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Serum concentrations of per- and polyfluoroalkyl substances (PFASs) among pregnant and delivering women: A comparison between the EMASAR study and existing literature

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Abstract

Background

Per- and polyfluoroalkyl substances (PFASs) is found ubiquitous in the environment and humans worldwide. These chemicals are proved to be toxic, persistent and has the capacity for long-range travels. PFASs are recognized as being responsible for adverse health effects in humans, especially fetuses and newborns in rapid development due to placental and lactational transfer.

Design and methods

This thesis is a descriptive part of the Estudio del Medio Ambiente y la Salud Reproductiva (EMASAR) study, with the aim to explore the PFASs concentrations in maternal serum. A study on environmental and reproductive health in two different regions in Argentina, Ushuaia (n = 193) and Salta (n = 496). Sampling of maternal serum, personal- and sociodemographic characteristics found place between 2011-2012. The PFASs levels from the EMASAR study is compared to delivering and pregnant women from other countries in different regions of the world.

Results

Salta's socioeconomic status as underdeveloped and fragmented and Ushuaia's status as the most prosperous in the country is reflected in the results, the women from Salta were younger, had on average more children and were less educated compared to the women in Ushuaia. Perfluorooctane sulfonate (PFOS) were the PFAS with highest concentration levels in delivering women both in Ushuaia and Salta (0.84 and 0.7 ng/mL, respectively), followed by pentafluorobenzoic acid (PFBA). The correlation between several of the PFASs were moderate to strong, with the strongest correlation between the PFASs in the two different regions were, r_s = 0.648 (p = 0.05) for PFOA and PFNA in Ushuaia and r_s = 0.656 (p = 0.05) between PFOA and PFHxA in Salta.

Conclusion

Among the PFASs concentrations from delivering women in Ushuaia and Salta, PFOS was the most prominent substance, followed by PFBA and PFOA, with a slight elevated level in Ushuaia compared to Salta. Compared to other regions of the world, the levels from the two study sites are low, but even relatively low levels of exposure to PFASs may have adverse effects on fetus and child health. Several of the PFASs correlates moderately to strong with each other.

Keywords: EMASAR; Per- and Polyfluoroalkyl Substances; PFAS; Maternal Serum; PFOA; PFOS; Human Exposure, Health Outcomes

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Abbreviations

ACCEPT	Adaptation to Climate Change, Environmental Pollution, and Dietary Transition
AFFF	Aqueous Film Forming Foam
AMAP	Arctic Monitoring and Assessment Programme
BMI	Body Mass Index
BRISA	Brazilian Ribeirão Preto
C	Carbon
°C	Celsius
C-F	Carbon-Fluorine
CHirP	Chemicals, Health and Pregnancy
CI	Confidence Intervals
EMASAR	Estudio del Medio Ambiente y la Salud Reproductiva
EPA	Environmental Protection Agency
EU	European Union
F	Fluorine
GDM	Gestational Diabetes Mellitus
GM	Geometric Mean
GMP	Global Monitoring Plan
GRULAC	Group of Latin American and Caribbean Countries
IGT	Impaired Glucose Tolerance
INMA	Infancia y Medio Ambiente
IUGR	Intrauterine Growth Restriction
LBW	Low birth weight
LOD	Limit of Detection
LOQ	Limit of Quantification
MISA	The Northern Norway Mother and Child Contaminant Cohort
MoBa	Norwegian Mother and Child Cohort
OR	Odds Ratio
PAHO	Pan American Health Organization
PFAS	Perfluoro- and Polyfluoroalkyl Substance
PFBA	Pentafluorobenzoic Acid
PFBS	Perfluorobutane Sulfonic Acid
PFDA	Perfluorodecanoic Acid

PFHxA	Perfluorohexanoate Acid
PFHxS	Perfluorohexane Sulfonate
PFNA	Perfluorononanoic Acid
PFOA	Perfluorooctanoic Acid
PFOS	Perfluorooctane Sulfonate
POPs	Persistent Organic Pollutants
PTE	Placental Transfer Efficiency
PTS	Persistent Toxic Substances
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
SELMA	Swedish Environmental Longitudinal, Mother and Child, Asthma and Allergy
SD	Standard Deviation
SDG	Sustainable Development Goals
S/N	Signal to Noise Ratio
UN	United Nations
UNDP	United Nations Development Programme
U.S	United States

1 Introduction

Human exposure to a group of man-made chemicals named per-and polyfluoroalkyl substances (PFASs) are being related to adverse health effects. PFASs are highly stable carbon-fluorine (C-F) compounds, widely used both industrially and commercially due to their unique surfactant properties. They are known to be highly resistant to water, heat, grease and oil and therefore used in a variety of products such as cooking ware, outdoor clothing, food handling equipment and aqueous film forming foam (AFFF), frequently used at airports and military bases for firefighting and training activities [1-3]. These are anthropogenic chemicals and have been produced since the late 1940's, but it is quite recent that these substances have received global attention due to their persistency, bio-accumulative and toxic properties both in the environment, but also in humans [4, 5]. Of special concern are maternal and prenatal health due to increased findings in placental and lactational transfer of PFASs from mother to child. Fetuses and newborns are in rapid development and studies show that early-life exposure to these chemicals may have adverse impact on development and health later in life [6].

The production and use of several of these substances has been targeted to be restricted and/or eliminated due to their properties through several international agreements, The Stockholm Convention is one of them [7]. Despite elimination and restriction of production and use of certain PFASs, traces of these substances are found ubiquitous in the environment and wildlife globally, even in the Arctic, which is far from industrialized regions and agricultural source centers [8, 9]. Regions worldwide are being monitored to see the effects from these agreements, but there is a lack of data to compare with in regions with developing countries such as the Group of Latin American and Caribbean Countries (GRULAC). Actions need to be taken to fill this information gap between the countries [10].

Estudio del Medio Ambiente y la Salud Reproductiva (EMASAR), is a study on environment and reproductive health and is designed to assess the maternal and fetal health risks related to both food security and exposure to persistent toxic substances (PTS), such as PFASs in two different regions of Argentina, Ushuaia and Salta.

2 Background

2.1 Per- and polyfluoroalkyl substances

To avoid confusion, this thesis will adopt and consistently use recommended PFASs terminology published by Buck *et al*. [11]. PFASs are aliphatic substances and consist of carbon (C) chains of different length with a perfluoroalkyl moiety (C_nF_{2n+1}-) [11]. They are often divided as short-chain and long-chain chemicals, where the long-chain analogues originally are characterized as the most hazardous, bio-accumulative and bio-magnifying, but studies now show that even though the short chain analogues have different properties, they are still as persistent, toxic and widely distributed in the world as the long chain analogues [12]. The hydrogen atoms are fully substituted with fluorine (F) atoms in perfluoroalkyl- and partly in polyfluoroalkyl substances. The C-F bond is strong and stable and can only be broken by high energy input which is why these compounds are not easily degradable in the environment [11].

There are two methods used for production of PFASs, electrochemical fluorination and telomerization. The electrochemical fluorination process produces a mixture of linear and branched isomers, while telomerization produces almost exclusively linear isomers [13]. A general information on the production and use of several PFASs is shown in Figure 1 [14]. Production showed increasing tendencies of these chemicals from the 1960s to the 1990s, before it stabilized up until approximately year 2000. One of the first and major global producer and manufacturer of these chemicals has been the 3M Company in the U.S, which started production with electrochemical fluorination in 1949 [15]. Production decreased after a phase out of a PFAS, perfluorooctane sulfonate (PFOS), whom was announced by the United States Environmental Protection Agency (EPA) and 3M Company itself [15].

PFASs is a large family of nearly 5000 synthetic chemicals and two of those most widely detected, studied and manufactured for the longest period of time is PFOS ($C_8F_{17}SO_3$) and perfluorooctanoic acid (PFOA:$C_8F_{15}O_2$) [16-18]. Olsen *et al*. estimated mean serum elimination half-life in humans of these long chain chemicals to be 5.4 years and 3.8 years, respectively [19]. PFOS are being released into the environment several ways, both through "direct" exposure, during manufacture, application and use, and "indirect" release, such as PFOS as chemical impurities which is formed during manufacture of perfluorooctane sulfonyl

fluoride (POSF), or from POSF-derivatives degrading in the environment to PFOS. In other words, a "direct" exposure has a clearly defined source, while "indirect" exposure is caused by a source that cannot be tracked to a specific product [15].

