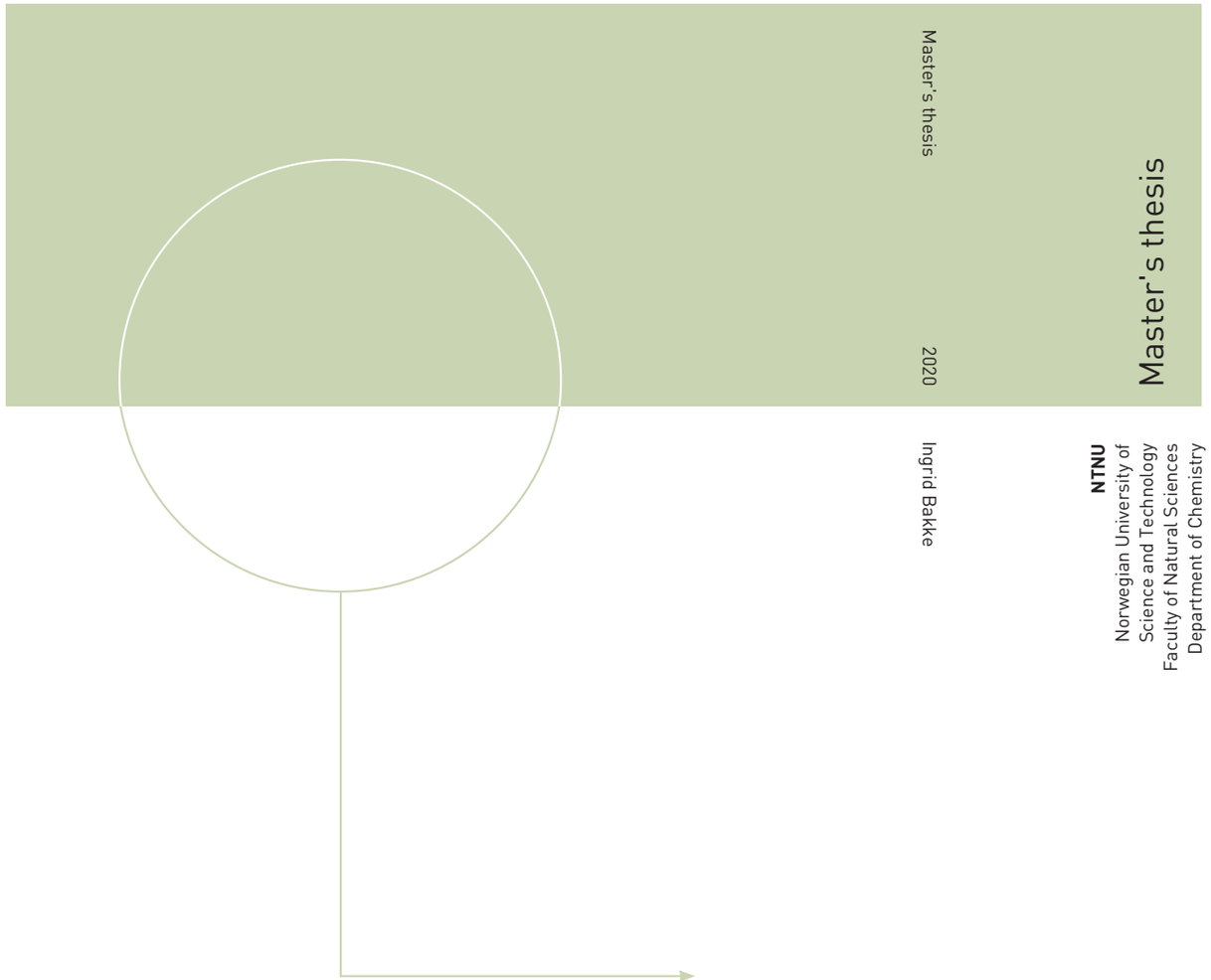

Master's thesis

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Ingrid Bakke

NTNU
Norwegian University of
Science and Technology
Faculty of Natural Sciences
Department of Chemistry

Ingrid Bakke

Endocrine Disrupting Substances in Infant Foodstuffs

Presence of Perfluoroalkyl Substances and Toxic Elements

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Ingrid Bakke

Chemical Engineering and Biotechnology
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Supervisor: Alexandros G. Asimakopoulos
Co-supervisor: Trond Peder Flaten

Norwegian University of Science and Technology
Department of Chemistry

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Abstract

Per- and polyfluoroalkyl substances (PFAS) have received a great deal of attention on account of their endocrine disrupting abilities. PFAS are found ubiquitously in nature and their presence in food for human consumption is therefore a matter of concern. As the introduction of complementary feeding into infants' diets may result in increased uptake of these substances their presence and adverse health affects must be studied. The objective of this Master's Thesis was therefore to determine the concentration of 15 PFAS and one similar unfluorinated substance in complementary infant foodstuffs purchased in Norway. To assess the overall risk associated with consumption of the infant foodstuffs elemental analysis was performed as well.

49 infant food products consisting mainly of *Dairy*, *Fruit*, *Corn*, *Grain*, *Meat* or *Vegetables* underwent extraction and UPLC-MS/MS analysis. The 15 PFAS recovered well from the extraction procedure, all with relative recoveries above 76% for 10 ppb spiked samples. Most PFAS had matrix effects below $\pm$ 60% for 10 ppb spiked samples. However, PFPA, PFHxS and PFOS exceeded this level, and were strongly affected by interfering substances in the infant food matrix. The unfluorinated substance DecaS did not recover well from the extraction procedure and was highly affected by interfering substances.

All PFAS were detected in the infant foodstuffs, and the products consisting of *Corn* generally contained PFAS in high concentrations. However, *Grain* products also contained high concentrations of some PFAS, such as PFPA. The three PFAS PFPA, PFHxA and PFTeDA were found in high concentrations in the infant foodstuffs. PFHxA was found in high concentrations in most main ingredient groups and PFTeDA in a *Corn* product. The restricted substance PFOS was one of only two PFAS found in lower concentrations than previously reported, indicating that the restriction has served its purpose and that further restrictions of PFAS may decrease their presence in foodstuffs. PFOS was also one of two PFAS found in lower amount in infant foodstuffs than in breast milk. The estimated daily intake (EDI) of all risk-assessed PFAS exceeded the minimum risk level (MRL), and some of the PFAS that were not previously risk assessed resulted in very high EDIs from infant food consumption. These were especially PFPA, PFHxA, PFDA and PFTeDA.

Among the 63 elements determined in the infant foodstuffs the thesis focuses on Cd, Hg, Pb, U, Cr, As and Sb. The toxic elements of highest concern were Hg and Sb, as they were found in higher average concentrations in the infant food than previously reported. However, these elements also had the highest uncertainty in the analytical procedure. The infant food products of special concern were the products 1, 3 (powdered grain porridge products), 7, 8, 10 (corn puff products), 11, 14 and 15 (concentrated fruit snack bars).

Sammendrag

Per- og polyfluorinerte stoffer (PFAS) har den siste tiden fått mye oppmerksomhet på grunn av deres hormonforstyrrende egenskaper. PFAS er påvist i naturprøver i mange deler av verden og det er derfor grunn til bekymring angående disse stoffenes tilstedeværelse i mat. Ettersom introduksjon av prosesserte produkter i barns kosthold kan føre til økt utsettelse for disse stoffene er det viktig å vurdere omfanget og alvorlige effekter av PFAS inntak. Hensikten med denne Masteroppgaven er derfor å bestemme omfanget av 15 PFAS og et lignende ikke-fluorinert stoff i komplementære barnematprodukter solgt i norske matbutikker. For å få et mer overordnet blikk over helserisikoen i disse produktene ble det også gjennomført en elementanalyse.

Det ble gjennomført ekstraksjon og UPLC-MS/MS analyse av 49 barnematprodukter med hovedingrediens *Melk*, *Frukt*, *Mais*, *Korn*, *Kjøtt* eller *Grønnsaker*. Generelt viste analysen god gjenopprettelse av PFAS analyttene, på over 76% gjenopprettet analytt for prøver spiket med 10 ppb standarder. Matriks effekter var lave (under $\pm$ 60%) for de fleste PFAS, med unntak av PFPA, PFHxS og PFOS, som da var sterkt påvirket av andre analytter i barnematmatriksen. Den ufluorinerte analytten DecaS viste dårlig gjenopprettelse og høy matriks effekt, og analysen var upassendefor denne analytten.

Alle PFASene ble funnet i barnematproduktene, og produtkene med hovedingrediens *Mais* inneholdt generelt høyest konsentrasjon av PFAS. *Korn*-produktene inneholdt også høye konsentrasjoner av noen PFAS, slik som PFPA. Spesiselt PFPA, PFHxA og PFTeDA ble funnet i høye konsentrasjoner i barnematproduktene. PFHxA i de fleste matgruppene og PFTeDA i *Mais*-produkter. PFOS, som har restriksjoner i forhold til dens bruk, ble funnet i lavere konsentrasjoner enn tidligere rapportert, mens de fleste andre PFAS ble funnet i høyere konsentrasjoner i denne analysen. Dette indikerer at restriksjoner fungerer og at en restriksjon av andre PFAS kan føre til lavere nivåer i mat. PFOS var også en av to PFAS som førte til et lavere Estimert Daglig Inntak (EDI) fra komplementær barnemat enn fra en brystmelk diett. EDI av alle risikovurderte PFAS overgikk Minimum Risiko Nivå (MRL), og noen av PFASene som ikke var risikovurdert førte til bekymringsverdig høy EDI. Mer spesifikt hadde PFPA, PFHxA, PFDA og PFTeDA svært høye EDI.

Av de 63 elementene analysert ble syv toksiske elementer valgt ut som hovedfokus: Cd, Hg, Pb, U, Cr, As og Sb. Hg og Sb ble funnet i høyere konsentrasjoner i barnematproduktene enn hva som var funnet tidligere. Disse to elementene hadde veldig høy usikkerhet i deteksjonen, som tilsa at resultatene kunne være misvisende. Barnematproduktene som hadde bekymringsverdig høye konsentrasjoner av PFAS eller toksiske elementer var: 1, 3 (grøtpulver av korn), 7, 8, 10 (maispuffs), 14 og 15 (korn- og fruktbarer).

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Abbreviations

AITD	Autoimmune thyroid disease
CLP	Classification, Labeling and Packaging regulation
DDT	Dichlorodiphenyltrichloroethane
DHT	Dihydrotestosterone
EDI	Estimated daily intakes
ESI	Electronspray ionization
ICP-MS	Inductively coupled plasma mass spectrometry
IS	Internal standard
ISM	Internal standard method
LLE	Liquid-liquid extraction
MRL	Minimum risk levels
MS/MS	Tandem mass spectrometry
PASF	Perfluoroalkyl sulfonyl fluoride
PCB	Polychlorinated biphenyl
PFAA	Perfluoroalkyl acids
PFAS	Per- and polyfluoroalkyl substances
PFCA	Perfluoroalkyl carboxyl acid
PFPE	Perfluoropolyether
PFSA	Perfluoroalkyl sulfonic acid
PFTE	Polyterafluoroethylene
RT	Retention time
SHBG	Sex hormone-binding globulin
SLE	Solid-liquid extraction
SRC	Single reaction chamber
TA	Target analyte
TDI	Tolerable daily intake
TTR	Thyroid transport protein
UPLC	Ultra-performance liquid chromatography
UV	Ultra-violet

Abbreviations of Endocrine Disrupting Substances

DecaS	Decasulfonate
PFBS	Perfluorobutansulfonic acid
PFDA	Perfluorodecanoic acid
PFDoDA	Perfluorododecanoic acid
PFHpA	Perfluoroheptanoic acid
PFHxA	Perfluorohexanoic acid
PFHxS	Perfluorohexansulfonate
PFNA	Perfluorononanoic acid
PFOSA	Perfluorooctanesulfamide
PFOS	Perfluorooctanesulfonate
PFOA	Perfluorooctanoic acid
PFPeA	Perfluoropentanoic acid
PFTeDA	Perfluorotetradecanoic acid
PFTrDA	Perfluorotridecanoic acid
PFUnDA	Perfluoroundecanoic acid
Sulf	Sulfluramide
^{13}**C-PFOS**	^{13}C-Perfluorooctanesulfonate
^{13}**C-PFOA**	^{13}C-Perfluorooctanoic acid

1 INTRODUCTION

In recent years there has been an increasing focus on the presence of endocrine disrupting chemicals such as per- and polyfluoroalkyl substances (PFAS) in our environment. PFAS are synthetic chemicals produced by humans since 1949 and they are found in rivers, agricultural soil, groundwater, in our homes and in our bodies [3]. Largely because of their surface protection properties PFAS are used in various fields, such as: food packaging, fire-fighting foam, ski wax, textiles, kitchen ware, electronics, cleaning agents, paints, biocides and emulsifiers [1][2]. Non-polymer PFAS are fluorinated carbon chains usually with carboxyl- or sulfonyl acid groups at one end. As non-polymer PFAS are resistant to decomposition and are both hydrophilic and hydrophobic they are prevalent both in aqueous environments and adsorbed to organic matter. From these environments PFAS can migrate to agricultural soil and further into plants for human and animal consumption. PFAS are found to be bioaccumulative, indicating that animals higher up in the food chain may be exposed to higher levels of PFAS [4].

