, and experimental research to get a full overview of how PFAS is acting in the human body. Also, the role of the placenta as a PFAS target should be investigated further, as the placenta decides the in-utero environment and has large implications on the fetus's development. Under the “developmental origin of health and disease” hypothesis, it must be a prioritized research area to understand the potential insults of today’s environmental pollution on the health of the next generations. For example, the Ronneby Mother-Child cohort has biobanked biological samples from mothers and children with background, intermediate, and high exposure. Here, the samples can be analyzed for both PFAS and biomarkers of effect to investigate both general mechanisms of PFAS in the human body, but also investigate the effect of PFAS exposure on the placenta.

In the future, research should also aim to clarify the effects after exposure in different sensitive exposure windows. In this thesis, the studies have focused on prenatal exposure, but the postnatal period is also a sensitive developmental period that could be susceptible to adverse health effects.

EFSA reported in 2020 that immunotoxicity was the most important adverse health outcome associated with PFAS making new guidelines. As we in Study IV could see associations between high prenatal exposure to PFAS and increased risk of

certain common infections, I agree that the association between PFAS and immunotoxicity should be investigated further in the future. More studies with clinically relevant outcomes, focusing both on antibody response from other vaccines, but also susceptibility to infections, could give more clarity to the associations.

The results from Study III showed an increased risk for developmental language disorders among girls associated with high prenatal PFAS exposure. As speech development in previous studies has been associated with emotional and behavioral difficulties and symptoms of Attention-Deficit/Hyperactivity Disorder (135, 136), it would be interesting to investigate neurodevelopmental disorders in a setting with high PFAS exposure in the Ronneby population. An investigation would be of high importance for other hotspot populations, as it could increase awareness and lead to difficulties being identified early to avoid suffering for the children.

In summary, there are many different outcomes associated with PFAS exposure that are interesting to investigate further in the future. But the basics in mechanisms, and also possible differences between prenatal exposure and later exposure would be interesting to gain a better understanding of. Also, research from hotspots is becoming increasingly important as the number of affected populations is increasing. As the PFAS profile differs between contamination sources, hotspots can provide valuable information regarding how different compounds can affect human health. Results from highly exposed populations would also be an important part of the puzzle to understand dose-response relationships.

Conclusions

This thesis investigated the health effects of PFAS pre-pregnancy and prenatal exposure, in two sensitive groups – pregnant women and children. The overall conclusion is that exposure to the endocrine disruptive characteristics of PFAS during the prenatal period may have adverse effects on the developing fetus that are expressed during childhood. From the results of this thesis, I can also draw the following conclusions:

- High exposure to prenatal AFFF contamination seemed to be associated with an increased risk of developmental language disorders in girls up to 7 years of age. The outcomes in my study were more clinically relevant compared to previous studies that used cognitive test instruments.
- High prenatal exposure to AFFF contamination seemed to be associated with the risk of diseases urinary tract infections and ear infections in children up to 7 years of age. This association strengthens the evidence that PFAS is immunotoxic, as it shows clinically relevant effects.
- High pre-pregnancy exposure to AFFF contamination was not associated with the risks of gestational hypertension, preeclampsia, or gestational diabetes mellitus. We observed the absence of effects in a high exposed population, even though some studies have reported associations at background exposure levels.
- Despite using a sophisticated method to adjust for confounding factors, prenatal exposure to PFAS was not associated with the risk of being overweight in 4-year-old children.
- Neurodevelopment and development of the immune system seem to be sensitive outcomes for prenatal PFAS toxicity.

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Exposure to perfluorinated alkyl substances and health effects in pregnant women and their children

Pregnant women all over the world have measurable levels of per- and polyfluorinated alkyl substances (PFAS), a group of environmental contaminants, in their blood. The exposure to PFAS already starts during pregnancy, as PFAS are transferred from the mother to the child. Pregnant women and their children are particularly vulnerable to PFAS insults, and research is needed to clarify health effects at different exposure levels. The overall aim of this thesis was to use epidemiological methods to investigate the effects of PFAS exposure on pregnancy complications, and the health and development of children after prenatal PFAS exposure.

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