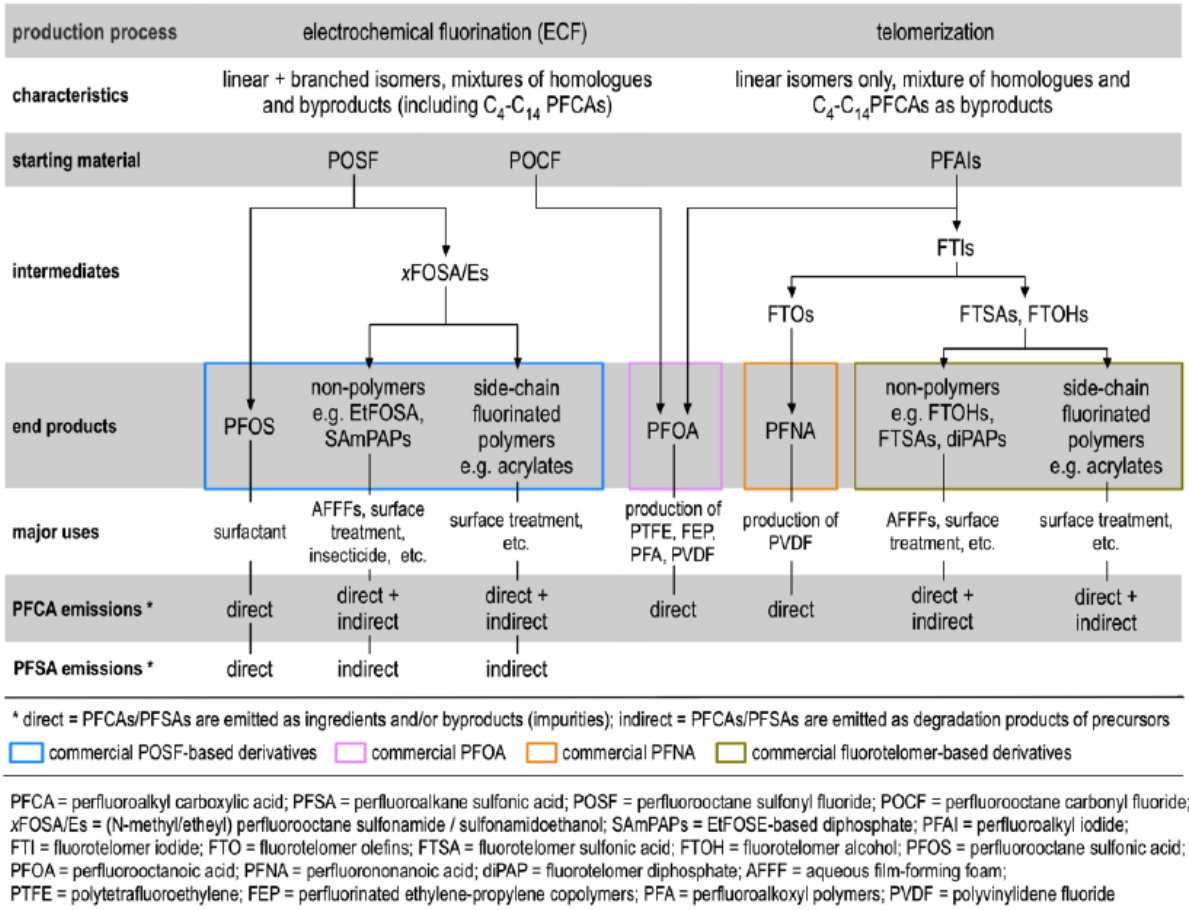


Figure 1. General information on the production and use of PFASs

Commercial PFOA, PFNA and POSF-and fluorotelomer based derivatives. Retrieved from OECD, Environment, Health and Safety Publications, Series on Risk Management No. 30 [14].

Perfluorohexane sulphonic acid (PFHxS:$C_6F_{13}SO_3$) is a long chain PFAS that has received less attention than PFOS and PFOA [20]. PFHxS is also known to be persistent in the environment and in humans with a potential of long-range transport. Properties of PFHxS is similar to PFOS but has a higher bio-accumulative potential. The estimated elimination half-life for PFHxS is approximately 8.5 years [21]. Perfluorononanoic acid (PFNA:$C_9HF_{17}O_2$) is known as a perfluorooctanoic acid or as a perfluorooctanoic derivative and are often found in wildlife and humans together with PFOA and PFOS. Perfluorodecanoic acid (PFDA:$C_{10}HF_{19}O_2$) is classified as a perfluoroalkyl carboxylic acid and have been frequently

used in products like Teflon and Gore-Tex due to its stain- and grease repellent properties [22].

A short chain PFASs is perfluorohexanoic acid (PFHxA:$C_6HF_{11}O_2$), a monocarboxylic acid, that also accumulate in the body, but has a shorter half-life due to shorter carbon length than PFOA. The chemical has been used in various products such as AFFF, food packaging and grease repellent products [23, 24]. Perfluorobenzoic acid (PFBA:$C_7H_5FO_2$) is also a PFASs known as short chain, it has mostly been used in analytical chemistry and has therefore had a minimal amount of analyses or toxicological evaluations [23].

PFASs have the capacity for long range transport, both in the atmosphere and in water [4]. The first study to demonstrate the global disperse of PFOS was published in 2001, where traces of PFOS was detected in the blood of polar bears and other wildlife animals in the Arctic and other remote areas. After this, restriction of production and use of several PFASs was initiated [25].

2.1.1 Restriction and elimination of chemicals

Manufacture of these chemicals, especially long chain PFASs changed globally after year 2000, these chemicals were found worldwide and suspected to have a negative impact on human health [25]. To minimize human and environmental exposure a multilateral international agreement to restrict and/or eliminate the production of persistent organic pollutants (POPs) was signed in 2001. POPs are defined as carbon-based chemicals of global concern. It is a widely used group of chemicals that is characterized as being persistent in various media, has the ability to bio-magnify, bio-accumulate and the potential for long-range transport [26, 27]. Studies has shown that exposure to POPs can be connected to adverse health effects and associated with certain cancers, birth defects, reproductive difficulties and damages to central and peripheral nervous systems [7]. The agreement was effective from 2004 and named The Stockholm Convention on Persistent Organic Pollutants [28].

Initially, 12 POPs were listed in the Stockholm Convention, but during recent years, new POPs has been targeted and added to the list [7, 26, 29]. The Stockholm Convention on Persistent Organic Pollutants (POPs) is an environmental treaty that aims to eliminate or restrict the production and use of POPs globally. Due to their properties, PFOS and PFOA

have been targeted in several regulatory initiatives worldwide [7, 11]. In 2008, The European Parliament restricted the use of PFOS in Europe [30]. Later, The Stockholm Convention listed the production and use of PFOS to be restricted (Annex B) in 2009 and PFOA to be eliminated (Annex A) in 2019 [7]. In 2017, PFHxS was nominated by the Norwegian Environmental Agency to the Stockholm Convention on Persistent Organic Pollutants and is now under review [21]. Another initiative to restrict and eliminate the production and use of these chemicals were the 2010/2015 PFOA Stewardship Programme by the United States Environmental Protection Agency (EPA). It was an agreement between eight major companies in the PFASs industry in the U.S and other countries. The aim was to reduce the production of PFOA by 95% before 2010 and eliminate the use within 2015. All of the participating companies state that they met the goals of this programme [31].

When the major manufacturer 3M phased out PFOS around year 2000, it was replaced by perfluorobutane sulfonic acid **(**PFBS:$C_4F_9SO_3$), a four carbon fluorocarbon chain [20]. Studies and reports published state that the substance was considered not to be persistent, bio-accumulative or toxic [20, 32]. 3M at the time believed that a short-chain chemical, such as PFBS was a good sustainable alternative to PFOS, which is a long-chain chemical [32]. It is used in carpeting, carpeting cleaners, floor wax and food packaging [24]. Until 2019, when Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), the European Union (EU) regulation, proposed PFBS to be a substance of very high concern due to scientific evidence that showed serious effects on the environment and human health [33].

The Global Monitoring Plan (GMP) is a programme under the Stockholm Convention which purpose is to identify changes in POPs levels over time and evaluate the effectiveness of the Convention. The aim is to use this data and compare all United Nations (UN) regions, but due to lack of information from developing countries there is a substantial gap between the regions. GRULAC is an example of a developing region where information is scarce. The third campaign for the GMP collection is ongoing and regional monitoring reports are expected to be released early 2021 [10, 28].

2.2 POPs in the context of the Sustainable Development Goals

United Nations Development Programme (UNDP) has been assisting Argentina and 83 other developing countries to manage their use, disposal and destruction of POPs since 2004. Until

now they have been expanding their work in promoting environmentally friendly alternatives that can promote achievement of the UNs Sustainable Development Goals (SDG) before year 2030. UNDPs monitoring and management of POPs are targeting several of the 17 SDGs, a selection of the goals is shown in Figure 2 [34].

Figure 2. Sustainable Development Goals targeted to monitor and manage POPs

Retrieved from https://sdg-tracker.org [34]

Sustainable Development Goals targeted by monitoring and management of POPs are nr. 3 good health and well-being, nr. 6 clean water and sanitation, nr. 9 industry, innovation and infrastructure, nr. 12 responsible consumption and production, nr. 14 life below water and nr. 15 life on land.

Several of the SDGs are relevant to prevent adverse health outcomes, illness and in worst case death. POPs such as PFOA and PFOS can lead to negative health effects worldwide by contaminating water, soil and air. To improve treatment of drinking supplies and increase availability to clean water is crucial to ensure good health. A resilient infrastructure promote inclusive and sustainable industrialization and foster innovation to achieve a greening holistic cleaner production and the use of less or non-harmful chemicals. Also, there is a need for a sustainable consumption and production pattern, this can be dealt with by redesigning products, phasing out toxic materials and minimizing waste generation.

Toxic releases from agriculture and industry leads to nutrient pollution and contamination in oceans and seas, this affects the food chain and is a cause of death worldwide for several marine mammals such as sea turtles and wales, but also for seabirds. Sustainably use of the oceans, seas and marine resources is important for a sustainable development. Therefore, improving waste management and reducing the release of toxic substances is an important intervention. There is a need for preventing or minimizing the release of hazardous chemicals into the environment to protect, restore and promote sustainable use of terrestrial ecosystems to combat desertification, reverse land degradation and biodiversity loss [34].

2.3 Human exposure to PFASs

The bio-accumulative properties of PFASs is the background of an increasing health concern. Even though restrictions and ban of several PFASs has been implemented globally, humans are being widely exposed to PFASs in their everyday life. And due to its high environmental persistence, it has become a public health problem.