The European Food and Safety Authority (EFSA) evaluated the risk of PFAS exposure in 2020, and found that parts of the European population are exposed to higher levels of PFAS than tolerated in regard to adverse health effects [2]. EFSA also found that European infants have double the daily PFAS intake per body weight as adults. In the first 6 months of infants' lifes the endocrine system is activated, and PFAS and other endocrine disruptors may disturb this activation. As this activation is under-researched the affects of this disruption is not necessarily comparable to well-researched effects in adults [5]. PFAS are also associated with other adverse health affects, such as liver damage, developmental effects and cardiovascular effects [3]. PFAS exposure may also diminish the effect of vaccines in the body, and this effect may be especially severe for infants younger than 6 months of age [6]. As a consequence, the presence of endocrine disrupting chemicals in foodstuffs intended for infants and the effect of these chemicals on infants' health and development are of great concern. Some PFAS have been restricted by the Stockholm convention in 2009, such as PFOS and its precursors [7]. However, as a consequence of consumerism and further need of surface protection substances other PFAS have taken their place in industry and products [8]. As a response to this trend Norway, Germany, Sweden, Holland and Denmark have initiated a proposal to ban the use of all PFAS in the EU by 2024 [9]. According to the Norwegian Environmental Agency this would result in the restriction of approximately 5000 per- and polyfluoroalkyl substances [9].

Another group of toxic substances that pose a potential adverse health risk for infants are toxic elements [10]. To obtain a complete review of the contamination of infant foodstuffs both elemental and trace organic analysis should be performed. The objective of this thesis was to inspect the occurrence of PFAS and toxic elements in infant food products, and to discuss the risk associated with consumption of these products.

2 BACKGROUND

2.1 Endocrine Disrupting Chemicals

Endocrine disrupting chemicals are chemicals that interfere with the endocrine system, and effect hormone biosynthesis, metabolism and action. The endocrine system is a bodily function that controls and coordinates hormone activity and secretion from specific organs [11]. Hormones are mainly produced in endocrine glands such as the thyroid, adrenal and gonad glands. Endocrine diseases originate from over- or underproduction of hormones, from altered tissue responses to hormones or from tumors on endocrine glands. Examples are Graves disease, caused by overproduction of thyroid hormones, type 1 diabetes, caused by autoimmune destruction of the insulin hormone and pseudohypoparatyroidism, caused by resistance to the thyroid hormone [11].

Abnormalities in the thyroid function are associated with reduced fertility in both men and women [12]. Hyperthyroidism and hypothyroidism, meaning over- and underproduction of the thyroid hormone, are both associated with changes in the sex hormone-binding globulin (SHBG) levels in the body. SHBG is a protein produced in the liver that carry the three reproductive hormones estrogen, dihydrotestosterone (DHT) and testosterone in the blood. Hyper- and hypothyroidism can cause disturbances in the menstrual cycle in women. Both hyper- and hypothyroidism has been linked to a decrease in fertility in women. Interferon induced autoimmune thyroid disease (AITD) is the most common endrocrine disease in women of reproductive age, and is just short of 10 times more common in women than in men. AITD is also often undiagnosed as it can occur at subclinical thyroid disfunction. Hypothyroidism is seen less in males than in females in general. In males, hypothyroidism leads to lower SHBG levels, which in turn leads to reduced free testosterone levels in plasma by 60%. Hyperthyroidism may lead to irregular changes in sperm mobility in men, while hypothyroidism may lead to irregularity in sperm morphology. Boys born with hypothyroidism that are treated early have normal sexual development and adult height, indicating that short-term hypothyroidism does not have adverse health effects on the male reproductive system. However, hypothyroidism in children is associated with growth retardation and changes in secretion and action of the growth hormones [12]. The thyroid hormone is also essential for brain development in the early life stages [13].

Some known chemicals that bind to the estrogen receptor are dichlorodiphenyltrichloroethane (DDT), chlordecone, alkylphenols and some PCBs [14]. The use of the pesticide DDT was restricted in 1972, but many new xenobiotic chemicals for use in production, agriculture and other fields have been synthesized since, and need to be identified and evaluated as environmental risks. Per- and polyfluoroalkyl substances (PFAS) have been suggested to have endocrine disrupting abilities and further studies on various aspects of PFAS are needed [15].

2.2 Per- and Polyfluoroalkyl Substances

PFAS are synthetic compounds found ubiquitously in the environment after having been produced industrially since 1949. PFAS are made up of a carbon backbone partly or completely fluorinated, and most PFAS contain a carboxyl- or sulfonic acid at the end of the carbon chain. The carbon-fluorine bonds are very strong and the three electron pairs surrounding the fluorine atom introduce a shielding effect on the carbon atoms, resulting in a very stable fluorinated carbon chain. This fluorinated carbon chain is hydrophobic while the acid in the other end of the molecule is hydrophilic, which gives rise to excellent surface protection properties [3].

PFAS can be divided into polymers and non-polymers [1]. Non-polymer PFAS are for example perfluoroalkyl acids (PFAA) which include, but are not limited to, perfluoroalkyl carboxyl acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs). Another group of non-polymers is perfluoroalkyl sulfonyl fluorides (PASFs), which are potential precursors of PFSAs. The grouping of non-polymer PFAS and some examples of molecular structures are shown in **fig. 1**.

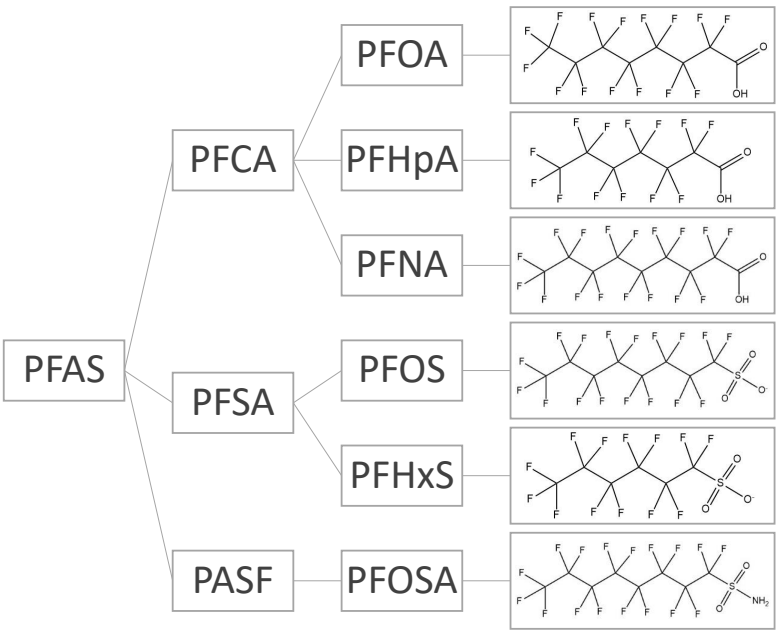

Figure 1: Grouping of non-polymer PFAS and examples of PFCAs, PFSAs and PASFs [1].

PFAS polymers are either telomerized fluorinated carbon chains (such as PFTE), non-fluorinated carbon polymers with fluorinated side-chains or fluorinated ether polymers (such as PFPEs). Polytetrafluoroethylene (PFTE) is a widely used PFAS polymer and is found in for example kitchen ware. Perfluoropolyethers (PFPEs) is a group of polymers used as lubricants in industry [16]. PFCAs are used as processing aids in the production of fluoropolymers, however, fluoropolymers are not made of PFCAs. The side-chain of PFPEs might be precursors of PFCAs.

The non-polymer PFAS groups PFCAs and PFSAs can be classified as short- or long-chained substances, depending on the length of their carbon backbone. PFCAs with carbon backbones longer than 7 and PFSAs with carbon backbones longer than 6 are classified as long-chained. PFCAs and PFSAs are resistant to decompositions such as photolysis, acid/base reactions and reduction/oxidation reactions. As a result, PFCAs and PFSAs are very persistant and are accumulated in nature and in humans.

2.2.1 Prevalence of Perfluoroalkyl Substances in Nature and in Foodstuffs

The Agency for Toxic Substances and Disease Registry (ATSDR) estimated a total global historical emission of PFCAs from 1951-2004 to be between 3200 and 7300 tonnes. Even though most industrial countries have ceased the production of PFOS and PFOA, some countries still produce these PFAS, and other PFAS that are not yet restricted are still being produced globally. The fact that PFAS are both hydrophilic and hydrophobic is convenient in surface treatment, but it also results in a wide prevalence of PFAS - as they are found both in aqueous environment and adsorbed to organic matter. PFAS are found in outdoor air, originating from industrial processes, but may also be found in indoor air and dust indicating that consumer products may be sources of contamination [3]. PFAS are present in groundwater, surface water and drinking water, possibly originating from industry run-off, waste water treatment plants, landfill sites and fire-fighter training facilities. Waste water treatment plants are found to been a main contributor to PFAS contamination in groundwater and surface water. PFAS are also found in agricultural soil, and may originate from biosolids used as fertilizer, atmospheric deposition, deposition from groundwater and surface water and biodegradation of fluototelomers. A study of biosolids from Chicago (2011) found a total PFAS concentration of 433 ± 121 ng/g [17], where PFOS was the main PFAS. The sorption of PFAS to soil depends on the interaction between the hydrophobic part of a PFAS and the organic matter in the soil, and consequently on the organic matter content in the soil. The sorption of PFOS in soil has been found to be irreversible, while the sorption of PFOA and PFBS is reversible [18]. PFOA is mobile in soil and will therefore not adsorb to suspended solids in for example groundwater. The affinity of some PFAS in soil is in the order of PFOS $>$ PFOA $>$ PFBS. PFAS also migrate from agrcultural soil to plants. Studies have shown that plant uptake of PFAS varies depending on chain length, functional group, plant species and part of the plant [19]. Short-chain PFAS accumulate in plants at higher levels than long-chained PFAS [20]. Ghisi *et al.* (2019) found low accumulations of PFOA and PFOS in peeled potato and cereal seeds and higher levels of short-chain PFAS in leafy vegetables and fruits [19]. PFOA and PFOS are reported in high concentration in food products containing fish, followed by egg, meat and fruit products [2]. PFNA has been reported in high concentrations in fruit products and in infant food, and PFHxS mainly in fruit products. Krippner *et al.* (2014) reported that short-chained PFAS were taken up in the roots of corn plants and transferred at high rates to the shoots of the plant, while long-chained PFAS tended to stay in the roots of the plant [21].

2.2.2 Endocrine Disrupting Properties of Perfluoroalkyl Substances

Organic fluorine was first found in human serum in 1968 and the linking of PFOA and PFOS to negative health effects in humans was first reviewed in 2002. Population studies have reported an association between PFOS and PFOA serum levels and risk of thyroid disease [3]. PFAS may bind to the thyroid transport protein (TTR) in humans [13]. The PFAS would then compete with the transportation form of the thyroid hormone, T_4-hormone. This contamination may lead to thyroid hormone levels decreasing, causing hypothyroidism. The trend in binding potency for different PFAS to TTR is PFHxS $>$ PFOS/PFOA $>$ PFHpA $>$ PFNA. The binding potencies were 12.5-50 times lower than that of the TTR protein itself [13]. PFOA has a half-life elimination of 4.4 years in human serum, and considering that PFOS has ben found to be more prevalent in human serum than PFOA the assumption that PFOS also has a high half-life elimination can be made [22]. It is also reported that PFOS seems to remain in tissue longer than PFOA [23]. In the case of low or non-existing internal degradation in humans the persistent compounds PFOA and PFOS will bioaccumulate in human serum. Additionally, longer chain PFAS have shown to be more toxic than short-chain PFAS on account of short-chain PFAS being eliminated into urine at faster rates. For instance PFDA has been shown to be more toxic than PFOA in rats [24]. Wilkens *et al.* found that levels of PFAS increase with increasing age as a result of absence of excretion [25]. However, infants may have increased contact with sources of PFAS (through toys, pacifiers, etc.) which may result in higher weight based internal PFAS contamination than adults [23]. Additionally, infants are in a much more sensitive phase of growth than adults and exposure to PFAS could have a larger negative effect on them. The method of uptake of PFAS is also of importance. Oral uptake is more efficient than inhalation or dermal exposure, and leads to almost complete assimilation into biological tissue in rat experiments [23]. Vestergren *et al.* (2012) found that PFOA and PFOS intake was dominated by dietary intake in the Swedish population, and that the total PFAS dietary intake had been almost constant from 1999-2010 [26]. PFAS have also been reported to have immunotoxic effects by suppressing the antibody response in the immune system or vaccination [6]. Other toxic health effects assiciated with PFAS are liver damage, developmental effects and cardiovascular diseases [3].

As a result of its endocrine disrupting properties and general toxicity the use of PFOS and its salts and precursors was restricted by the Stockholm Convention of 2009 [7]. Precursors of PFOS such as PFOSA and Sulfluramide are also restricted in their manufacturing and use. PFOA is restricted by the Classification, Labeling and Packaging regulation (CLP), and its manufacturing will be restricted as of July 4^{th} 2020 [2][9]. Other PFAS are under investigation by the European Commission to be restricted or classified as Substances of Very High Concern (SVHC). Details of the restrictions and applications of 15 perfluorinated substances and one similar unfluorinated substance are listed in **table 1**. In addition to the restrictions and evaluations listed in **table 1** Norway, in cooperation with Germany, Holland, Denmark and Sweden has suggested a general

ban of PFAS in the EU by 2024 [9].