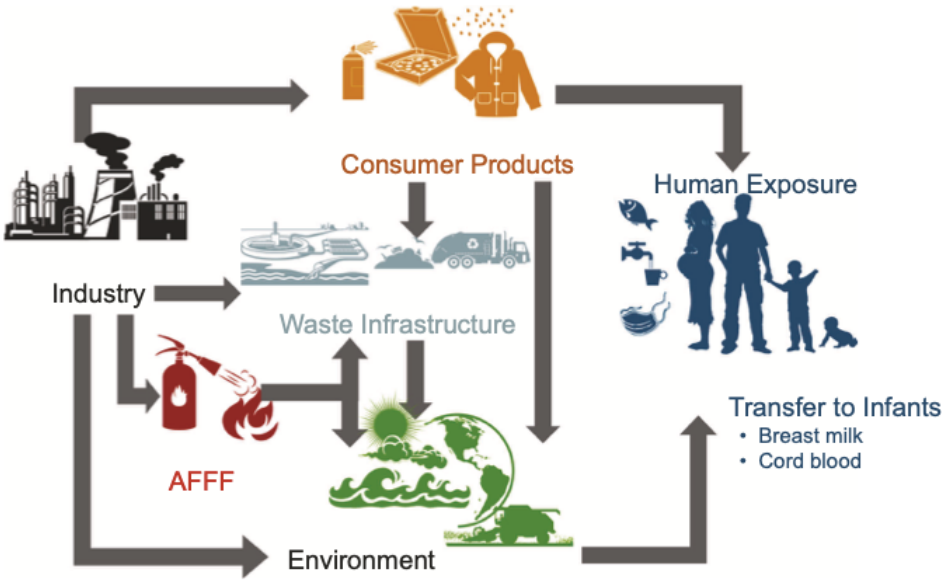


Figure 3. Pathways for human exposure to PFASs

Retrieved from Sunderland *et al*. [3]

The most common pathway to human exposure is ingestion of contaminated food and drinking water, but the exposure pathways also includes inhalation of fumes and dust from PFASs-containing products as shown in Figure 3 [6, 35, 36]. A shift in production of toxic chemicals can change the direct exposures, but due to the properties of PFASs, accumulation in the ocean, marine food chain, and contaminated groundwater will persist for a long time [3]. From production, product use and disposal of industrial and consumer products, during their life-cycle, PFASs are being released into the aquatic environment [4]. Groundwater contamination from AFFF is causing contamination of the drinking water and has for some time been a major problem in several countries. This is a substantial source of exposure to PFASs, especially for populations situated near a contaminated site or populations that frequently consume seafood due to leakage of these chemicals into the ocean and cause contamination of the food chain. High serum concentrations have been found in serum samples from populations where seafood is consumed frequently such as Inuit men on Greenland or whaling men in the Faroe Island [3]. Recommended health advisory levels for

both PFOA and PFOS are therefore proposed for drinking water in certain countries, such as the U.S [37]. Berg *et al*. found that main predictors of dietary exposure to PFOS, PFNA and PFDA is marine food, PFOS in white meat and PFOA in beef [38].

While the human exposure to PFASs such as PFOA and PFOS are declining due to regulatory interventions, production has replaced these chemicals to short chain PFASs. This is causing a global concern due to toxic and persistent properties, in addition these PFASs are often difficult to detect by using standard methods [3].

2.3.1 Risks of maternal and prenatal exposure to PFASs

The most common complications during pregnancy are hypertensive disorders and gestational diabetes mellitus (GDM). Hypertensive disorders are often classified into four categories; (1) chronic hypertension, (2) gestational hypertension, (3) preeclampsia (4) and preeclampsia superimposed on chronic hypertension [39, 40]. GDM is defined as glucose intolerance with onset or first recognition during pregnancy and is connected to short- and long-term adverse health outcomes both for mother and offspring. Affected women are at higher risk of developing diabetes mellitus type 2 post pregnancy and the offspring is at higher risk of developing childhood obesity and glucose intolerance in adulthood [40]. Etiology suggest that the prevalence of hypertensive disorders and GDM is multifactorial, in addition to body mass index (BMI), lack of physical activity combined with large calorie consumption and parity, exposure to environmental chemicals such as PFASs also is considered as a risk factor [39, 41]. Due to the adverse health effects on both mother and child and increase in prevalence, these pregnancy complications are a growing health concern globally.

Maternal exposure to PFASs during pregnancy may have adverse neurodevelopmental effect on the fetus. An animal study with mice prenatally exposed to PFOS and PFOA showed alterations in the offspring when it comes to behavior and motor functions, but also in the levels of proteins required for a normal brain development [36]. Fetuses and newborns are regarded as the most vulnerable group to these chemicals and their potential harmful effects due to rapid development [42]. Liu *et al*. and other studies points out a high transport rate for PFOA and PFOS from mother to neonate through placenta and lactation. Early exposure to PFASs may lead to an increased risk of chronic toxic effects than exposure later in life [42,

43]. Studies also indicate that PFASs may have adverse health effects such as fetal growth and development [13, 44].

2.4 Argentina and health care

Argentina, officially The Argentine Republic, has a total area of 2 780 400 km² and has a population of approximately 44 million people (2017). It is the second largest country in South America after Brazil and boarders towards Chile in west and south, Paraguay and Bolivia in the north and Brazil and Uruguay in the east [45]. Argentina is divided in 23 provinces, whom all function independently and has constitutional responsibility for leadership, finance and delivering health services. All of the 23 provinces are in charge of providing health service for its inhabitants. The Argentinian Public Health System offers coverage to all, included those covered by social- and private insurance [45, 46].

According to Pan American Health Organization (PAHO) the health care in Argentina is one of the most fragmented and segmental in the region of the Americas and includes public, private and social security sectors [45]. In an article about the Argentinian health care, Novic *et al*. states that the country is experiencing epidemiological transitions, ageing population and are currently facing a silent epidemic of non-communicable diseases. Biased allocation of resources and segmented financial structures by the government in the health care spending in the regions results in basic or minimum care, especially for the unemployed or poor. Conclusively, even though access to health care should be greatly inclusive and covered for all by The Argentinian Health System, due to lack of integration, complexity and fragmentation, it is not. There are a great need for capacity building and health care reforms to avoid inefficiencies and inequities [47].

3 Rationale

3.1 Purpose

Traces of PFASs are found ubiquitous in the environment and humans all over the world. This has raised serious concerns due to the potential harmful effects this might have on living organisms due to the properties such as toxic, persistent and bio-accumulative. Maternal exposure to PFASs are suspected to cause adverse health effects in the fetus and newborns due to placental and lactational transfer. There is inconsistent literature on the burden of PFASs in maternal health, especially in developing countries such as Argentina.

3.2 Aim and objectives

This thesis is a descriptive part of the EMASAR study, with the aim to explore the PFASs concentrations in maternal serum. The specific objectives of this study will be (1) to assess the maternal PFASs levels in Ushuaia and Salta from the EMASAR study: (2) to compare PFASs levels in maternal blood samples from the EMASAR study and other countries in different regions of the world: (3) evaluate the correlation between PFASs from the EMASAR study and other studies. Review of the health outcomes according to PFASs in pregnant and delivering women and their offspring is difficult with the existing database.

3.2.1 Research questions

1. How are the concentrations of PFASs in Ushuaia compared to Salta?
2. How is the level of PFASs in pregnant or delivering women in the EMASAR study compared to other regions of the world?
3. How is the correlation between PFASs in the EMASAR study compared to existing literature?

4 Materials and methods

4.1 Geographical information

The two study sites in Argentina where the fieldwork was conducted in the EMASAR study is shown in Figure 4, Salta and Ushuaia. The City of Salta (24.78°S, 65.42°S) is situated northwest in Argentina at the foothills of the Andes Mountains located in Lerma Valley, 1187 meters above sea level [48]. The population for the province Salta is approximately 1 210 000 and 620 000 around the metropolitan area. The climate is subtropical, hot and dry, and the agricultural profile depend on the altitude, main crops are grape, tobacco and corn [49]. Due to its inland location, livestock and tradition, the main component of the diet is beef. The economy in the region is diverse, but relatively underdeveloped with large socioeconomic inequalities and widespread poverty [50].

Figure 4. Map of South America with the study areas Salta and Ushuaia, Argentina Retrieved from Økland *et al*. [50]

Tierra del Fuego is the name of the province where Ushuaia is located. Ushuaia (54.80° S, 68.30° W) is the southernmost city in Argentina and the world and is based at sea level. Population in the province is approximately 130 000, and in the city of Ushuaia 60 000. Unlike Salta, Ushuaia has a sub-polar oceanic climate where fishing, sheep farming, natural gas and oil extraction and ecotourism are main economic activities. The salaries in the city is above national standards due to the attraction of different industries on the background of its status as free port. Socioeconomic standards in Ushuaia are therefore the most thriving in the country [50, 51].

4.2 Study design

This is a descriptive part of the EMASAR study from two different regions in Argentina, Salta in the north and Ushuaia in the south. EMASAR is an observational study with a cross-sectional design to investigate maternal and fetal health risks related to exposure to PTS and food security in the two regions as mentioned above. EMASAR is a collaboration project between UiT, University of Tromsø (The Arctic University in Norway), Stavanger University Hospital (Norway) and the two Argentinian partners Hospital Público Materno Infantil in Salta and at the Clínica San Jorge in Ushuaia [50]. The EMASAR study design is based on and compatible with the circumpolar programme, Arctic Monitoring and Assessment Programme (AMAP) [52].

The field work was conducted in the two hospitals in Argentina mentioned above. The hospital of Salta, Hospital Público Materno Infantil, is a public institution. It is responsible for all in-hospital deliveries in the City of Salta and is also the referral hospital for the whole province of Salta. Clínica San Jorge, the hospital in Ushuaia is a private institution cooperating with a public hospital for the in-hospital deliveries in the city, but also for surrounding areas in the region Tierra del Fuego [50].

4.3 Study population

The women recruited to the EMASAR study were either about to give birth or had given birth within the last 48 hours in one of the two hospitals mentioned earlier. A total of 717 were recruited, 200 from Ushuaia and 517 from Salta, but due to lack of biological samples, 19 mothers from Salta were excluded from the study. The final study sample was 698.

4.4 Data collection

Data for the EMASAR study was obtained between April 2011 and March 2012 at Hospital Público Materno Infantil in Salta and at the Clínica San Jorge in Ushuaia. This data included questionnaire, clinical information and maternal blood samples. Sampling kits included printed questionnaires and blood sampling equipment. Sample transfer vials were prepared and pre-numbered in Norway. In Argentina, the local staff received a written comprehensive instruction and the researchers were thoroughly informed about the protocol and sampling procedures [50].

4.4.1 Clinical information and questionnaire

The clinical information about the women was based on hospital records and a standardised form completed by a medical doctor. Information from the current delivery was gestational age and pre- and postpartum BMI. To collect additional information a questionnaire was compiled in English and subsequently translated to Spanish. Information collected through this questionnaire was maternal age, previous history of earlier pregnancies, socioeconomic factors (place of birth, housing, education), lifestyle (smoking) and dietary information before and during pregnancy.