Table 1: Classification [1], restrictions [2] and applications [1] of 15 PFAS and DecaS.

PFAS	Class.	Restrictions	Application of each PFAA
DecaS	Long	-	Replacing PFAS as emulsifier
Sulf	Long	Restricted precursor of PFOS	Ant/termite bait
PFOSA	Long	Restricted precursor of PFOS	-
PFDA	Long	CLP[b]. Restriction proposal in EU	-
PFDoDA	Long	Restriction proposal in EU	-
PFHpA	Long	Substance evaluation in Belgium	-
PFHxA	Short	Restriction proposal in Germany December 2019	Emulsifier in polymer manufacturing
PFNA	Long	CLP[b]. Restriction proposal in EU	Emulsifier in polymer manufacturing
PFOA	Long	CLP[b]. Restricted manufacturing after 4 July 2020	Emulsifier in polymer manufacturing
PFPeA	Short	-	-
PFTeDA	Long	Restriction proposal in EU	
PFTrDA	Long	Restriction proposal in EU	-
PFUnDA	Long	Restriction proposal in EU	-
All PFCAs			Paper and packaging industry, active ingredients in firefighter-foams, ski wax, polymerization aid
PFBS	Short	SVHC[a] by REACH, RMOA[c] by Norway	Flame retardant in polycarbonate resins used in electronics
PFHxS	Long	SVHC[a] by REACH, Restriction proposal in Norway	-
PFOS	Long	Manufacturing and use restricted since 2009	Hydraulic fluids, metal plating, semiconductor in manufacturing, surfactant in oil/gas and mining
All PFSAs			Investigated as fuel cell and battery, electrolyte, polymerization aid
[a] Substance of Very High Concern			-
[b] Classification, Labelling and Packaging regulation			-
[c] Risk Management Option Analysis			-

2.3 Elements

Most elements can be classified as either essential or toxic elements. Essential elements are essential in human nutrition and necessary in various biological processes. Some essential elements may be toxic if they are absorbed in excessive concentrations [27]. Toxic elements are elements that are toxic to human health even in low concentrations. Determination of toxic elements in

foodstuffs is of importance because of the chronic exposure consumers have as a consequence of mining and other human activities. As infants are in a developmental phase their organs are especially susceptible to toxic elements. Additionally, infants have a high consumption of food relative to their body weight and underdeveloped excretion routes compared to adults, which may lead to higher accumulation of toxic elements. Infants also have under-developed blood-brain barriers which may lead to more adverse health effects from toxic element exposure than for adults [10]. Examples of toxic elements are arsenic (As), cadmium (Cd), antimony (Sb), lead (Pb), uranium (U), mercury (Hg) and chromium (Cr(VI)) [28][29]. Symptoms and effects of toxicity as well as and main sources of these elements are shown in **table 2**.

Table 2: Toxic effects and some main sources of contamination of seven toxic elements.

	Toxic effects [30]	**Main sources**
Cd	Effects metabolism of elements, probably carcinogenic, kidney main target organ.	Steel industry and in plastic. Also in food and tobacco. Oral daily intake of 10-35 µg.
Hg	Mainly damage in kidney that can lead to digestive disorders, necrosis, proteinuria and hypoalbuminaemia.	Production of chlorine, dental equipment and electrical appliances. Mean daily dietary intake is 2-20 µg.
Pb	Decreased IQ, impaired renal function, hypertension, impaired fertility, cardio-vascular diseases and neurodevelopmental effects.	Lead-acid batteries, alloys and solders. Contamination in food decreased because of less use in oil. Drinking water concentration below 5 µg/L.
U	Nephritis (in kidneys), insufficient data on carcinogenicity of U.	Fuel in nuclear power plats. Daily intake through food is 1-4 µg, drinking water also a source.
Cr	Cr(VI) is carcinogenic in rats, but oral intake can reduce to Cr(III) in the stomach.	Food main source as it is prevalent in earth crust, Cr(III) is essential element. Drinking water concentration ranges from 2-120 µg/L.
As	Nausea, vomiting and diarrhea. Chronic toxicity is carcinogenic and may lead to dermal and cardiovascular diseases.	Earth's crust, fish, shellfish (only about 25 % in the toxic inorganic form). Drinking water concentration above 10 µg/L (dominant source)
Sb	Diarrhea, vomiting, pneumocconiosis and altered electrocardiogram, no data indicate carcinogenicity of oral intake.	Used as replacement for lead in solders, in alloys (with tin, copper and lead). Low food and drinking water contamination at less than 5 µg/L.

Chromium is found mostly in two oxidation states, Cr(III) and Cr(VI), were Cr(III) is essential and Cr(VI) is toxic [31]. Cd, Cr(VI) and As are found to be carcinogenic, while Cd, Hg and U toxicity mainly target the kidneys [30]. Pb toxicity is suggested to decrease IQ and negatively affect fertility, in addition to known causes like neurodevelopmental effects. Sb toxicity may lead to lung diseases and lung cancer in the case of inhalation. However, there is no data indicating carcinogenicity of Sb by oral uptake. According to World Health Organization (2017) the toxic elements As and Cr occur in drinking water at levels higher than 10 µg/L, while Sb and Pb occur

at levels less than 5 µg/L. The daily dietary intake of U has been approximated to 1-4 µg, Hg to 2-20 µg and Cd to 10-35 µg.

2.4 Infant Foodstuffs

Postnatal exposure of PFAS has been studied by regarding PFAS concentration in human breast milk. However, in most high-income countries the prevalence of breastfeeding after 12 months is less than 20 %, and the exposure through infant foodstuffs is therefore important [32]. A complete list of previously reported element concentrations is listed in **table 3**. The toxic elements were reported in infant foodstuffs in ng/g ranges, and Cr had the highest reported average concentration in infant foodstuffs, at 124 ng/g. This is consistent with the fact that chromium is both an essential and a toxic element. A complete list of previous studies is found in **table 4**. As can be seen from **table 4** infant foodstuffs are previously found to contain PFAS in the ng/g range. PFOS was found in the highest average concentration of 1.5 ng/g.

Table 3: Amount of seven toxic elements in infant foodstuffs previously analyzed.

		Average concentration [ng/g]						
Ref	**Product type**	**Cd**	**Hg**	**Pb**	**U**	**Cr**	**As**	**Sb**
[33]	Fish and chicken	<20	<10	<14	-	320	<10	<6
	Fish	-	-	-	-	120	-	-
	Chicken	-	-	-	-	510	-	-
	Formula	-	-	-	-	22	-	-
[34]	Formula	-	-	-	-	0.20	-	-
	Dinners	-	-	-	-	65	-	-
	Fruit pate	-	-	-	-	63	-	-
	Porridge	-	-	-	-	627	-	-
	Cereal food, conv.	-	0.29	-	-	-	-	-
	Cereal food, org.	-	1.6	-	-	-	-	-
[35]	Cereal food, org.	-	-	-	-	1.6	-	-
	Formula, conv.	-	-	-	-	0.64	-	-
	Formula, org.	-	-	-	-	0.29	-	-
	Baby foods, conv.	-	-	-	-	0.29	-	-
	Baby foods, org.	-	-	-	-	0.50	-	-
[29]	Milk	0.28	-	1.2	0.30	-	0.23	0.04
	Semolina	3.6	-	1.1	0.47	-	1.0	0.14
	Spelt flour	3.8	-	1.8	0.53	-	2.4	0.97
	Oat	2.4	-	3.1	0.22	-	3.3	0.18
	Wholegrain rice	1.7	-	1.2	0.49	-	33	0.14
	Wholegrain rice	0.39	-	1.3	0.02	-	30	0.14
	Rice + banana	1.3	-	3.3	0.67	-	17	0.26
	Rice + banana	4	-	13	3.2	-	18	2.8
	Rice + locust bean	11	-	3.1	2.6	-	32	1.0
Average [ng/g]		2.2	0.95	3.3	0.74	124	13	0.58
Range (min-max)		(0.28-11)	(0.29-1.6)	(1.1-13)	(0.02-3.2)	(0.2-627)	(0.23-33)	(0.04-2.8)

Table 4: Amount of 14 PFAS in infant foodstuffs previously analyzed.

			Average concentration [ng/g]												
Ref.		**N**	**PFPA**	**PFBS**	**PFHxA**	**PFHpA**	**PFHxS**	**PFOA**	**PFNA**	**PFOSA**	**PFOS**	**PFDA**	**PFUnDA**	**PFDoDA**	**PFTrDA**	**PFTeDA**
[36]	Formula[a]	16	3.356-3.356	0.025-0.280	0.284-1.236	0.012-2.127	0.143-0.201	0.027-2.490	0.017-0.195	-	0.017-0.563	0.041-0.398	0.026-0.189	0.057-0.117	0.069-0.069	-
	-	-	0.21	0.039	0.194	0.492	0.034	0.415	0.029	-	0.061	0.155	0.042	0.018	0.004	n.d.
	Cereal[a]	13	-	0.117-0.179	0.066-0.207	0.050-0.017	0.165-0.429	0.059-0.557	0.162-0.165	-	0.023-9.795	0.026-0.269	0.032-5.744	0.059-0.386	0.018-0.018	-
		-	n.d.	0.148	0.134	0.1	0.265	0.179	0.164	-	1.321	0.203	1.505	0.218	0.018	n.d.
	Pots[a]	12	2.997-2.997	0.020-0.056	-	0.222-0.222	-	0.151-0.356	0.165-0.224	-	0.036-0.186	0.252-0.387	0.191-0.227	0.167-0.168	0.156-0.156	-
		-	0.25	0.006	n.d.	0.019	n.d.	0.216	0.076	-	0.019	0.247	0.068	0.028	0.013	n.d.
[37]	Fish	16	-	-	-	-	-	-	-	-	7.65	-	-	-	-	-
	Meat	8	-	-	-	-	-	-	-	-	1.43	-	-	-	-	-
	Dairy	12	-	-	-	-	-	-	-	-	0.36	-	-	-	-	-
[38]	Formula[a]	-	-	<0.7	-	<1.2	<1.34-3.59	<48.3	<2.20	-	<11.0-11.3	-	-	-	-	-
[39]	Cereal	3	-	-	-	-	-	0.302	0.091	-	0.31	0.251	-	-	-	-
	Formula	2	-	-	-	-	-	0.528	0.166	-	0.58	0.980	-	-	-	-
[40]	Formula[a]	6	<0.020	<0.009	<0.009	<0.009	<0.005	<0.009-0.010	<0.009-0.011	<0.005	<0.007-0.019	<0.009	<0.009	<0.009	<0.009	<0.009
[2]	Review[b]	11	-	-	-	-	0.000-0.240	0.000-0.150	0.126-0.240	-	0.000-0.240	-	-	-	-	-
	Average		0.23	0.064	0.16	0.20	0.15	0.27	0.090	-	1.5	0.37	0.54	0.088	0.012	-
Range (min-max)			(<0.020-3.4)	(<0.009-0.28)	(<0.009-1.2)	(<0.009-2.1)	(<0.005-3.59)	(<0.009-48)	(<0.009-2.2)	(<0.005)	(<0.007-11.3)	(<0.009-0.98)	(<0.009-5.7)	(<0.009-0.39)	(<0.009-0.16)	(<0.009)

n.d.: not detected.

[a] range values (min-max).

[b] EFSA panel review article reporting upper and lower bound.

2.5 Exposure and Risk Assessment

To assess the risk associated with dietary intakes of PFAS and toxic elements through infant food consumption one could compare estimated daily intakes (EDI) to minimum risk levels (MRLs) or tolerable daily intakes (TDIs) not causing adverse health effects. A risk assessment of infant foodstuffs and breast milk samples previously studied is shown in **table 5**.

Table 5: EDIs, MRLs and TDIs of PFAS and toxic elements previously reported in infant foodstuffs and breastmilk.

µg/*day*	**EDI**		**MRL**[a]**/TDI**[b]
	Infant food	**Breast milk**	
PFHxS	0.065	0.027	0.17
PFOA	0.12	0.14	0.026
PFNA	0.039	0.023	0.026
PFOS	0.65	0.12	0.017
PFDA	0.16	0.10	-
PFUnDA	0.23	0.040	-
As	5.6	2.1	18
Cd	0.93	0.14	7.1
Cr	53	0.24	20-60[c]
Hg	0.41	0.32	31
Pb	1.4	1.4	60
Sb	0.25	0.83	8.6
U	0.32	0.039	17

References [a] [2], [b] [30], [c] [31]

Equations to calculate EDIs of infant food and breast milk are found below.

$$EDI(\textit{infant food}) = C \cdot 3m_{if} \tag{1}$$

Where C is the concentration (µg/g) of PFAS or toxic element in infant food and m_{if} is the average amount of infant food in a meal (g/meal). The amount of complementary food meals per day is assumed by WHO (2002) to be 3 (meals/day) [41].

$$EDI(\textit{breast milk}) = \frac{C \cdot V_{bm}}{bw} \tag{2}$$

Where C is the concentration (µg/L) of PFAS or toxic element in breast milk, bw is the infants body weight (kg) and V_{bm} is the average volume of breast milk consumed daily by an infant

per body weight (L/kg bw/day). MRLs and TDIs contain a lot of uncertainty as the toxicity and biological uptake of toxic substances need more research, particularly those of PFAS. MRLs are based on intermediate oral exposure of the PFAS [2]. According to Tripathis *et al.* (1999) the average breast milk consumption of an infant is 150 mL/kg bw/day [42]. The average body weight of infants at 9 months is assumed by WHO (2006) to be 8.6 kg (average of boys and girls) [43]. The complete list of previously determined toxic element and PFAS concentrations in breast milk are shown in **table 6** and **table 7**, respectively.

Table 6: Amount of seven toxic elements in breast milk previously analyzed.

		Average concentration [ng/L]						
Ref.	**N**	**Cd**	**Hg**	**Pb**	**U**	**Cr**	**As**	**Sb**
[44]	-	0.018	0.38	0.44	-	0.19	0.027	1.2
[42]	30	0.090	-	1.9	-	-	-	-
[29]	-	0.060	-	0.50	0.030	-	0.60	0.12
[45]	19	-	-	0.94	-	-	5.8	-
[46]	120	0.27	0.12	1.5	-	-	0.20	-
Average [μg/L]		0.11	0.25	1.1	0.030	0.19	1.7	0.65

Table 7: Amount of 15 PFAS in breast milk previously analyzed.

Ref.	Year	N	Average concentrations [ng/L]														
			F-DecaS	PFPeA	PFBS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOSA	PFOS	PFDA	PFUnDA	PFDoDA	PFTrDA	PFTeDA
[7]	2018/19	174	-	6.8	n.a.	41	n.d.	n.a.	87	12	-	25	12	13	-	-	-
[47]	2014	63	-	-	-	-	-	-	54	41	-	-	24	29	21	-	-
[48]	2011-2013	100	-	-	-	-	-	26	41	14	-	40	11	37	-	-	-
[36]	2012	10	-	n.d.	2.0	6.0	98	n.d.	177	4.0	-	50	43	88	-	-	-
[49]	2011	293	-	0.040	0.26	13	6.3	7.6	56	19	-	57	0.88	24	1.6	0.70	0.38
[50]	2004-2011	2-203	-	-	-	-	-	-	96	-	-	121	-	-	-	-	-
[51]	2008-2010	30	-	-	-	-	-	-	94	32	-	-	21	37	<10	15	-
		30	-	-	-	-	-	-	65	15	-	-	<15	20	<10	119	-
		30	-	-	-	-	-	-	52	15	-	-	<15	16	<10	88	-
[40]	2010	50	-	-	-	-	-	-	50	-	-	33	-	-	-	-	-
[52]	2009	50	-	-	-	-	-	-	181	26	-	56	20	26	-	-	-
[53]	2009	30	-	-	-	-	-	-	57	-	-	74	-	-	-	-	-
[39]	2008	20	54	-	-	-	-	-	332	-	-	122	666	-	-	-	-
[54]	2007/08	302	-	-	-	-	-	10	80	-	-	60	-	-	-	-	-
[55]	2007	1237	-	-	-	-	-	6.3	116	16	-	56	9.9	38	-	-	-
[56]	2007	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[57]	2007	48	-	-	-	-	-	49	82	-	-	92	-	-	-	-	-
[58]	2006, 1996/97	70	-	-	-	-	-	-	77	-	-	158	-	-	-	-	-
[59]	2006	22	-	-	-	-	-	-	300	-	-	2900	-	-	-	-	-
[60]	1999-2005	24	-	-	-	-	-	7.6	78	-	-	232	-	-	-	-	-
		13	-	-	-	-	-	6.5	-	-	-	121	-	-	-	-	-
		24	-	-	-	-	-	16	-	-	-	98	-	-	-	-	-
		20	-	-	-	-	-	-	-	-	-	84	-	-	-	-	-
		40	-	-	-	-	-	6.8	-	-	-	76	-	-	-	-	-
		24	-	-	-	-	-	-	-	-	-	67	-	-	-	-	-
		39	-	-	-	-	-	-	-	-	-	46	-	-	-	-	-
[61]	2004	12	-	-	-	-	-	85	-	17	13	201	-	-	-	-	-
[62]	2004	45	-	-	-	-	-	15	44	7.3	-	131	-	-	-	-	-
[63]	2003	19	-	-	2.0	-	4.9	20	106	18	-	121	7.2	19	-	-	-
Average [ng/L]			54	3.4	1.4	20	36	21	106	18	13	92[a]	82	31	11	56	0.38

n.d.: not detected. n.a.: not available.

[a] average value from [59] left out as it was over ten times higher than other values.

2.6 Organic Analysis

Various organic chemicals can be found in nature and in our environment in low concentrations. Some of these compounds are naturally occurring in nature, and others are xenobiotic substances from industrial production and other human activities. The compounds may have toxic effects on the environment and it is therefore imperative to develop sensitive analytical procedures to detect them. In organic analysis of environmental samples concentrations of target analytes are usually very low and the sample bulk matrix is complex and of unknown composition. This calls for isolation of the target analytes to a less complicated matrix, as well as enrichment and clean-up of the samples before analysis.

2.6.1 Extraction

Extracting analytes from a sample matrix can be performed in several ways. The two main procedures are liquid-liquid and solid-liquid extraction. In liquid-liquid extraction (LLE) the target analytes are moved from one solvent to another by their affinity to the solvents. The two solvents need to have different polarities, and normally one aqueous solvent and one organic solvent is used. LLE can be performed in a separating funnel or simply by centrifuging and separating the supernatant from the rest of the liquid. It is very important to ensure high purity throughout the procedure. In solid-liquid extraction (SLE) the target analytes are retained on a sorbent from a liquid phase or the opposite direction. This method is based on the target molecules' sorption/desorption properties in the presence of different solvents. SLE can be carried out using a column containing a sorbent with either polar (normal phase) or non-polar (reversed phase) functional groups. There are many forms of SLE, such as soxhlet, ion exchange and ASE.

2.6.2 UPLC-MS/MS

Chromatography is a separation technique based on the target analytes' partitioning between a mobile phase and a stationary phase. The interplay between elution by the mobile phase and retardation by the stationary phase is what causes the separation of analytes [64].

HPLC (High-Performance Liquid Chromatography) consists of an injector and a pump pumping the sample into a separation column and further through a detector where the analytes are detected and analyzed with a data handling system. UPLC (Ultra-Performance Liquid Chromatography) is a more sensitive version of HPLC where the signal-to-noise ratio is larger because of less band broadening [65]. This arrangement is shown in **fig. 2**. In HPLC the stationary phase is a solid that works as an absorbent, often silica with or without added functional groups. The mobile phase is a liquid chosen depending on the analytes' solubility in the liquid, delivered at a high pressure to ensure constant flow rates [64]. In UPLC-MS systems the column is connected to an evaporative ionizing interface, which in turn is connected to a mass spectrometer (MS) [66].

The MS contains a sample introduction method, methods for ion production and separation, an ion detector and a data handling system [67]. This arrangement is also shown in **fig. 2**.

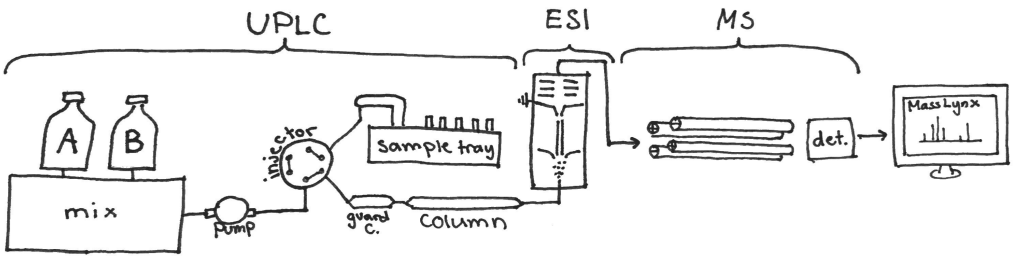

Figure 2: Simplified model of the UPLC-MS/MS system.

There are many types of detectors available for UPLC, e.g. UV, fluorescence, electrochemical and conductivity detectors [67]. Mass spectrometry has many advantages compared to other UPLC detectors, such as absolute identification and sensitivity. The MS provides the molecular weight of the analytes which reduces the library of possible structures dramatically [67].

Before introducing the analytes into the mass analyzer the volatile solvent and its additives must be removed from the flow out of the UPLC column [66]. This is because the mass spectrometer operates as 10^{-5} to 10^{-7} torr vacuum, and the high flow rates from UPLC would overwhelm this system. Solvent removal and ionization is performed by heaters, pressure reducers and gas nebulizers, which we find in the UPLC-MS interface [66]. To ensure not only molecular mass determination, but also structural information, soft-ionization techniques with little fragmentation are needed [67]. The two ways of obtaining this are cone-voltage fragmentation by either electrospray or atmospheric pressure ionization, or tandem mass spectrometry (MS/MS), which is applicable to all types of ionization [67]. The instrument illustrated in **fig. 2** contains an electronspray ionization (ESI).

The MS/MS has two stages of mass spectrometry, where one isolates the ion of interest and the other compares this ion to other ions that might either be the precursor or the decomposition product of the first ion. The most widely used hardware for the MS/MS is a triple quadrupole, which consists of three sets of quadrupoles (four rods) in series. The second quadrupole is used for fragmentation and focusing, while the first and last are used for separation of the ions based on their trajectories in the electric field [67]. The advantage of the the MS/MS triple quadrupole is that it can give high-resolution spectra, which are sometimes necessary when analyzing complex matrices [67].

2.7 Element Anaysis

A widely used method applied to determine elements in various sample matrices is Inductively Coupled Plasma Mass Spectrometry (ICP-MS). ICP-MS mainly performs analysis on liquid samples, and it is therefore necessary to prepare the samples before the analysis [68]. Some ways of dissolving the sample are: Acid/pressure digestion, dissolving in strong bases, dissolving in organic solvents or heating with fusion mixtures [68]. In recent years, the most used method is acid digestion with pressure and high temperature, e.g. by using UltraClave digestion.

2.7.1 UltraClave Digestion

The UltraClave digestion uses a Single Reaction Chamber (SRC) technology, which involves a chamber with room for up to 40 samples at the time, pressurized and heated by a micro wave oven to oxidize organic material. The UltraClave provides high throughput, good reproducibility and reduces usage of chemicals and labor [69]. As the temperature is increased by a microwave oven the vessels need to be of polymer material, e.g. PFA (perfluoroalkoxy alkanes) [70]. PFA has an ability to absorb and desorb metal cations and therefore must be stored in weak acid and operated with in stronger acid.

2.7.2 ICP-MS

A major reason why ICP-MS is a preferred analytical technique is that the detection limits range from ppq up to ppm levels. This makes it possible to analyze many different materials with different element contents [68]. There are different types of ICP-MSs, but all have the same components [68]. These are a nebulizer, a spray chamber, a plasma torch and a detector, as shown in **fig. 3**.

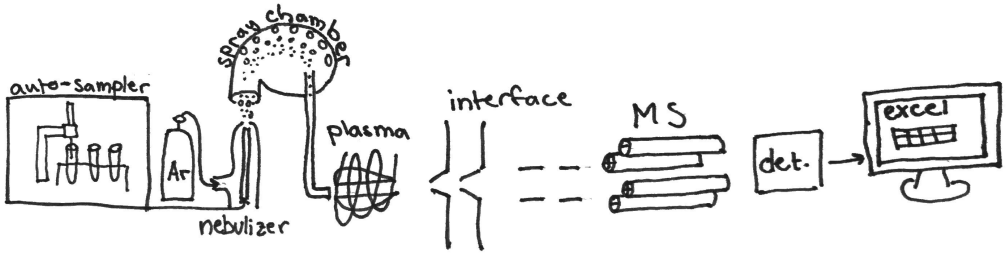

Figure 3: Simplified model of the ICP-MS system.

The sample is introduced, in liquid form, into the nebulizer, where it is sprayed at a certain flow rate (e.g. 200 µL/min). Argon gas is also sprayed into the chamber with a larger flow rate (e.g. 1 L/min) in order to convert the liquid sample into a fine aerosol. The spray chamber is made so that the fine droplets (about 1-2% of the sample) are separated from the larger droplets. These fine droplets are introduced to the ICP Torch. The torch generates positively charged ions to be analyzed by the MS detector. This by producing an intense magnetic field on a tengient

flow of argon gas to ionize the sample aerosol. Before the ions reach the MS detector they are sent through an interface region in a vacuum and through ion lenses. This step minimizes the formation of interfering species formed from potential differences in the torch and focuses the beam of ions onto the MS detector. The MS detector can also be of different types, the four most common being quadrupole, magnetic sensor, time of flight and collision/reaction cell technology [68]. These four methods perform the same separation of the sample cations based on their mass-to-charge ratio. When the ions reach the detector, they are converted to electrical signals to be processed by the data handling system. The ions can be converted to electrical signals with a discrete dynode detector, which contain metal dynodes that change the ions into a stream of detectable electrons.

2.8 Quantification and Quality Assurance

2.8.1 Limit of Detection and Lower Limit of Quantification

The Limit of Detection (LOD) is the miniumum concentration of a TA where it is distinguishable from a blank sample. The Lower Limit of Quanitification (LLOQ) is the lowest concentration of a TA with acceptable precision and accuracy [71]. From UPLC-MS/MS analysis LODs are calculated by the following equation:

$$LOD = \frac{3 \cdot (\textit{Average noise height of calibration solutions})}{\textit{(Slope of calibration heights)}} \tag{3}$$

The heights used in LOD calculation must be determined before smoothing of the chromatograms. The LLOQ is found by multiplying the LOD by three. LODs for ICP-MS analysis can be found by graphically determining the concentration of element at relative standard deviation (RSD) of 25% and correcting this concentration for baseline distortion.