4.4.2 Blood sampling

Maternal blood samples were obtained at 36±12 hours after delivering the baby. This was non-fasting venous blood collected from the antecubital vein. The blood samples collected for contaminant analysis were frozen immediately and stored at -20°C at the local hospital until they were shipped frozen to Norway and stored in the EMASAR biobank at the University in Tromsø, The Arctic University of Norway, at -35°C until analysis. The total study population in the EMASAR study were originally 698, but due to missing values only 689 blood samples were assessed for PFASs concentrations. The missing values were caused by glass vials too full prior to freezing and therefore the glass cracked under frozen conditions.

4.5 Chemical analysis and quality control

Sample preparation, instrumental analysis, quantification method, validation and quality control have been described in detail by Huber and Brox [53]. Analysis of the blood samples was done at the Laboratory for Analysis of Environmental Pollutants, Department of Laboratory Medicine, University Hospital of North-Norway.

Tecan Freedom Evo 200 (Männedorf, Switzerland), an automated liquid handler prepared the samples combined with a solid phase micro-extraction technique on a Waters Oasis WAX 96-well plate. The instrumental analysis by the PFASs was done by ultrahigh pressure liquid chromatography triple quadrupole mass spectrometry (UHPLC-MS/MS). A Waters Acuity UPLC system (Milford, MA, USA) performed the analysis, consisting of a binary solvent manager, an autosampler and a column manager coupled to a Xevo TQ-SMS (Waters, MA, USA) with an atmospheric electrospray interface. By using a programmed flow and solvent gradient of 2 mM NH_4OAc in MilliQ-water and 2mM NH_4OAc in methanol (MeOH) as eluents to separate the target analytes, an Acquity UPLC HSS 3 T column (2.1x100 mm, 1,8 µm) (Waters, Milford, MA, USA) were used. Quantification was conducted using the Masslynx and Targetlynx software (Version 4.1, Waters, Milford, MA, USA) and achieved by the internal-standard method with isotope-labelled PFASs.

For quality control of the analysis Milli-Q-water blanks (x4), bovine serum (3x) and standard reference material (SRM) 1957 (x4) and 1958 (x4) were analysed together with every batch of real-samples (81). The PFAS method performance is continuously controlled and tested in a Quality Assurance/Quality Control (QA/QC) by participating in interlaboratory comparison studies. Comparison with the AMAP interlaboratory is used to evaluate the precision by analysis of certified standard reference material and samples. Spiked concentrations and the assigned concentrations of the reference material was compared to measured concentrations to assess accuracy and precision.

The Targetlynx-software calculated the limit of detection (LOD) for each individual sample and each individual analyte with a signal to noise ratio (S/N) of 3 divided by the related sample amount. LOD was calculated as an average of the blanks multiplied by three times of their standard deviation where blank contamination was detected. If the LOD of the individual sample were lower than the LOD calculated from the blank contamination the LOD calculated based on the blank samples was used. Limit of quantification (LOQ) was defined as 10 times the S/N [53].

Altogether 30 different PFASs were assessed in the EMASAR study, this thesis is restricted to LOD above 50% of the samples and will investigate seven PFASs, PFOS, PFHxS, PFNA, PFOA, PFHxA, PFDA and PFBA. Sum of branched and linear species (sum) were quantified

for PFOS and PFHxS. These PFASs was chosen due to comparison with other studies. When discussing PFOS and PFHxS results, it is the sum of linear and branched isomers unless otherwise is specified.

4.6 Statistical analysis

Statistical analyses were carried out using the IBM SPSS Statistics for Windows (version 26; SPSS Inc., Chicago, IL, USA). Statistics was focused on the PFASs found above the limit of detection in more than 50% of the samples: PFOS, PFHxS, PFNA, PFOA, PFHxA, PFDA and PFBA. Data were given as arithmetic means, standard deviation (SD), median and minimum and maximum or proportion (%) for describing clinical and sociodemographic characteristics of the study population. Arithmetic means, geometric means, median and minimum and maximum were used for the maternal blood PFASs levels descriptive analysis. The interrelationships between PFASs were explored using Spearman's rank correlation analysis. Complete case analysis was used for handling missing data, which means that participants with any missing data were excluded in the statistical analyses. A significance level of $p < 0.05$ (two tailed) was used for all analyses.

4.7 Ethical consideration

The EMASAR study was conducted in accordance to the ethical principles for medical research involving human subjects of The World Medical Associations, The Declaration of Helsinki [54]. The women participated voluntarily, and a written informed consent was obtained. The study was approved by both Ethics Committee of the Salta Medical Association and the Ministries of Health in both Salta and Ushuaia (#2010/7317) and The Norwegian Regional Committee for Medical and Health Research Ethics (REC North) (#2011/706) [50].

5 Results

5.1 Sample characteristics

The personal and sociodemographic characteristics, such as pre- and post-pregnancy BMI, parity, gestational age, education and smoking, from the two study sites, Ushuaia and Salta, are shown in Table 1. The study consists of 698 participants between 14 and 45 years of age. The mean age in Ushuaia was on average 4 years older than in Salta with a mean of 28.8 (SD=6.5) and 24.7 (SD=6.2) years of age, respectively. The pre-pregnancy BMI was approximately the same in both cities (24 kg/m^3), but women in Ushuaia had a higher postpartum BMI at 28 kg/m^3, compared to 26.1 kg/m^3 in Salta. The mean for gestational age was 39 weeks in both sites. The proportion of women carrying their first child were slightly higher in Salta with 44.4% compared to women in Ushuaia 41%.

Nearly half of the women from Ushuaia obtained tertiary or university education (48%), while the group only accounted for 10.4% in Salta. The proportion of current smokers were higher in Salta, compared to Ushuaia (9.6% vs. 4.5%). A total of 97.3% (n = 679) of the study sample had Argentinian nationality and the remaining 2.6% (n = 18) came from Chile, Peru, Korea, Bolivia or Paraguay.

Table 1. Maternal characteristics of the study population from Ushuaia and Salta

	Total (*n* = 698)			**Ushuaia (*n* = 200)**			**Salta (*n* = 498)**		
	n **(Missing data)**	**Mean (SD) or *n* (%)**	**Median (Min-Max)**	***n*** **(Missing data)**	**Mean (SD) or *n* (%)**	**Median (Min–Max)**	***n*** **(Missing data)**	**Mean (SD) or *n* (%)**	**Median (Min-Max)**
Age (years)	698 (0)	25.9 (6.6)	25.0 (14.3-44.5)	200 (0)	28.8 (6.5)	28 (16–45)	498 (0)	24.7 (6.2)	23 (14-44)
Pre-pregnancy BMI (kg/m^3)	639 (59)	23.5 (4.1)	22.7 (14.8-40.8)	192 (8)	23.5 (4.0)	22.6 (16.1–40.7)	447 (51)	23.5 (4.2)	22.8 (14.8-39.6)
Postpartum BMI (kg/m^3)	689 (9)	26.7 (4.2)	26.2 (16.4-44.1)	200 (0)	28.0 (3.8)	27.5 (18.6-44.1)	489 (9)	26.1 (4.2)	25.5 (16.4-43.4)
Gestational age (weeks)	660(38)	38.8 (1.3)	32-42	199 (1)	38.8 (1.4)	39.0 (32-41)	461 (37)	38.8 (1.3)	38.8 (33-42)
Parity	698 (0)			200 (0)			498 (0)		
Para 1		303 (43.4)			82 (41)			221 (44.4)	
Multiparity		395 (56.6)			118 (59)			277 (55.6)	
Education	697 (1)			200 (0)			497 (1)		
Primary		168 (24.1)			7 (3.5)			161 (32.3)	
Secondary		381 (54.6)			97 (48.5)			284 (57.0)	
Tertiary		96 (13.8)			56 (28.0)			40 (8.0)	
University		52 (7.4)			40 (20.0)			12 (2.4)	
Missing data		1 (0.1)			/			1 (0.2)	
Smoking, current	698 (0)			200 (0)			498 (0)		
Yes		57 (8.2)			9 (4.5)			48 (9.6)	
No		641 (91.8)			191 (95.5)			450 (90.4)	
Nationality	698 (0)			200 (0)			498 (0)		
Argentina		679 (97.3)			197 (98.5)			482 (96.8)	
Other*		18 (2.6)			3 (1.5)			15 (3.0)	
Missing data		1 (0.1)			/			1 (0.2)	

* Chile, Peru, Korean (Ushuaia); Bolivia (*n* = 13) and Paraguay (*n* = 2) (Salta)

5.2 Distribution of dominating PFASs

The highest PFASs median concentration found in the EMASAR study were PFOS, both in Ushuaia and Salta (0.84 ng/mL and 0.7 ng/mL, respectively), this was also the PFAS with the highest detection rate (100%) in both sites. PFBA had a lower detection rate in Salta than Ushuaia (94% vs. 73%) but was the compound with the highest concentration that followed after PFOS with a median concentration of 0.56 ng/mL in Ushuaia and 0.53 ng/mL in Salta. PFOA had a lower detection rate in Salta than Ushuaia (92% vs. 100%) but had the third highest concentrations with a median concentration of 0.33 ng/mL in Ushuaia and 0.22 ng/mL in Salta. The lowest median concentration levels of all the PFASs assessed from the EMASAR study in this thesis were PFDA, with a detection rate of 86% both in Ushuaia and Salta (0.03 ng/mL and 0.02 ng/mL, respectively).

Supplementary Table S1 present the LOD of maternal serum of 30 the PFASs assessed in the EMASAR study. A total of seven PFAS components were detectable in more than 50% of the blood samples are used for the statistical analysis in this thesis. Descriptive statistics of the dominating PFASs in maternal serum concentrations (ng/mL), stratified by the two study sites, are shown in Table 2 and Figure 5. The level of PFOS is the highest median concentration among the selected PFASs both in Ushuaia and Salta (0.84 ng/mL, 0.7 ng/mL, respectively), followed by PFBA (0.56 ng/mL, 0.53 ng/mL) and PFOA (0.33 ng/mL, 0.22 ng/mL). The average mean levels of PFHxS, PFNA, PFHxA, PFDA in both study sites were less than 0.1 ng/mL.