2.8.2 Internal Standards Method

In the internal standards method (ISM) internal standards are added to all samples to correct for inaccuracies and provide higher precision than the calibration curve method. An internal standard (IS) is a chemical that is not present in the sample of concern, but will act similarly to the target analyte (TA) in the sample matrix and in analysis. Carbon-13 isotopes of similar chemical structure to the TAs are normally used. The IS corrects for minor instrumental variations and compensates for losses of TA, making it possible to quantify the TAs with better precision. The TA signals are divided by the IS signals, defining a new ratio variable, R.

$$R = \frac{S_{TA}}{S_{IS}} \tag{4}$$

S_{TA} and S_{IS} in **eq. (4)** denote the signals of TA and IS, respectively. This ratio variable, R, is then used in a calibration curve to determine the concentration of TA.

$$R = \alpha \cdot C + \beta \tag{5}$$

In **eq. (5)**, α denotes the slope of the curve, C the concentration of TA and β the intercept of the curve. The concentration of unknown samples can thus be calculated by rearranging **eq. (5)**, although intercepts close to zero are typically ignored. As the IS signal is normally stronger than the TA signal, and the ratio consequently a proper fraction, the standard error is usually lower than that of the calibration curve method.

2.8.3 Recovery and Matrix Effects

Developing an analytical technique for a specific group of target analytes requires quality assurance analysis to determine whether or not the procedure is sufficiently accurate and reproducible. This analysis can consist of calculations like matrix effects and recovery of analyte. Matrix effects evaluate effects on accuracy and reproducibility by interfering compounds in the sample matrix and recovery measures how detection of an analyte is affected by sample preparation. Different sample matrices will introduce different interfering compounds that may enhance or diminish the TA result, so using equally and correctly spiked samples as well as calibration solutions is very important [72]. To determine the recovery of a TA in an analytical technique **eq. (6)** is used [73].

$$\textit{Recovery} = \frac{S_s - S_u}{MM - S_u} \cdot 100\% \tag{6}$$

Here, S_s is the signal of the target analyte in a sample spiked with TA and IS before the sample preparation, for example extraction. *MM* is the signal of the TA in a matrix match sample, spiked with TA and IS after extraction. S_u is the signal of the TA in a sample spiked with IS but not with TA, and a blank sample could be used in its place. Relative recovery, as shown in **eq. (7)**, is the recovery corrected for analyte losses as the samples S_s and *MM* are divided by their corresponding internal standard samples.

$$\textit{Relative Recovery} = \frac{(S_s - S_u)/S_{IS}}{(MM - S_u)/MM_{IS}} \cdot 100\% \tag{7}$$

S_{IS} is the IS signal of a sample spiked before sample preparation and MM_{IS} is the IS signal of a matrix match sample, spiked after extraction.

The matrix factor (*MF*) is a measurement of the effect of other analytes on the detection of the TA, and the calculation is shown in **eq. (8)**.

$$MF = \frac{MM - S_u}{CS} \tag{8}$$

CS is the signal of a calibration solution sample of the same concentration as the *MM* spike. The matrix effect is calculated as shown in **eq. (9)** and expresses the percentage effect of other analytes on the signal of the target analyte.

$$ME = (MF - 1) \cdot 100\% \tag{9}$$

3 MATERIALS AND METHODS

3.1 Samples

49 infant food products were chosen and bought at Coop Obs grocery store in September 2019. The products consisted of 15 solid samples (in the form of snack bars, puffs and powders) and 34 semi-solid samples (smoothies, dinner mixes, milk and porridge). The 49 products were chosen from eight different producers. One of the products was intended for infants from 0 months of age and four products for infants from four months of age. The remaining 44 products were intended for infants/children of above 6 months. The products had different main ingredients, and most consisted mainly of fruit, as shown in **table 8**. Products containing fruit and grain were characterized as fruit, products containing dairy and corn/grain were characterized as dairy and all products containing meat were characterized as meat products. Detailed information, including the exact recommended minimum age, texture, package material and producer number are listed in **table B.1**.

Table 8: Main ingredients of the infant food products analyzed.

Product main ingredients	#
Grain (1 grain and vegetable)	7
Dairy (1 dairy and corn, 1 dairy and grain)	5
Corn	5
Fruit (2 fruit and grain, 4 concentrated fruit)	24
Vegetable	2
Meat (3 cattle, 2 chicken and 1 ham)	6

3.2 Chemicals

The internal standards utilized in the determination of PFAS in infant food were ^{13}C-isotopes of perfluorooctanoic acid (^{13}C-PFOA) and perfluorooctane sulfonate salt (^{13}C-PFOS). All target analytes used for the quality assurance analysis are listed in **table 9**, as well as where they were purchased, purity, retention time and the mass-to-charge ratio of the molecular ion and most detected fragment ion (m $>$ m/z). The chemical DecaS is not fluorinated, but was analyzed to compare a similar long-chained alkyl sulfonate with the PFAS.

Working solutions of IS and TA were made in the following manner:

- A mix of the ISs was made to have a concentration of 1 ppm. The IS solutions both had original concentrations of 50 ppm, so to make 500 µL of 1 ppm IS solution 10 µL of each IS were mixed, and dilluted with 480 µL MeOH.

- A mix of all 16 TAs was made to have 1 ppm of each TA in a total volume of 1 mL MeOH. The original concentration of each of the TA solutions was 100 ppm. The data for preparation of the TA mixture are shown in **table C.1** in **Appendix**.

Table 9: Purchasing information, purity, RT and m/z-ratios of TAs and ISs.

	Chemical Information		**Detection Parameters**	
PFAS	**Purchased from**	**Purity**	**RT**	**m > m/z**
DecaS	Sigma Aldrich	98%	2.10	221>80
PFPA	Sigma Aldrich	97%	1.63	263>219
PFBS	Sigma Aldrich	98%	1.69	299>80
PFHxA	Sigma Aldrich	97%	1.83	313>119
PFHpA	Sigma Aldrich	99%	1.98	363>169
PFHxS	Sigma Aldrich	98%	1.98	399>80
PFOA	Sigma Aldrich	96%	2.09	413>169
^{13}C-PFOA	Cambridge Isotope Labs	99%	2.09	421>172/223
PFNA	Sigma Aldrich	97%	2.20	463>219
PFOSA	Chiron AS	-	2.47	498>78
PFOS	Sigma Aldrich	95%	2.19	499>99
^{13}C-PFOS	Cambridge Isotope Labs	99%	2.20	507>80/172
PFDA	Sigma Aldrich	95%	2.29	512>468
Sulf	Sigma Aldrich	-	2.72	526>169
PFUnDA	Sigma Aldrich	98%	2.39	562>269
PFDoDA	Sigma Aldrich	95%	2.47	612>169
PFTrDA	Sigma Aldrich	97%	2.54	663>169
PFTeDA	Sigma Aldrich	96%	2.60	712>668

3.3 Organic Analysis

To extract the PFAS from the infant food products a combination of LLE and SLE was performed. The samples were subsequently analyzed using UPLC-MS/MS, and determined using the Internal Standard Method.

3.3.1 Extraction

The extraction protocol was developed in the pre-project of this master's thesis, performed during the fall of 2019 [74]. The procedure was performed with slight modifications, described below.

Infant food samples were freeze dried before the extraction procedure was carried out. After salt addition, spiking and solvent addition the samples were treated with Ultra-Sonication for 30

minutes. The extraction procedure was then continued as described in [74]. Blank samples underwent the exact same procedure as the infant food samples. After concentration of the samples to near dryness, they were reconstituted to 1 mL with pure MeOH. No syringe filters were used in the procedure, as these may be a source of contamination of perfluorinated substances. In the case of particulate precipitate, the samples were centrifuged and the supernatant was transferred to new UPLC vials.

3.3.2 UPLC-MS/MS Analysis

The UPLC-MS/MS analysis was carried out with an Aquity UPLC system connected to a tandem quadrupole XevoTQS instrument from Waters. The Aquity UPLC system consisted of a Binary Solvent Manager, a Sample Manager and a Column Manager, all from Waters. The ionization method used was ESI in negative mode. The UPLC column was a Kinetex C18 column (30 x 2.1 mm, 100 Å Phenomenex), serially connected to a guard column (C18, Phenomenex). The separation was performed using a gradient elution program with two solvents, namely MilliQ water with 2 mM ammonium acetate and methanol of gradient grade (99.93% pure), and the gradient elution program is shown in **table D.1** in appendix. The flow rate was 0.4 µL/min and the injection volume used was 4 µL. Additional instrument parameters are listed in **table D.2** in **Appendix D**. Retention times and mass-to-charge ratios of most detected fragment ions of TAs are listed in **table 9**. The data from the analysis was obtained from MassLynx and TargetLynx processing, and further treated according to internal standard method (ISM) in Excel. The IS with RT closest to the target analyte RT was used to correct for analyte losses in relative recovery and concentration calculations. PFNA was an exeption, where the ^{13}C-PFOA was used for correction despite it having a RT closer to ^{13}C-PFOS, since PFNA is a PFCA similar to PFOA. Additionally, the fragment ion mass/charge-ratio of IS which was closest to the mass/charge-ratio of the TA fragment ion in question was used to calculate the ratio for ISM.

3.3.3 Quality Assurance

In order to investigate the quality of the extraction method, 15 samples of a mix of 1 g of each of 23 semi-solid infant food products were extracted and analyzed by the procedure described above. The 23 products were the following: 19, 21-24, 26, 28-34, 36-39, 42-46 and 49. These 15 samples were spiked in the order described in **table 10** below. Additionally, three regent blank samples were analyzed in the same manner to evaluate background contamination in the laboratory. This procedure was performed to calculate the recovery of TAs and the matrix effects in the extraction procedure. Calibration solutions of concentrations 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, and 50 ppb were prepared using the TA solution of 1 ppm. These calibration solutions were all spiked with IS to a concentration of 10 ppb.

Table 10: IS and TA addition to the 15 QA samples.

	Sample	Spiked Sample	Matrix Match	Blank
#	3	8	4	3
IS	b	b	a	b
Spike	10 ppb	10 ppb	10 ppb	10 ppb
TA	n	b	a	n
Spike	10 ppb	4 x 10 ppb	2 x 10 ppb	10 ppb
		4 x 20 ppb	2 x 20 ppb	

b: added before extraction

a: added after extraction

n: not added

3.4 Element Analysis

Element concentrations of 63 elements in the 49 infant food products were determined using ICP-MS after sample preparation by UltraClave digestion.

3.4.1 Sample Preparation by UltraClave Digestion

To remove the organic content in the infant food all samples were digested in a Milestone UltraClave instrument. The digestion procedure is described in the pre-project of this thesis [74]. The temperature profile used in the UltraClave digestion is shown in **fig. E.1**.

3.4.2 ICP-HR-MS Analysis

The ICP-MS analysis was carried out with ICP-HR-MS Element 2 from Thermo Scientific, by the instrument owner at the Dept. of Chemistry. The ICP-HR-MS Element 2 includes a sample introduction system with an SC2-DX Autosampler and a prepFAST system. The sample uptake parameters of the ICP-HR-MS system are listed in **table F.1**.

3.4.3 Quality Assurance

Blank samples, repeating tests, extra parallels and certified reference material were used to ensure accuracy of the analytical procedure. The certified reference material used was Polish Virginia Tobacco Leaves (INCT-PVTL-6) from the Institute of Nuclear Chemistry and technology, Poland. Three samples of reference material, six blank samples, three repeating tests and two extra parallels of samples were prepared and analyzed to ensure the quality of the results. The recovery of elements from certified reference material, also reffered to as the accuracy, was found using **eq. (10)**.

$$Recovery = \frac{Average\ concentration}{Certified\ value} \cdot 100\% \tag{10}$$

Two sets of solutions were used for calibration and quality assurance of the ICP-MS instrument, these from two different producers. These were primary solutions for chlorine, bromine and iodine, and primary solutions for 70 elements. The two solutions from producer 1 were used as calibration solutions and the two solutions from producer 2 were used as quality solutions. The content of all primary solutions is shown in **Appendix G**. In the Element 2 software, three different resolutions were used to avoid interference in the spectra. The three resolutions available were Low (400), Medium (5 500) and High (10 000). 36 elements were quantified at low resolution, 26 at medium resolution and one element at high resolution.

3.5 Statistics and Data Treatment

Descriptive statistics of PFAS and toxic elements analyzed were computed in excel. Bivariate Pearson Correlation was performed using IBM SPSS statistics 25.

4 RESULTS AND DISCUSSION

4.1 Quality Assurance

4.1.1 Organic Analysis

LODs and LLOQs for the 16 target analytes are shown in **table 11**. LODs and LLOQs were calculated from noise heights of chromatograms of 0.1, 0.2 and 0.5 ppb samples before smoothing. To calculate LODs for TAs with no visible noise in the chromatogram (i.e. chosen transitions of PFNA, PFOSA and PFTrDA) the lowest noise height out of all TAs was used (Sulf 527>169). Concentration results lower than the LOD were removed from the dataset. Sulf had the lowest LOD of 0.23 pg/g, while PFHxA had the highest LOD value of 1.8 ng/g. The detection rate (DR) of each TA is also shown in **table 11**. All TAs were detected in infant foodstuffs. Eight TAs had detection rates above 50% and were therefore detected in over half of the infant food samples. PFBS, PFHxS and Sulf were detected in all samples. PFDA was only detected in 4% of the samples. However PFDA had a high LOD of 0.99 ng/g, which may indicate that some samples contained PFDA but in concentrations that were not distinguishable from blank samples. PFPA and PFHxA also had high LOD values, but these PFAS had higher detection rates of 30 and 36%, respectively, indicating that they were detected in high concentrations in about a third of the infant food products.

Table 11: LLOQ, LOD and DR of the 16 target analytes determined in the infant food samples.

ng/g	**LLOQ**	**LOD**	**DR** [%]
DecaS	0.35	0.12	94
PFPA	1.8	0.61	30
PFBS	0.094	0.031	100
PFHxA	5.3	1.8	36
PFHpA	0.15	0.051	88
PFHxS	0.037	0.012	100
PFOA	0.050	0.017	92
PFNA	0.0072	0.0024	44
PFOSA	0.011	0.0038	44
PFOS	0.12	0.038	98
PFDA	3.0	0.99	4
Sulf	0.00070	0.00023	100
PFUnDA	0.016	0.0053	26
PFDoDA	0.0036	0.0012	14
PFTrDA	0.0024	0.00081	42
PFTeDA	0.37	0.12	58

From the quality assurance analysis using 10 and 20 ppb spiked samples the recovery, relative recovery and matrix effects of infant food samples were calculated. As can be seen from **table 12** all TAs except DecaS and Sulf had relative recoveries between 70% and 130% at spiking concentration 10 ppb. This indicates that most target analytes in infant food products with PFAS concentrations of about 10 ppb recovered well from the extraction procedure. At spiking concentration 20 ppb all TAs except DecaS, PFPA and PFTrDA had relative recoveries within the same range. Consequently, most PFAS recovered well from the extraction procedure when correcting for analyte losses with the internal standard method. Additionally, the detection of 10 ppb spiked samples was least affected by sample preparation, and samples at this concentration recovered slightly better than those at 20 ppb. The exception was PFOSA and Sulf, which had relative recoveries closer to 100% for 20 ppb spiking concentration. Previously reported PFAS concentrations in infant foodstuffs were closer to 10 ppb than to 20 ppb, so the fact that the analytical procedure was a better fit for 10 ppb concentrations than for 20 ppb was appropriate.

For spiking concentration 10 ppb the PFAS that recovered especially well (within 100 $\pm$ 10%) were PFBS, PFHxA, PFHpA, PFHxS, PFOA, PFNA, PFOS and PFDoDA. That is, PFSAs of chain lengths four, six and eight and PFCAs of chain lengths six-nine and twelve. For the higher spiking concentration of 20 ppb PFBS, PFOA, PFNA and PFOSA had relative recoveries within this range.

Table 12: Absolute recoveries, relative recoveries and matrix effects of 16 TAs at 10 and 20 ppb.

	Absolute Recovery		**Relative Recovery**		**Matrix Effect**	
%	**10 ppb**	**20 ppb**	**10 ppb**	**20 ppb**	**10 ppb**	**20 ppb**
DecaS	2.7	3.8	15	13	218	152
PFPA	33	27	76	60	-66	-60
PFBS	38	47	100	108	50	39
PFHxA	43	50	105	112	20	-30
PFHpA	40	54	107	124	2.6	-15
PFHxS	43	51	104	112	66	38
PFOA	38	44	101	100	4.5	-6.9
PFNA	36	37	109	92	22	12
PFOSA	45	47	120	100	51	30
PFOS	38	41	102	88	80	48
PFDA	38	32	112	72	-5.7	7.4
Sulf	52	54	138	115	-14	-35
PFUnDA	33	35	120	89	-53	-62
PFDoDA	25	28	103	74	-12	-24
PFTrDA	27	26	77	56	-6.1	-3.0
PFTeDA	26	29	89	70	-16	-24

Matrix effects were quite high for TAs DecaS, PFPA, PFBS, PFHxS, PFOSA, PFOS and PFUnDA. For these TAs matrix effects were outside the range ± 40% for one or both of the spiking concentrations, indicating that other analytes in the infant food sample matrix greatly diminished or enhanced the detection of these TAs. Especially DecaS had high matrix effects, and adding in the low recovery it can be concluded that the extraction procedure was a poor fit for DecaS. This was a quite natural result, considering that DecaS is not a PFAS. PFBS, PFHxS, PFOSA and PFOS only had positive matrix effects ranging from 30-80%. Among these are all analyzed PFSAs and one of the two PASFs. Consequently, the detected concentrations of the PFSAs and of PFOSA may be enhanced by interferring analytes. The other PASF, Sulf, on the other hand, had negative matrix effects. The TAs with strong negative matrix effects were PFPA and PFUnDA. These PFAS had matrix effects of about -60% or both spiking concentrations, indicating that detected concentrations may have been diminished by interferring analytes.

PFAS with matrix effects of less than ± 10% for both spike concentrations are PFOA, PFDA and PFTrDA. These were least affected by other analytes in the infant food matrix. For spike concentration 10 ppb PFHpA also had a low matrix effect of only 2.6%.

4.1.2 Chromatography

Chromatograms of two main fragment ions of all TAs except PFPA and of the two ISs were obtained. The chromatogram of only one fragment ion was obtained for PFPA. Total Ion Chromatography of all 18 TAs and ISs is shown in **Appendix H**. **Figure 4** shows chromatograms of PFOA 413<169 and PFOS 499<99 at 50 ppb spike concentration. The chromatograms of TAs

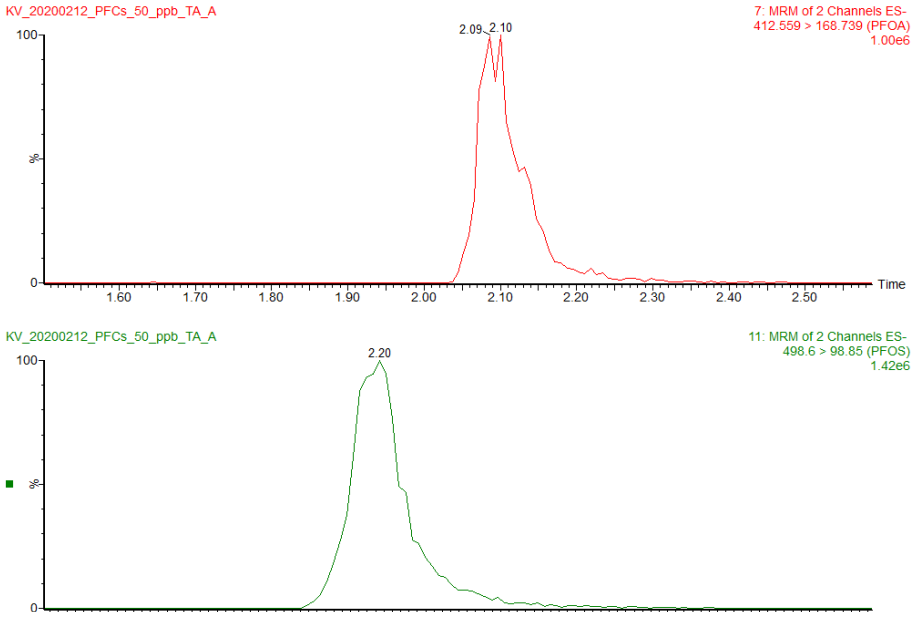

Figure 4: Chromatogram of PFOA and PFOS at 50 ppb spike concentration.

and ISs at high concentrations (50 ppb) had very low noise-heights, while at lower concentrations some were less sensitive and had higher noise heights, such as DecaS. The DecaS TIC is shown in **fig. I.1** in appendix and corresponds with the poor recovery and high matrix effects obtained for DecaS. Calibration curves for all 16 TAs are shown in **Appendix J**. All TAs had R^2-values above 0.99. Intercepts of the calibration curves were subtracted when calculating the concentrations, as they were not close to zero.

4.1.3 Inorganic Analysis

Limits of detection for all 63 elements analyzed are shown in **table 13**. Essential elements such as Ca, Mg, K, Si, Na and S have high LODs, owing to their high concentrations in nature and inevitable contamination of the samples. Toxic elements and rare earth elements have lower LODs. The toxic elements Cd, Hg, Pb, U, Cr, As and Sb were in focus in this thesis.

Table 13: LODs for all 63 elements analyzed.

	LOD μg/g				
Al	0.089	**Hf**	0.00045	**Sc**	0.0018
Sb	0.00089	**Ho**	0.000089	**Se**	0.022
As	0.0045	**Fe**	0.0089	**Si**	4.5
Ba	0.0058	**Ir**	0.00022	**Ag**	0.0089
Be	0.00089	**La**	0.00022	**Na**	4.5
Bi	0.00045	**Pb**	0.00089	**Sr**	0.011
B	0.022	**Li**	0.0022	**S**	8.9
Cd	0.00089	**Lu**	0.000089	**Ta**	0.000089
Ca	0.89	**Mg**	0.22	**Tb**	0.000089
Ce	0.000089	**Mn**	0.0027	**Tl**	0.00011
Cs	0.00022	**Hg**	0.00089	**Th**	0.00022
Cr	0.0089	**Mo**	0.0089	**Tm**	0.00022
Co	0.0018	**Nd**	0.000089	**Sn**	0.00045
Cu	0.013	**Ni**	0.0067	**Ti**	0.0089
Dy	0.00036	**Nb**	0.00045	**U**	0.00011
Er	0.00013	**P**	0.18	**V**	0.0013
Eu	0.00036	**Pt**	0.00045	**W**	0.00045
Gd	0.00045	**K**	0.45	**Yb**	0.00018
Ga	0.0031	**Pr**	0.00013	**Y**	0.00018
Ge	0.0089	**Rb**	0.0054	**Zn**	0.011
Au	0.000089	**Sm**	0.00022	**Zr**	0.00045

Certified reference material analyzed showed a recovery ranging from 2% (for element Hf) to 142% (for element Al). However, about half of all certified elements analyzed showed good accuracy, of between 85-115% recovered analyte. 25% of the elements were not certified. All the seven toxic elements in focus were certified (U and Cr only with info values), and accuracy ranged from 72-114%. This indicates high to moderate recovery of the toxic elements. All 63 elements except Hg, Zr, Mg, Al, Ca, Ti, Fe and Zn had no detected concentration in the blank samples. However, over 80% of the elements had relative standard deviation (RSD) of the blank concentrations above 10%. Repeating tests of three of the infant food samples (28, 32 and 25) showed a large variability in the detection of trace elements, and a small variation in the detection of major elements. However, the RSDs of average concentrations of the repreating tests were below 10% for most of the toxic elements listed above. Only Sb and Hg had RSD values above 10% for all three repeating tests. Hg and Sb also had high RSD values for the extra parallels, of 118 and 100% for sample 45 and 63 and 31% for sample 46, respectively. The extra parallels of samples 45 and 46 showed adequate precision of most remaining detected elements, as the RSDs ranged from 0 to 95%.

4.2 Determination of Perfluoroalkyl Substances

Concentrations of the 16 target analytes in all 49 infant food products are given in ng PFAS per gram sample and are shown in **table 14**. Some of the infant food products were not completely dried in the freeze drying process. Product number 18 was analyzed twice as there was some uncertainty in the weight of the first sample due to a difficult consistency. Mean, median, minimum and maximum concentrations of the 15 PFAS and DecaS are shown in **table 15**. The number of outlying concentrations (exceeding the third quartile pluss 1.5 times the inter quartile range) for each PFAS is also shown. As can be seen from **table 15** the substances PFPA, PFHxA, PFDA and PFTeDA have the largest mean concentrations in the infant food products, indicating that these PFAS were found in highest concentrations in the products. However, the three PFAS PFPA, PFHxA and PFDA all had low detection rates and high LODs, indicating that there may have been undetected levels of these PFAS in the other infant food products. In the case of better sensitivity and detection of PFPA, PFHxA and PFDA the mean values may be brought down by lower concentrations in other samples. However this is not possible to discuss without further analysis. PFPA and PFTeDA both had much larger maximum values than their mean, median and minimum values, resulting from one product of concern, containing especially large amounts of these PFAS. Product 3 contained the maximum concentration of PFPA, and was a *Grain* powder intended for infants above 8 months of age. PFPA was previously detected in infant foodstuffs in an average concentration of 0.23 ng/g. This was lower than the minimum detected value of PFPA in this analysis. Additionally, PFPA had a high negative matrix effect, indicating a signal reduction from interfering analytes. The *Corn* roduct 7 contained the maximum concentration of PFTeDA, and PFTeDA was previously not detected in infant foodstuffs.

Table 14: Concentrations of 15 PFASs and DecaS in the 49 infant food products.