Table 2. Maternal serum concentrations (ng/mL) of PFASs in the EMASAR study Stratified by region, Ushuaia (n = 193) and Salta (n = 496)

PFASs	Ushuaia (n = 193)			Salta (n = 496)		
	% > LOD	Mean[a] (SD)	Median (Min-Max)	% > LOD	Mean[a] (SD)	Median (Min-Max)
PFOS	100	1.02 (1.18)	0.84 (0.28-14.4)	100	0.97 (2.83)	0.7 (0.11-59.8)
PFHxS	99.5	0.23 (0.27)	0.18 (0.01-3.3)	98.0	0.26 (0.17)	0.22 (0.002-1.28)
PFNA	99.5	0.07 (0.05)	0.05 (0.01-0.42)	92.7	0.04 (0.05)	0.04 (0.004-1.14)
PFOA	100	0.39 (0.21)	0.33 (0.14-1.39)	92.1	0.23 (0.16)	0.22 (0.08-3.02)
PFHxA	58.5	0.1 (0.1)	0.09 (0.004-0.47)	96.4	0.08 (0.04)	0.07 (0.002-0.27)
PFDA	85.5	0.03 (0.02)	0.03 (0.005-0.2)	85.5	0.03 (0.11)	0.02 (0.004-0.55)
PFBA	93.8	0.54 (0.09)	0.56 (0.26-0.78)	73.2	0.53 (0.11)	0.53 (0.26-0.87)

[a]Arithmetic Mean with standard deviation (SD)
The limit of detection (LOD) > 50% of the samples

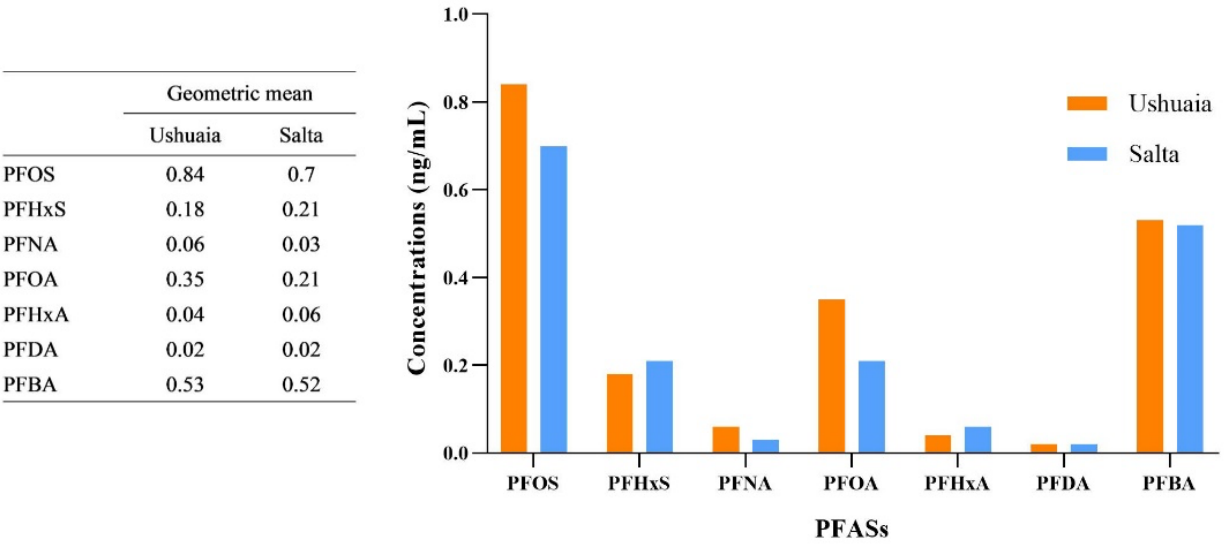


Figure 5. Geometric mean of the PFASs concentrations (ng/mL) in maternal serum from the EMASAR study

The bars present the geometric means of selected maternal blood PFASs levels (ng/mL) in delivering women from the study population, stratified by region (n = 689).

5.3 Correlation between PFASs

Table 3. Spearman's rho correlations (ρ) for interrelationships between serum correlations, stratified by study region

		PFHxS	PFNA	PFOA	PFHxA	PFDA	PFBA
Ushuaia (*n* =193)	**PFOS**	0.495**	0.558**	0.593**	0.483**	0.344	-0.179*
	PFHxS		0.370**	0.381**	0.502**	**0.089**	-0.177*
	PFNA			0.648**	0.419**	0.627**	-0.164**
	PFOA				0.445	0.428**	-0.192**
	PFHxA					**0.131**	-0.292**
	PFDA						**-0.020**
Salta (*n* =496)	**PFOS**	0.280**	0.534**	0.497**	0.423**	0.548**	0.228*
	PFHxS		0.249**	0.261**	0.196**	0.214**	0.119*
	PFNA			0.544**	0.435**	0.497**	0.193**
	PFOA				0.656**	0.331**	0.232**
	PFHxA					0.281**	0.289**
	PFDA						0.214**

*Correlation is significant at 0.01

** Correlation is significant at 0.05

Table 3 show the Spearman correlation coefficients between the seven PFASs components stratified by study sites, Ushuaia and Salta. In Ushuaia the lowest significant correlation observed was between PFBA and PFNA (r_s = -0.164, p = 0.05), and the highest was between PFOA and PFNA (r_s = 0.648, p = 0.05). The correlations were not significant between PFDA and PFHxS (r_s = 0.089) and PFDA and PFHxA (r_s = 0.131) and PFBA and PFDA (r_s = -0.020). In Ushuaia all of the correlations with PFBA were negative.

The correlation between all of the 7 PFASs were significant in Salta. The lowest significant correlation observed was between PFBA and PFHxS (r_s = 0.119, p = 0.01) and the highest was between PFOA and PFHxA (r_s = 0.656 p = 0.05)

6 Discussion

Data from the cross-sectional EMASAR study were used in this thesis to evaluate PFASs levels in maternal serum from the two Argentinian regions, Ushuaia and Salta. A descriptive analysis found distinct socioeconomic differences between the study sites. While Salta has a rather underdeveloped socioeconomic status, Ushuaia has one of the most prosperous in Argentina [50]. In the EMASAR study the women from Salta were younger, had more children and were less educated compared to the women in Ushuaia [50]. The analysis of the PFASs concentrations showed that PFOS followed by PFOA was the most prominent compounds among the PFASs assessed in this thesis. The results also showed elevated levels of PFHxS and PFBA compared to PFNA, PFHxA and PFDA, with slightly higher concentrations observed in Ushuaia, except from PFHxS, where the levels were slightly higher in the women from Salta.

Characteristics concerning housing and family situation from the study population show that there are differences in living situation between the two study sites, in Ushuaia 99% of the participants was either living in an urban or semi-urban area, while the proportion was 93% in Salta. The amount of people living in the same house together was doubled for the Salta population (7 vs. 4 in Ushuaia). Maternal and newborn characteristics showed that the proportion of women with a caesarian section was 43% in Ushuaia compared to 6% in Salta. The proportion of first time mothers was similar in the two sites, slightly higher in Salta (44% vs. 41%), but the number of children differed with 31% being para 3 or more (22% in Ushuaia) [50] Souza *et al*. reports that the serum concentrations of PFOA and PFOS general are higher in developed countries, compared to those reported in developing countries. This implies that socioeconomic status appears to be related to the level of PFASs in the population [18]. The observed differences between the two study sites assessed in this thesis indicates that the socioeconomic status might affect the concentrations of PFASs of a population.

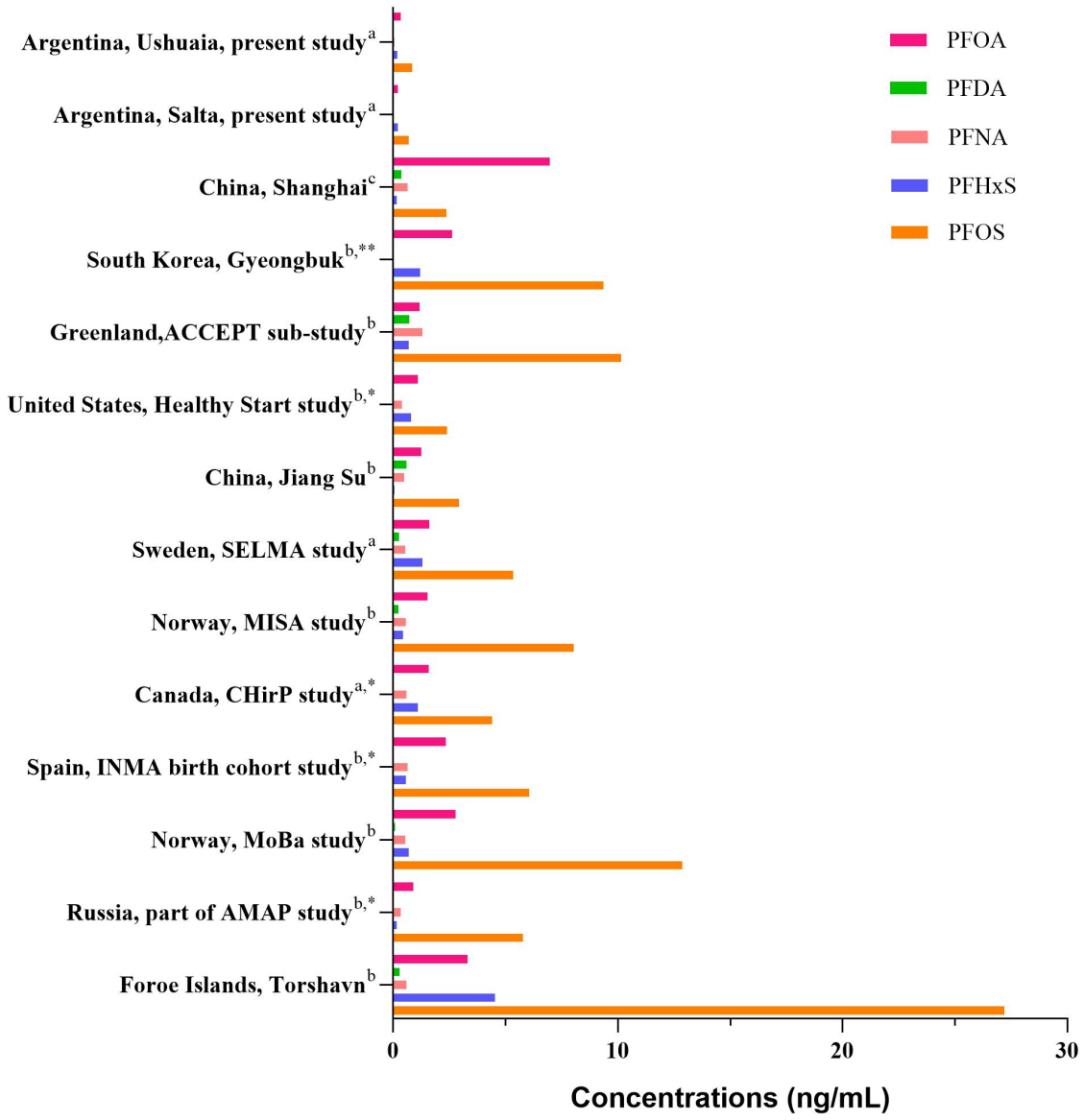
6.1 Comparison between the EMASAR study and existing literature

In the range of studies concerning pregnant and delivering women and the levels of PFASs, the measure for collection of blood varies. While the EMASAR study used maternal serum concentrations as a measure for PFASs, some other studies used maternal plasma, cord blood

or whole blood as a measure for these substances. For comparison reasons, studies containing only whole blood measures were excluded in the comparison in this thesis.