#	DecaS	PFPA	PFBS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOSA	PFOS	PFDA	Sulf	PFUnDA	PFDoDA	PFTrDA	PFTeDA
1	3.9	-	0.66	-	0.49	0.50	0.37	0.20	-	0.45	-	0.48	-	-	-	0.18
2	-	-	0.63	-	0.58	0.48	0.33	-	0.10	0.54	-	0.47	-	-	-	-
3	-	46	1.9	-	-	4.6	1.4	-	-	0.40	-	0.66	-	-	-	-
4	0.72	-	0.65	-	0.59	0.48	0.30	-	-	0.44	-	0.42	-	-	-	0.13
5	0.41	1.7	0.73	-	0.35	0.53	0.32	-	-	0.65	-	0.53	-	-	-	0.30
6	0.61	5.4	0.89	6.2	0.18	1.3	0.13	-	0.087	0.90	-	0.44	-	-	-	0.47
7	1.2	3.2	3.8	7.1	0.38	4.3	0.61	1.4	0.13	1.1	1.2	0.67	-	3.6	5.63	18
8	-	3.2	0.88	5.8	-	0.30	0.57	-	-	-	-	0.93	-	-	-	9.4
9	0.80	2.2	0.83	2.9	-	0.72	0.71	1.3	-	0.45	-	0.56	-	0.14	-	0.93
10	1.5	9.6	1.1	3.2	0.45	0.91	0.19	4.7	-	0.52	1.4	0.49	-	0.035	0.31	0.81
11	4.1	-	0.83	-	0.32	0.90	0.31	0.64	0.11	0.55	-	0.51	-	0.073	0.55	1.4
12	1.5	-	0.67	2.5	0.28	0.44	0.25	0.52	0.083	0.50	-	0.41	-	0.068	-	0.53
13	0.21	-	0.68	2.0	0.25	0.48	0.26	1.1	-	0.50	-	0.44	-	-	0.06	1.6
14	0.40	-	0.48	7.5	-	0.38	0.29	-	0.083	0.42	-	0.39	-	-	-	-
15	0.35	-	0.41	-	0.13	0.33	0.26	-	-	0.24	-	0.27	-	-	-	-
16	0.32	-	0.35	-	0.49	0.33	0.074	-	0.061	0.26	-	0.28	0.020	-	0.06	-
17	0.67	-	0.89	-	0.74	0.50	0.14	-	0.10	0.41	-	0.48	-	-	0.43	-
18	0.41	-	0.50	-	0.68	0.43	-	-	-	0.52	-	0.34	-	-	0.29	-
18-2	0.54	-	0.79	-	0.24	0.51	0.24	-	0.094	0.45	-	0.45	0.15	-	0.25	-
19	0.94	-	0.62	-	0.35	0.54	0.23	-	-	0.42	-	0.44	0.10	-	0.23	-
20	1.1	-	0.66	2.9	0.30	0.51	0.20	-	0.10	0.50	-	0.47	0.075	-	0.27	-
21	0.93	-	0.58	-	0.47	0.46	0.22	-	0.084	0.42	-	0.40	0.057	-	0.35	-
22	1.5	-	0.48	2.7	0.30	0.38	0.15	-	-	0.38	-	0.36	0.017	-	0.11	-
23	2.3	-	0.61	-	1.4	0.62	0.58	-	0.093	0.52	-	0.43	0.085	0.12	0.43	-
24	1.5	-	0.59	-	1.2	0.61	0.29	-	-	0.48	-	0.46	0.072	-	0.29	-

#	DecaS	PFPA	PFBS	PFHxA	PFHpA	PFHxS	PFOA	PFNA	PFOSA	PFOS	PFDA	Sulf	PFUnDA	PFDoDA	PFTrDA	PFTeDA
25	1.1	-	0.66	1.8	1.3	0.54	0.27	-	-	0.49	-	0.48	0.015	0.26	0.36	-
26	1.4	-	0.67	4.0	1.1	0.63	0.26	-	-	0.44	-	0.51	-	-	0.78	-
27	0.73	-	0.33	-	1.0	0.40	0.12	-	-	0.18	-	0.23	0.18	-	0.09	-
28	0.35	-	0.41	-	0.95	0.36	0.28	-	-	0.27	-	0.34	-	-	-	-
29	1.5	-	0.42	-	0.86	0.45	0.10	-	-	0.37	-	0.37	-	-	2.28	-
40	0.54	-	0.48	-	1.0	0.37	0.17	-	-	0.31	-	0.30	0.011	-	0.14	-
31	2.0	-	0.79	-	0.32	0.58	0.30	0.92	0.10	0.46	-	0.45	0.039	-	-	0.60
32	1.3	-	0.62	-	0.17	0.50	0.33	0.12	-	0.28	-	0.45	-	-	-	0.18
33	0.82	-	0.76	3.0	0.16	0.56	0.32	1.0	0.10	0.27	-	0.43	-	-	-	0.50
34	1.7	-	0.57	-	0.26	0.63	0.32	0.22	0.090	0.32	-	0.41	-	-	1.62	0.15
35	1.4	-	0.69	-	0.31	0.55	0.28	0.23	0.086	0.37	-	0.41	-	-	-	0.30
36	1.1	0.61	0.70	2.0	0.42	0.56	0.34	0.28	0.10	0.45	-	0.49	-	-	-	0.28
37	2.3	0.82	0.75	-	0.49	0.55	0.33	0.44	0.10	0.45	-	0.46	-	-	-	0.32
38	1.2	0.79	0.64	2.4	0.31	0.56	0.35	0.17	-	0.65	-	0.44	-	-	0.03	0.23
39	0.83	0.81	0.49	2.4	0.25	0.46	-	-	-	0.40	-	0.40	-	-	-	0.16
40	0.49	-	0.45	-	0.57	0.42	0.32	0.052	0.084	0.32	-	0.44	-	-	-	-
41	0.66	-	0.61	-	0.55	0.52	0.30	0.19	0.094	0.39	-	0.48	-	-	-	0.32
42	0.79	3.3	0.64	-	-	0.57	0.34	-	0.095	0.46	-	0.45	-	-	-	0.19
43	1.1	1.8	0.73	-	0.065	0.63	-	-	-	0.42	-	0.46	-	-	-	0.33
44	1.7	4.4	0.52	-	0.50	0.51	0.43	0.082	-	0.39	-	0.47	-	-	-	0.18
45	1.3	4.8	0.57	4.8	-	0.65	0.43	-	-	0.53	-	0.48	-	-	-	0.32
46	1.9	-	0.60	-	0.20	0.48	0.28	0.14	-	0.37	-	0.40	-	-	-	0.22
47	0.88	-	0.41	-	0.15	0.39	0.23	0.20	0.069	0.30	-	0.33	0.0092	-	-	0.16
48	0.72	-	0.58	-	0.27	0.57	0.36	0.37	-	0.42	-	0.47	-	-	-	0.19
49	2.7	-	0.59	1.9	0.15	0.51	-	0.07	-	0.41	-	0.43	-	-	-	0.21

PFOSA and PFUnDA had low values for mean, median, min and max, and were consequently found in low concentrations in all infant food samples. PFOSA was previously not detected in infant foodstuffs. PFUnDA, however, had been reported in higher average concentrations than the maximum concentration detected of 0.18 ng/g. This may indicate a decrease of PFUnDA concentrations in infant foodstuffs. On the other hand, PFUnDA had high negative matrix effects and its detected signal may have been diminished by interfering analytes. The remaining PFAS (excluding DecaS) had mean concentrations ranging from 0.32 ng/g to 0.72 ng/g, and all except PFOS had higher mean concentrations in the infant foodstuffs than previously reported levels. This may demonstrate a shift in the use of perfluorinated substances from PFOS to other PFAS, following the restriction of PFOS and its precursors by the Stockholm convention in 2009.

Table 15: Descriptive statistics of TAs in the 49 infant food products.

ng/g	**Mean ± Std.**	**Median**	**Min**	**Max**	**Outliers**
DecaS	1.2 ± 0.83	1.1	<LOD	4.1	3
PFPA	5.9 ± 11	<LOD	<LOD	46	1
PFBS	0.72 ± 0.50	0.63	0.33	3.8	3
PFHxA	3.6 ± 1.8	<LOD	<LOD	7.5	0
PFHpA	0.49 ± 0.34	0.37	<LOD	1.4	4
PFHxS	0.69 ± 0.80	0.51	0.30	4.6	5
PFOA	0.32 ± 0.20	0.30	<LOD	1.4	5
PFNA	0.65 ± 1.0	<LOD	<LOD	4.7	1
PFOSA	0.092 ± 0.013	<LOD	<LOD	0.13	1
PFOS	0.44 ± 0.15	0.42	<LOD	1.1	2
PFDA	1.3 ± 0.12	<LOD	<LOD	1.4	-[a]
Sulf	0.45 ± 0.11	0.44	0.23	0.93	3
PFUnDA	0.064 ± 0.055	<LOD	<LOD	0.18	0
PFDoDA	0.62 ± 1.3	<LOD	<LOD	3.6	1
PFTrDA	0.69 ± 1.3	<LOD	<LOD	5.6	3
PFTeDA	1.3 ± 3.7	0.30	<LOD	18	4

[a] Not enough data to compute quartile statistics

The average sum of the 15 PFAS (excluding decaS) in the 49 infant food products was 7.7 ng/g. However, the products number 3 and 7 contained a sum of PFAS of over 50 ng/g and the products 8 and 10 over 20 ng/g. As mentioned above, product 3 was a *Grain* product while product 7 was a *Corn* product. Products 8 and 10 were also made predominantly of *Corn*, indicating that especially *Corn* products, and also *Grain* products, generally contained high levels of PFAS.

4.2.1 Concentrations in Main Ingredient Groups

To consider the risk associated with consumption of each infant food group, average PFAS concentrations in main ingredient groups were found. As described in **section 3.1** the infant food products were divided into six main ingredient groups: *Corn*, *Dairy*, *Fruit*, *Grain*, *Meat* and *Vegetable*. The average concentrations of PASFs, PFCAs and PFSAs in these six food groups are shown in **fig. 5**. Concentrations of DecaS in the six food groups are not included in the figure, as DecaS is not fluorinated, and additionally had low recovery and high signal enhancement from sample matrix. As can be seen from **fig. 5** PASFs were found in relatively low average concentrations below 0.5 ng/g in all food groups. PFCAs were found in high concentrations, especially in *Corn* and *Grain* food, where they were found in an average concentration of 2.7 ng/g. All main ingredient groups contained more PFCAs than the two other PFAS groups except for *Dairy* products. **Figure 5** indicates that *Grain* and *Corn* products generally contain more PFAS than the other food groups.

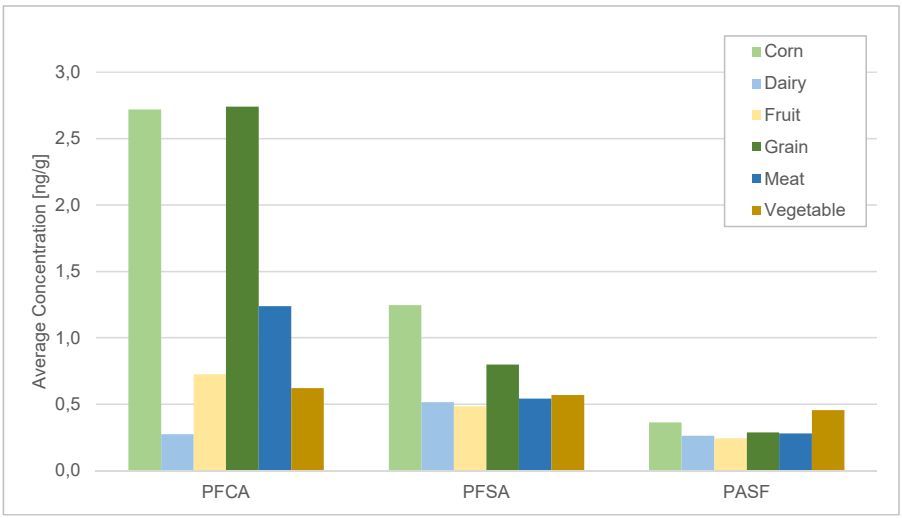

Figure 5: Average concentrations of PASFs, PFCAs and PFSAs in the six main ingredient groups.

Average concentrations of all 15 PFAS and DecaS in the six product groups are listed in **table 16**. The average concentrations are shown graphically in **fig. 6**. *Dairy* products contained low concentrations of all the detected PFAS, and is therefore not a food group of great concern. Although *Fruit* products contained most PFAS they were mostly found in low average concentrations. The exception was PFHxA, which were found in uniformly high concentrations. The same can be said for the food group *Vegetable*. *Corn* products contained the maximum of many of the PFAS, *Grain* products contained very high levels of PFPA and *Meat* products contained quite high levels of PFPA and PFHxA.

PFPA was found in high average concentrations in the three food groups *Corn*, *Grain* and *Meat*, in

low concentration in *Vegetable* and was not detected in the remaining two. This may be explained by PFPAs low relative recovery and its high negative matrix effect, as well as its high LOD. PFPA concentrations may have been highly affected by the extraction procedure and analyte signals may have been diminished by interferring analytes in the infant food matrix. PFPA was found in especially high concentrations in *Grain* products, at 13 ng/g, and as previously mentioned the maximum PFPA concentration of 46 ng/g was found in a *Grain* product. PFHxA was found in high concentrations in all food groups except *Dairy*, where it was not detected. PFHxA had a maximum average concentration in *Corn*, of 5.0 ng/g. When disregarding the *Corn* concentrations, PFBS, PFNA and PFOS were found in quite steady concentrations in the food groups, ranging from 0.12 ng/g to 0.83 ng/g. However, these three PFAS were found in higher average concentrations in *Corn* foodstuffs. PFHpA, PFOA and Sulf were detected in all food groups, but in low average concentrations in the range 0.25-0.62 ng/g. PFHxS was found in similarly low concentrations in all food groups except *Corn* and *Grain*, where it was found in higher average concentrations of 1.5 and 1.1 ng/g, respectively. PFOSA and PFUnDA were found in very low concentrations, around 0.1 ng/g average concentration in the food groups were they were detected. PFDA was only found in *Corn* products, in an average concentration of 1.3 ng/g. As previously discussed, PFDA had a low detection rate of 4% and a high LOD of 0.99 ng/g, which may be the reason why it is only detected in one food group. The three last PFAS, PFDoDA, PFTrDA and PFTeDA had very similar concentration trends, with low concentrations for all food groups except *Corn*. The concentrations also increased for increasing carbon chain length for these three PFCAs in the food groups *Corn* and *Fruit*.