Figure 6 show median or mean PFASs concentrations (ng/mL) of PFOA, PFDA, PFNA, PFHxS and PFOA from the present EMASAR study and results from other studies from countries in different regions of the world. It is clear that the PFASs concentrations among delivering women from the study population in both Salta and Ushuaia are in the lower range compared to other regions of the world.

Selected studies presented in Figure 6 with additional information in Table 4 covers a 17-year period, from 1997-2014 [55]. As the studies are arranged by the initial start of study periods, from the oldest at the bottom to the most recent on top, the results in this thesis is consistent with other literature. There has been a decrease in PFASs detection, especially for PFOA and PFOS, most likely due to international restriction on production and use of these chemicals [3, 38, 56, 57].



[a]Geometric mean, [b]Median, [c]Umbilical cord blood sample, *PFDA not measured in the selected reference study, **PFDA and PFNA not measured in the selected reference study

Figure 6. Comparison of PFASs concentrations (ng/mL) in maternal serum or plasma Concentrations of PFOS, PFDA, PFNA, PFHxS and PFOA with a study period between 2014-1997.

Table 4. General information of selected studies from Figure 6

Authors, location, study period and number of participants (*n*)

Authors	**Location/study**	**Study period**	***n***
Present study	Salta, Argentina	2011-2012	496
Present study	Ushuaia, Argentina	2011-2012	193
Huang *et al*. (2019) [39]	Shanghai, China	2011-2012	674
Lee *et al*. (2013) [43]	South Korea	2011	70
Long *et al*. (2015)	Greenland ACCEPT study	2010-2011	207
Starling *et al*. (2017)	U.S, Healthy Start study	2009-2014	628
Liu *et al*. (2011) [42]	Jiang Su, China	2009	50
Wickström *et al*. (2019) [58]	Sweden, SELMA study	2007-2010	1773
Berg *et al*. (2014) [38]	Norway, MISA	2007-2009	391
Webster *et al*. (2014) [59]	Canada, ChirP	2007-2008	152
Matilla-Santander *et al*. (2017) [60]	Spain, INMA study	2003-2008	1240
Starling *et al*. (2014) [61]	Norway, MoBa	2003-2007	976
Hanssen *et al*. (2013) [62]	Russia, part of AMAP study	2001-2004	7
Valvi *et al*. (2017) [55]	Faroe Island	1997-2000	604

As shown in Table 4, the number of participants varied in the studies used for comparison, the two studies with the larges sample size were in the Swedish Environmental Longitudinal, Mother and Child, Asthma and Allergy (SELMA) study from Sweden (*n* = 1773) [58] and the Spanish Infancia y Medio Ambiente (INMA) study (*n* = 1240) [60]. A number of three studies in the comparison had a study population <100 (*n* = 7, 50 and 70, Russia, Jiang Su, China and South Korea, respectively) [42, 43, 62].

6.1.1 Detection rate and PFASs concentrations

PFASs are found everywhere in the environment, PFOS, PFOA, PFNA and PFHxS are detected in nearly 100% of maternal blood samples worldwide, included the results from the present study [39, 42, 60, 61]. Other PFASs that has a high detection rate in several studies are PFDA, PFBS [39, 61].

During the same time period as the EMASAR study, umbilical cord blood samples were collected in a cross-sectional Chinese study from Shanghai (*n* = 674). Plasma from these samples showed that PFOA (median 6.98 ng/mL) not only was the most abundant compound

in the study, but also with the highest median concentration of PFOA in the comparison between all of the selected countries in this thesis, included the EMASAR study [39]. This is the only study in this thesis that has analyzed plasma from umbilical cord blood samples, rather than maternal serum. Compared to the median levels of the EMASAR study, the elevated concentration of PFOA in this study are high (Salta = 0.22 ng/mL, Ushuaia = 0.33 ng/mL).

A small study conducted a few years earlier (2009) from a neighboring region of Shanghai in China, the Jiang Su province (*n* = 50), PFOS had the highest median maternal serum concentration (2.92 ng/mL) of PFASs measured in the study sample followed by PFOA (0.12 ng/mL) [42]. In Norilsk, Russia, a study part of AMAP (*n* = 7), Hanssen *et al*. reported low PFASs levels of PFOA, PFNA and PFHxS in maternal blood samples from plasma collected between 2001-2004, with a median of 1.61, 0.60, 0.26 ng/mL, respectively. The level of PFOS were in the medium range in the comparison with a concentration of 11 ng/mL compared to the other studies [62].

In 2011 from South Korea, Gyeongbuk county (*n* = 70), results showed a detection of PFOS, PFOA and PFHxS in all (100%) of the blood samples (median = 9.37, 2.62 and 1.21 ng/mL, respectively). The levels of concentration from PFDA and PFNA were not measured in this study [43]. The Greenlandic Adaptation to Climate Change, Environmental Pollution, and Dietary Transition (ACCEPT) study from 2010-2011 found levels of PFOS with a median concentration of 10.15 ng/mL, similar to the Norwegian Mother and Child Cohort (MoBa) study and the South Korean study. The study from Greenland show the highest median concentration of PFNA (1.30 ng/mL) compared to the rest of the studies included in this thesis.

In the Spanish INMA study (*n* = 1240) Matilla-Santander *et al*. report that PFOS and PFOA were detected in all samples in the study collected from 2003-2008, with levels within the medium range compared to the selected reference countries [60]. PFOS had the highest median concentration (6.05 ng/mL) followed by PFOA (2.35 ng/mL). PFHxS and PFNA had median concentration levels of 0.58 ng/mL and 0.65 ng/mL.

Valvi *et al*. report from Torshavn, Faroe Island (*n* = 604) the overall highest maternal serum concentration of PFOS, with blood samples collected between 1997-2000, a median level of

27.2 ng/mL. In addition, the same study also report the highest median level of PFHxS among the selected studies (4.54 ng/mL). It is important to note, that the study were obtained in the first study period among the selected countries from 1997-2000, this was before the phase out and restriction of production and use of several PFASs in the beginning of year 2000 [7, 31, 55].

Due to the amount of PFHxS in the blood samples found in South Korea and other similar studies, Lee *et al*. suggest that next to PFOS and PFOA, PFHxS is the most detected PFAS in human blood from the general population [43]. PFHxS had the third highest concentration among several of the selected studies in this thesis [55, 58, 59], but in Ushuaia and Salta, the median levels of PFHxS were low in comparison (0.18 ng/mL and 0.22 ng/mL). The third highest level of PFASs in the EMASAR study after PFOS and PFOA were PFBA (0.56 ng/mL and 0.53 ng/mL, respectively).

There has been a decrease in PFOS exposure due to extensive restrictions on production and use of PFOS and PFOA during the past year, simultaneously, exposure to other PFASs, such as PFNA and PFHxS has emerged [55]. Comparing the studies from the Arctic region, the Norwegian MISA study and the study from Russia, PFASs levels are higher in the ACCEPT study from Greenland, this may be related to the high seafood consumption in the population of Greenland [38, 55, 62, 63].

6.2 Blood collection

All of the studies selected in the comparison measured PFASs concentrations from maternal serum or plasma, except one, the study from Shanghai, China, which used cord blood [39]. While concentrations of PFOA and PFHxS increased in the samples from the umbilical cord, the PFNA and PFOS decreased, the results indicate that the carbon-chain length is influential [42, 64].

The time of collecting the maternal blood samples differed between the various studies used for comparison in this thesis. PFASs concentrations from maternal blood serum/plasma tend to decrease according to the length of the pregnancy (gestational age), due to placental and lactational transfer [65]. An example is the Northern Norway Mother and Child Contaminant Cohort (MISA) study, the PFOS and PFOA concentration in the women recruited within the

first 100 days of the study had higher concentrations, 25 % and 26 %, respectively, compared to those recruited in the last 266 days of the study (867 days in total) [38]. In comparison to the EMASAR study, several other studies collected the maternal blood samples within the first days after delivery [36, 42, 62], while others collected the samples within the first [58-60] or second trimester [38, 55, 61, 63, 66].

The Chemicals, Health and Pregnancy (CHirP) study in Vancouver, Canada, chose time of collection according to the aim of the research. The aim was to assess PFASs levels and thyroid hormones. Therefore the time of collection was early second trimester between week 15 to 18 of gestation to avoid the 10 week peak of maternal plasma chorionic gonadotropin levels, which suppress the thyroid stimulating hormone in the first trimester [59].

6.3 Maternal characteristics and PFASs levels

The mean age of the study population in the EMASAR study were 26 (14-45) years of age, lower than several other studies. The Canadian CHirP study had the highest mean age of 34 (25-43), while the study conducted in Russia had the lowest median age of 24 [59, 62].

Parity is a factor that can affect the PFASs levels in pregnant and delivering women. Studies show that the concentrations of some of these substances in maternal blood seem to decrease with parity. Among several studies, Lee *et al*. and Shu *et al*. observed that the concentrations of PFOA were significantly higher in nulliparous, than multiparous women [43, 57]. Reasons for this might be lactation and placental transfer to decrease the level of maternal PFASs [42, 43].

Also, studies show that nulliparous women have a higher risk of hypertensive disorders, as preeclampsia than parous women [38]. The Norwegian MoBa study is the only study that restricted their study population to nulliparous women to avoid this as a confounding factor. This study has the second highest observed level of PFOS among the selected reference countries with a median concentration of 12.87 ng/mL [61]. The majority of the studies assessed had a proportion of approximately 50% nulliparous women, with some exceptions. In Shanghai, China, 92% of the study population were nulliparous [39], while the number was 42% in the Swedish SELMA study [58]. This is similar to the EMASAR study, who had a total of 43% nulliparous women (41% in Ushuaia and 44% in Salta).

6.3.1 PFASs exposure and maternal health effects

As mentioned earlier, one of the most common complications of pregnancies are hypertensive disorders. Inconsistent results have been reported from epidemiological studies concerning PFASs and preeclampsia. The C8 Health Project found a weak association between preeclampsia and PFOA and PFOS [67], while another study from Shanghai, China, found no association between preeclampsia and PFOA or PFOS, but a positive association between preeclampsia and PFBS in plasma drawn from cord blood [39]. Studies on prenatal PFBS exposure and hypertensive disorders are scarce, due to this and a detection level below 50% of PFBS in the EMASAR study this compound has not been assessed in this thesis. Inconsistent findings when it comes to preeclampsia may be due to different methods of diagnosing preeclampsia. As an example, the C8 Health Project used self-reported preeclampsia as a measure, while the study from Shanghai and Norway used validated medical records to determine this condition [36, 39, 61].

Matilla-Santander *et al*. obtained information from 1240 mothers on metabolic outcomes and reported that the overall prevalence of GDM and impaired glucose tolerance (IGT) were 4.3% and 11%, respectively. Both GDM and IGT were positively associated with maternal plasma concentrations of PFOS, PFHxS were also positively associated, but the odds ratio (OR) were closer to null. The study provide evidence for associations between PFASs exposure and metabolic outcomes in pregnant women [60].

As mentioned earlier the MoBa study from Norway was restricted to nulliparous women in their study population, due to this inclusion criteria, this group of pregnant women had another set of risk factors for developing preeclampsia than multiparous women. The results can therefore not be compared to those with both nulliparous and multiparous women, thus the study found no association between PFASs levels and preeclampsia [61].

6.4 PFASs exposure and birth outcomes

A common measure of fetal growth is birth weight, especially when gestational age is taken into account. A fetal growth restriction might be present when the fetus fails to obtain the genetically determined growth. Several epidemiological studies report inverse association between PFASs and birth weight, but not all of them statistically significant [68]. Studies show a clear indication of placental transfer of PFASs between mother and fetus [43] and due

to evidence of accumulation of PFASs in placenta, fetal exposure seems to increase with gestational age [65].

A study from China reported that their findings indicated that factors as maternal age, weight and BMI could influence the placental transfer efficiency (PTE) of PFASs. A statistically significant association was found between maternal weight and PTE for PFOS and a possible association for PFNA. A suggested reason for these results might be an interference of the function of placenta due to maternal age and maternal weight affecting the development of placental vascular [44].

Several studies assessed in this thesis found an association, significant or non-significant, between PFOS and birth weight [55, 60]. The INMA study of Spanish women found that higher concentrations of PFHxS, PFOA and PFNA were non-significantly associated with birth weight. The same study also reported that PFOS and low birth weight (LBW) seemed to be dependent on the sex of the baby. An increase in concentration of PFOA and PFOS was associated with an increased OR of being LBW (OR = 1.90 (95% Confidence Interval (CI): 0.98, 3.68)) for boys, but not for girls (OR = 0.73 (95% CI 0.46, 1.19)) [2]. These results were consistent with a study from the Faroe Island, where they also found an association between PFOS and birth size in boys [55]. Not all of the studies found an association, the Brazilian Ribeirão Preto (BRISA) study found no significant association between PFOS and PFOA, neither for LBW or preterm birth, but a significant positive association was found between PFAS and PFOS and fetal growth restriction (PFOA p = 0.015 and PFOS 0.0003) in maternal whole blood samples [18].

From the C8 Health Project with self-reported health outcomes, Stein *et al*. observed an increased risk for LBW connected to an increase of PFOS exposure, consistent to some other studies [67, 68]. Same study found no association between PFOA and birth weight [67], which is consistent with Gao *et al*. who report no association between PFOA or PFOS in maternal cord blood and birth weight/length [44]. In South Korea, Lee *et al*. found significant inverse association of PFHxS in the blood samples from the umbilical cord and the birth weight of the newborns. Despite limitations due to sample size, this study also suggest that prenatal exposure to PFASs may have an effect on birth outcomes [43]. In Bejing, China, a study showed a positive association with PFBA and birth weight and they suggest that both

PFBA and PFBS might affect the birth outcomes (median concentrations 0.10 ng/mL, 0.14 ng/mL, respectively) [44].

For birth defects, Stein *et al*. report a weak association for PFOA, but no association for PFOS. Further, there were no association between these chemicals and miscarriage or preterm birth [67]. Few studies assessing neurodevelopment in children are included in this thesis, but Spratlen *et al*. evaluated the association between prenatal PFASs exposure and the cognitive outcomes, such as memory, problem solving, hand-eye coordination, imitation and early language and measures as fine and gross development in children. The study population were prenatally exposed to the World Trade Center disaster 9/11-year 2001, they found sex-specific associations between the concentration of PFASs and childhood neurodevelopment. The associations were positive among females, but not males [36]. While the Hokkaido birth cohort from Japan, found a negative association between mental development for girls at 6 months of age [56].

A study among Japanese women found that thyroid hormone levels were more influenced by PFOS than PFOA [56]. The CHirP study suggested that pregnant women with high levels of thyroid peroxidase anti-bodies were more susceptible to PFASs-induced thyroid disruption, who can affect the fetus. Relatively small changes in maternal thyroid hormone levels are known to affect the fetal brain development [59].

To summarize, when it comes to PFASs exposure and birth outcomes several studies suggest that PFASs exposure may have an effect on birth outcomes [43, 44]. A few studies found an association with PFASs levels and LBW [68], some of these studies indicates that this association is dependent on gender, with a higher risk of LBW in boys than in girls [55, 60].

6.5 Correlation between PFASs

In the Norwegian study MoBa, the PFASs were moderately to high correlated with one another, the highest observed correlation was between PFNA and PFDA (r_s = 0.75), categorized as a strong correlation, while the lowest correlation was between PFDA and PFHxS (r_s = 0.18) [61, 69]. In comparison correlations observed in the EMASAR study is lower than correlations reported from the MoBA study, where the highest significant

correlation was found between PFOA and PFHxA, a strong correlation, in Salta (r_s = 0.656, p = 0.05) and the lowest were between PFBA and PFHxS in Ushuaia (r_s = 0.119, p = 0.01).

The correlation between PFOS and PFOA were moderate in the EMASAR study as shown in Table 3, both in Ushuaia and Salta (r_s = 0.593 and r_s = 0.497), while the correlation was labeled as strong with a correlation of r_s = 0.64 in the Norwegian MoBa study [61]. The correlation between the PFASs in the study from Torshavn, Faroe Island were similar to the MoBa study with a correlation at r_s = 0.63 for PFOS and PFNA [55].

6.6 Strengths and limitations

Data from the EMASAR study which is presented in this thesis gives a novel insight in PFASs levels in the two study sites, Ushuaia and Salta. By collecting data from two geographically different study sites it is possible to assess the similarities and differences within the regions. Ushuaia is located in the south of Argentina, a coastal city where the diet mainly is based on agricultural products as well as meat and fish, while the City of Salta is located inland at the foot of the Andes mountains, with a high-altitude population where the diet is based on agricultural products and meat. Different location, altitude, socio-economic status and lifestyle characteristics gives a good overview of the current situation. An estimation of 90% of the women invited to participate in the EMASAR study consented. The representativeness of the sample size is therefore acceptable (n = 689) and suitable for comparison to other population monitoring studies. Several studies included in the comparison in Figure 6 had a similar study design and study population to make the comparison reliable.

In addition, the EMASAR study design is conducted to be compatible with the circumpolar programme, AMAP [52]. The EMASAR study has adopted and used the methodological and analytical approach as the AMAP study. The Laboratory for Analysis of Environmental Pollutants, Department of Laboratory Medicine, University Hospital of North-Norway participated successfully in the AMAP Analytical Ring Test [51].

Published materials on PFASs concentrations among pregnant and delivering women are scarce in developing regions, such as Latin America and Argentina. Global monitoring is failing to compare results worldwide due to lack of data [10]. The findings of the EMASAR

study addresses the gap of knowledge in these areas and are providing a theoretical basis for further studies within this field of research, especially PFASs concentrations and potential health effects.

Despite the strengths, there are several limitations in this thesis. The literature on PFASs concentrations in pregnant and delivering women show inconsistent methods for collection of blood samples. The EMASAR study collected maternal blood serum within 36±12 hours after delivery, while others have collected these samples during the first or second trimester of the pregnancy. Evidence of placental and lactational transfer of PFASs from mother to fetus can be found in a number of studies [43, 65] and might have influenced the PFASs levels collected in postpartum women, compared to blood samples collected at an earlier stage in the pregnancy. Therefore, the burden of PFASs exposure on the fetus may not be accurately reflected by the blood sample collected postpartum in the present study. Due to sampling methods, women were recruited as they were about to deliver or just delivered, so this was not possible in the EMASAR study. To assess the total PFASs exposure from mother to fetus, this thesis suggest that blood samples could have been collected twice, early in the pregnancy (first or second trimester), as well as postpartum.

As a routine check, a blood sample was collected from the newborn children at the hospital immediately after birth, but these are not yet assessed for PFAS levels. The blood sampling method might also have affected the PFOS levels (in comparison to blood samples with maternal serum/plasma), a study from Norway and China observed differences in plasma from maternal- and umbilical cord and found different efficiencies of various PFASs and placental transfer. While concentrations of PFOA and PFHxS are increased in the samples from the umbilical cord, the PFNA and linear PFOS has decreased, the results indicate that the carbon-chain length is influential [42, 64]. This indicate a need for further assessments of the PFOA and PFHxS level in newborns in the future.

In addition, parity is an important factor when observing maternal PFASs concentrations, this is due to higher levels in nulliparous, than multiparous women [61]. Due to a limitation in the datafile used in this thesis, it was not possible to assess the differences of PFASs levels in nulliparous (43%) and multiparous women (57%) in the EMASAR study.

The EMASAR study used two reference hospitals, a public hospital in Salta, responsible for all in-hospital deliveries versus a private hospital, co-responsible with a public hospital for in-hospital deliveries in Ushuaia. Significant gaps and problems in the health system, especially regarded to access can be reflected in the number of caesarian sections in Ushuaia (44% total, 23% elective), compared to Salta (6% total, 2% elective) [46, 50]. There are clear differences between the two regions, both when it comes to socioeconomic status and lifestyle. But even though Ushuaia has one of the most prosperous socioeconomic conditions in Argentina and a difference between the two study sites were expected, two public hospitals should have been used to avoid bias, especially in a country with a health system as fragmented as in Argentina [47].

Observations from the MISA study, which is categorized as a short study, reports inconsistent decline in concentration level, especially in PFNA. This is pointing out the importance of an extension of recruitment period when investigating predictors of PFAS concentration [38]. Several of the studies in this review of literature are cross-sectional, included the EMASAR study, which might have limited causal interpretation compared to cohort studies.

7 Conclusion

The EMASAR study has allowed this thesis to explore and present PFASs concentrations among delivering women in Ushuaia and Salta, then previously explored in this region. Among the PFASs concentrations from delivering women in Ushuaia and Salta, the most prominent substance is PFOS, followed by PFBA and PFOA, with a slight elevated level in Ushuaia compared to Salta. Compared to other regions of the world, the PFASs levels assessed in this thesis from the two study sites in Argentina are low. In accordance to existing literature, this thesis concludes that implementation of regulatory initiatives to restrict and/or eliminate production and use of several PFASs such as PFOA and PFOS is beneficial for the detection of these chemicals in maternal blood samples, while other PFASs are emerging, such as PFHxS and PFNA [55]. PFASs assessed in the EMASAR study and existing literature find several PFASs to be statistically significant positive correlated with each other.

Recommendations

In several of the studies reviewed in this thesis, a limited amount of PFASs are being assessed, which means that the total PFASs burden is expected to be higher than reported. These observations indicate a need for better mapping of the adverse health effects and the levels of PFASs in humans, especially pregnant women and their offspring. PFASs replacing PFOA and PFOS, are currently more detected in humans and wildlife, this needs attention and need to be considered in future studies [17].

Due to restrictions in production of several PFASs, humans are now being exposed to many new, unidentified compounds. There has been a decrease in PFOS exposure due to extensive restrictions on production and use of PFOS and PFOA during the last years, simultaneously, exposure to other PFASs, such as PFNA and PFHxS has emerged [55]. The manufacturers of these chemicals have claimed that the PFASs used for replacement not is associated with adverse health effects because of shorter chain analogues, shorter half-lives and less bio-accumulative. However, recent research disagree, ongoing work suggest that short chain compounds have different properties, but still as persistent, toxic and widely distributed in the world as long chain analogues [12]. Further research is needed to understand the exposure pathways and the health outcomes associated with emerging PFASs [3].

PFHxS is a PFAS that has received less attention than PFOS and PFOA. Not only is this compound as persistent, but it also has a higher bio-accumulative potential than PFOS and an estimated elimination half-life of approximately 8.5 year in humans [19, 21]. In addition, a study has shown that the compound increases from maternal blood samples to umbilical cord blood [42, 64]. PFHxS should therefore in the future be monitored for its global presence and hazardous potential, especially in newborns.

A positive association between preeclampsia and PFBS in plasma drawn from cord blood was found in a Chinese study. Studies on prenatal PFBS exposure and hypertensive disorders are scarce, further investigation on PFBS and maternal health is recommended [39].

Relatively low levels of PFASs may have adverse effect on fetus and child health. There is a need for further studies on maternal and fetus exposure to these chemicals [56].

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Appendix

Table S1: Limit of detection (LOD) of maternal PFAS serum samples in Argentina (*n* = 698) and stratified by the city of Ushuaia (*n* = 193) and Salta (*n* = 496)

	Total			**Ushuaia**			**Salta**		
	% > LOD	**Mean (SD)**	**Median (Min-Max)**	**% > LOD**	**Mean (SD)**	**Median (Min-Max)**	**% > LOD**	**Mean (SD)**	**Median (Min-Max)**
lin PFOS	100	0.009 (0.008)	0.006 (0.000-0.077)	100	0.008 (0.007)	0.006 (0.000-0.039)	100	0.009 (0.009）	0.006 (0.001-0.077)
sum PFOS	100	0.009 (0.008)	0.006 (0.000-0.077)	100	0.008 (0.007)	0.006 (0.000-0.039)	100	0.009 (0.009)	0.006 (0.001-0.077)
lin PFHxS	98	0.007 (0.006)	0.005 (0.001-0.081)	98.4	0.006 (0.005)	0.005 (0.002-0.063)	97.2	0.007 (0.006)	0.006 (0.001-0.081)
Sum PFHxS	98.4	0.007 (0.006)	0.005 (0.000-0.081)	99.5	0.006 (0.005)	0.005 (0.002-0.063)	98.0	0.007 (0.006)	0.006 (0.000-0.0810
PFNA	94.5	0.009 (0.003)	0.009 (0.004-0.020)	99.5	0.010 (0.003)	0.010 (0.004-0.019)	92.7	0.009 (0.003)	0.009 (0.004-0.020)
PFOA	94.2	0.121 (0.028)	0.119 (0.059-0.178)	100	0.101 (0.027)	0.098 (0.059-0.132)	92.1	0.128 (0.025)	0.119 (0.059-0.178)
PFHxA	85.8	0.012 (0.005)	0.011 (0.003-0.046)	58.5	0.012 (0.005)	0.011 (0.006-0.029)	96.4	0.012 (0.005)	0.011 (0.003-0.036)
PFDA	84.2	0.010 (0.004)	0.009 (0.003-0.060)	85.5	0.010 (0.005)	0.009 (0.003-0.054)	85.5	0.010 (0.004)	0.009 (0.003-0.060)
PFBA	78.7	0.504 (0.073)	0.484 (0.363-0.645)	93.8	0.465 (0.051)	0.484 (0.363-0.531)	73.2	0.520 (0.075)	0.517 (0.363-0.645)
lin PFHpS	33.4	0.004 (0.002)	0.003 (0.001-0.011)	55.4	0.004 (0.002)	0.003 (0.001-0.008)	24.8	0.004 (0.002)	0.003 (0.001-0.011)
sum PFHpS	33.5	0.004 (0.002)	0.003 (0.001-0.011)	55.4	0.004 (0.002)	0.003 (0.001-0.008)	25.0	0.004 (0.002)	0.003 (0.001-0.011)
PFUDA	28.9	0.009 (0.003)	0.009 (0.002-0.022)						
PFHpA	28.6	0.009 (0.002)	0.009 (0.003-0.018)	19.7	0.009 (0.002)	0.008 (0.005-0.015)	32.1	0.010 (0.002)	0.010 (0.003-0.018)
PFDoDA	11.3	0.010 (0.003)	0.009 (0.003-0.018)	5.7	0.010 (0.003)	0.010 (0.006-0.018)	13.5	0.009 (0.002)	0.008 (0.003-0.018)
PFPeA	3.5	0.013 (0.004)	0.013 (0.003-0.026)	1	0.016 (0.003)	0.016 (0.008-0.026)	4.4	0.011 (0.004)	0.011 (0.003-0.026)
PFTrDA	2.6	0.015 (0.006)	0.014 (0.004-0.034)	2.6	0.014 (0.005)	0.014 (0.007-0.028)	2.6	0.015 (0.006)	0.014 (0.004-0.034)
PFTeDA	1.2	0.031 (0.016)	0.026 (0.003-0.087)	1	0.032 (0.019)	0.021 (0.011-0.079)	1.2	0.031 (0.014)	0.030 (0.003-0.087)
PFPS	0.4	0.004 (0.002)	0.004 (0.001-0.019)	0	0.003 (0.001)	0.003 (0.001-0.007)	0.6	0.004 (0.003)	0.004 (0.002-0.019)
4:2 FTS	0.4	0.009 (0.007)	0.006 (0.002-0.046)	0.5	0.008 (0.006)	0.008 (0.002-0.020)	0.4	0.009 (0.008)	0.006 (0.002-0.046)
6:2 FTS	4.2	0.007 (0.005)	0.006 (0.002-0.035)	3.6	0.008 (0.006)	0.009 (0.002-0.035)	4.4	0.007 (0.004)	0.006 (0.002-0.022)
8:2 FTS	4.5	0.007 (0.004)	0.006 (0.002-0.028)	1.6	0.009 (0.006)	0.009 (0.002-0.028)	5.6	0.006 (0.003)	0.006 (0.002-0.026)
10:2 FTS	0.1	0.007 (0.004)	0.007 (0.001-0.020)	0.5	0.008 (0.006)	0.006 (0.002-0.020)	0	0.006 (0.003)	0.007 (0.001-0.012)
PFBS	0	0.005 (0.002)	0.004 (0.002-0.019)						
lin PFNS	0	0.003 (0.002)	0.003 (0.001-0.011)						
sum PFNS	0	0.003 (0.002)	0.003 (0.001-0.011)						
lin PFDS	0	0.003 (0.003)	0.003 (0.001-0.020)						
sum PFDS	0	0.003 (0.003)	0.003 (0.001-0.020)						
PFDoDS	0	0.003 (0.002)	0.003 (0.001-0.016)						
lin PFOSA	0	0.004 (0.002)	0.004 (0.001-0.009)						
sum PFOSA	0	0.004 (0.002)	0.004 (0.001-0.009)						

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