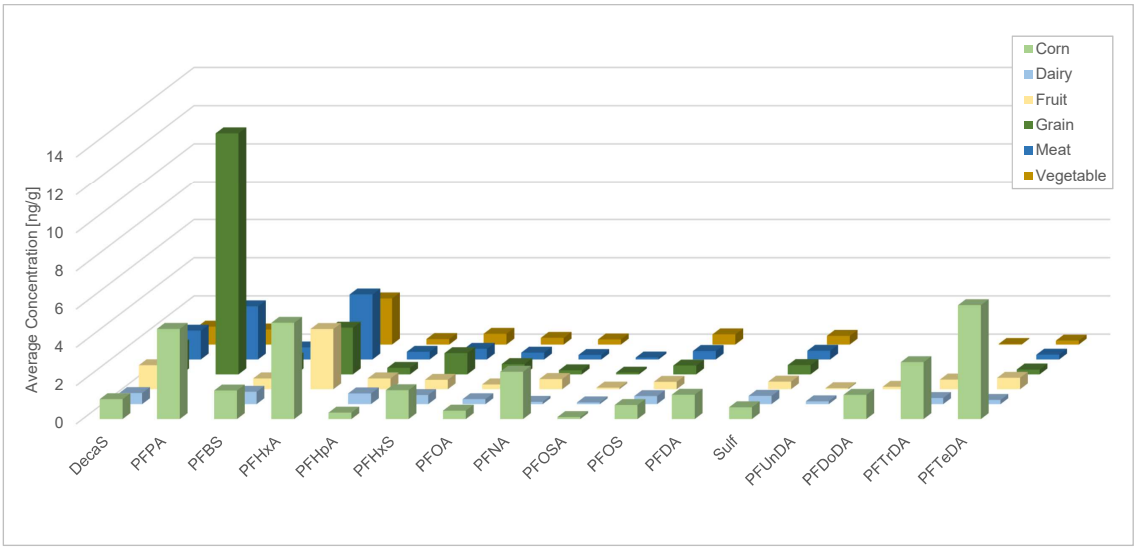

Figure 6: Average amount of each PFAS in the six food groups.

When comparing the PFAS concentrations to previously analyzed infant food listed in **table 4** in **section 2.4**, it can be concluded that the 49 infant food samples analyzed generally contained

Table 16: Average concentration (ng/g) of PFAS in food groups.

	Corn	Dairy	Fruit	Grain	Meat	Vegetable
#	5	5	24	7	6	2
DecaS	1.0 ± 0.42	0.58 ± 0.12	1.3 ± 0.89	1.5 ± 1.4	1.5 ± 0.56	0.94 ± 0.31
PFPA	4.7 ± 3.0	-	-	13 ± 22	2.8 ± 2.0	0.79 [a]
PFBS	1.5 ± 1.3	0.65 ± 0.17	0.57 ± 0.14	0.83 ± 0.47	0.63 ± 0.084	0.61 ± 0.043
PFHxA	5.0 ± 1.9	-	3.2 ± 1.8	2.4 [a]	3.4 ± 1.9	2.4 [a]
PFHpA	0.34 ± 0.14	0.56 ± 0.17	0.57 ± 0.44	0.34 ± 0.18	0.40 ± 0.14	0.29 ± 0.023
PFHxS	1.5 ± 1.6	0.48 ± 0.040	0.50 ± 0.13	1.1 ± 1.5	0.56 ± 0.058	0.56 ± 0.0048
PFOA	0.44 ± 0.26	0.26 ± 0.074	0.25 ± 0.10	0.54 ± 0.47	0.36 ± 0.060	0.35 ± 0.0081
PFNA	2.5 ± 1.9	0.12 ± 0.10	0.54 ± 0.41	0.21 ± 0.016	0.24 ± 0.16	0.27 ± 0.14
PFOSA	0.11 ± 0.028	0.092 ± 0.0056	0.087 ± 0.014	0.091 ± 0.0069	0.10 ± 0.0049	-
PFOS	0.73 ± 0.29	0.42 ± 0.068	0.39 ± 0.10	0.46 ± 0.10	0.44 ± 0.054	0.53 ± 0.17
PFDA	1.3 ± 0.12	-	-	-	-	-
Sulf	0.62 ± 0.19	0.43 ± 0.0517	0.40 ± 0.075	0.49 ± 0.089	0.46 ± 0.033	0.46 ± 0.025
PFUnDA	-	0.15 [a]	0.057 ± 0.050	-	-	-
PFDoDA	1.3 ± 2.0	-	0.13 ± 0.091	-	-	-
PFTrDA	3.0 ± 3.8	0.32 ± 0.10	0.51 ± 0.63	-	-	0.032 [a]
PFTeDA	6.0 ± 7.8	0.22 ± 0.14	0.60 ± 0.56	0.25 ± 0.08	0.25 ± 0.063	0.21 ± 0.027

[a] st.d. not possible, only one detected sample in group.

higher concentrations of all PFAS except PFOS and PFUnDA. As previously mentioned, PFUnDA had high negative matrix effect and the detection may have been diminished by interfering analytes. PFOS had positive matrix effect, and if anything the detected concentration of PFOS was higher than the actual concentration in the foodstuffs. The TAs DecaS and Sulf were not previously analyzed in infant foodstuffs. PFOSA and PFTeDA were not detected in previously analyzed infant foodstuffs. Sulf and PFOSA are precursors to PFOS and in this analysis Sulf was found at similar concentrations to PFOS, while PFOSA was less prevalent in the foodstuffs. Considering that PFOSA was previously not detected, the low concentrations detected in the 49 products may indicate an increase in PFOSA levels in foodstuffs. However, it may also indicate a development of more accurate detection of PFOSA. The average concentration of PFOS in infant food samples previously analyzed was 1.5 ng/g, while none of the PFOS concentrations in this report surpassed 1.1 ng/g. Lorenzo et al. (2016) found PFOS concentrations of 1.321 ng/g in cereal foods, which is much higher than in the analyzed *Grain* products, containing 0.46 ng/g PFOS. Additionally, PFOS had a high positive matrix effect leading to signal enhancement, indicating that the PFOS levels in foodstuffs may be lower than reported. This apparent decrease in PFOS concentrations in infant foodstuffs may be a result of the restriction of the manufacturing and use of PFOS since 2009, and thus the decrease in environmental concentrations and spreading of PFOS. However, it is not possible to conclude this with certainty as other factors may play a part in the results. PFOA was previously found in quite similar concentrations, as the average

concentration in previously analyzed foodstuffs was 0.27 ng/g, compared to 0.32 ng/g found in this analysis. This may indicate almost unchanged environmental concentrations of PFOA. PFPA, PFBS, PFHxA and PFTrDA were all found in over ten times higher average concentrations in the 49 infant food products compared to previously reported infant foodstuffs concentrations. With the exception of PFTrDA this may indicate a shift towards using shorter-chain PFAS, as these are found to be less toxic to human health. In *Grain*, *Fruit* and *Meat* foods PFAS with chain-lengths 5 and 6 appear to be the most accumulative, as PFPA, PFHxA and PFHxS were found in highest levels in these food groups. This corresponds with findings of Ghisi et al. (2019), stating that short-chain PFAS accumulate more in plants than long-chained PFAS [19]. However, this does not hold for *Corn* products, as PFTeDA, a 14-C chain PFAS, was the PFAS of highest concentration in *Corn* foods. PFOS and PFOA have previously been reported in high levels in *Meat*, *Fish*, *Egg* and *Fruit* products. However, in this analysis they were found in higher concentrations in *Corn* and *Grain* products. On the other hand, the concentrations of PFOS and PFOA were similar in the six main food groups analyzed. PFNA and PFHxS were previously reported in high concentrations in *Fruit*, while in this analysis they were both most present in *Corn* products, followed by *Fruit* and *Grain*, respectively. The reason for this difference may be lack of reports on *Corn* foodstuffs for infants. However, short-chain PFAS are previously reported to accumulate at higher rates in the edible parts of corn-plants than long-chained PFAS [21]. With the exception of PFTeDA, this corresponds to the results in this thesis, as PFPA and PFHxA were most present in the *Corn* products.

4.3 Determination of Elements

Detection resolution, detection rate [%], mean, median, minimum, maximum, standard deviation and relative standard deviation [%] of the 63 elements analyzed by ICP-MS are listed in **table 17**. As mentioned in **section 2.3** the elements of special concern in regard to consumption of infant foodstuffs are toxic elements. More specifically, the elements Cd, Hg, Pb, U, Cr(VI), As and Sb are previously focused on in infant foodstuffs. As can be seen from **table 18** the toxic elements were found in the infant food in the ng/g range. Cr and Sb were found in high concentrations of 72 and 545 ng/g, respectively, while the other elements in concentrations below 11 ng/g. The toxic elements had quite large standard deviations of the mean. Especially Pb, Cr and Sb had large standard deviations, and these three toxic elements had the most outliers out of the seven, with 5, 4, and 8 outliers, respectively. Outliers are concentrations above the third quartile + 1.5 times the interquartile range. These outliers are interesting to take a closer look at as they represent infant food samples with especially high concentration of toxic elements. However, Sb had large relative standard deviations in the average concentrations of repeating tests and extra parallel samples, which may indicate that the outlying concentrations were results of analytical inaccuracy. The average Sb concentration in all infant food products was 1.9 ± 1.0 ng/g when subtracting all 8

outliers, and the maximum concentration was 5.5 ng/g in product number 3. All toxic elements except for As and Cr had LODs below 1 ng/g. Chromium had a high LOD and and a high DR of 89%. As Cr is an essential element in its third oxidation state, and it is not possible to distinguish Cr(III) form Cr(VI) in the analysis, it is to be expected that Cr is detected in higher concentrations. Arsenic had a high LOD and it has been reported in higher concentrations in drinking water than most other toxic elements. However, As had a low detection rate of only 26% in the infant food products. Cadmium, mercury, lead and antimony all had LODs of 0.89 ng/g. Out of these four toxic elements, Pb was detected in most samples (89%) followed by Sb (74%). Comparing the results to toxic element concentrations previously reported the infant foods analyzed contained higher concentrations of all toxic elements except Cr. The product with the highest number of outlying concentrations was product 11, a raspberry and apple fruit bar.

Table 17: Element descriptive statistics.

$\mu g/g$	^{7}**Li**	^{9}**Be**	^{11}**B**	^{82}**Se**	^{89}**Y**	^{90}**Zr**	^{93}**Nb**	^{114}**Cd**	^{98}**Mo**
Resolution	LR	LR	LR	LR	LR	LR	LR	LR	MR
DR	72	20	98	44	98	43	33	67	85
Mean	0.011	0.0018	2.9	0.062	0.0027	0.0023	0.0029	0.0049	0.13
Median	0.0082	<LOD	1.6	<LOD	0.00055	<LOD	<LOD	0.0038	0.058
Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Max	0.037	0.0044	19	0.31	0.021	0.018	0.025	0.027	0.99
Std.	0.0096	0.0010	3.9	0.060	0.0053	0.0038	0.0060	0.0047	0.20
RSD	85	53	137	96	199	170	203	97	155
	^{118}**Sn**	^{133}**Cs**	^{139}**La**	^{140}**Ce**	^{141}**Pr**	^{146}**Nd**	^{147}**Sm**	^{151}**Eu**	^{157}**Gd**
Resolution	LR	LR	LR	LR	LR	LR	LR	LR	LR
DR	87	100	100	100	57	100	48	9	26
Mean	0.020	0.0046	0.0018	0.0028	0.00060	0.0017	0.00066	0.00052	0.0012
Median	0.0019	0.0024	0.00068	0.0012	0.00027	0.00065	<LOD	<LOD	<LOD
Min	<LOD	0.00036	0.00023	0.00010	<LOD	0.00012	<LOD	<LOD	<LOD
Max	0.22	0.023	0.016	0.0318	0.0040	0.018	0.0028	0.00088	0.0039
Std.	0.049	0.0056	0.0028	0.0050	0.00079	0.0029	0.00057	0.00022	0.0011
RSD	246	121	157	179	131	174	87	42	92
	^{159}**Tb**	^{163}**Dy**	^{165}**Ho**	^{166}**Er**	^{169}**Tm**	^{172}**Yb**	^{175}**Lu**	^{178}**Hf**	^{181}**Ta**
Resolution	LR	LR	LR	LR	LR	LR	LR	LR	LR
DR	13	20	19	28	4	20	7	4	15
Mean	0.00024	0.0012	0.00030	0.00066	0.00028	0.00076	0.00021	0.00087	0.00023
Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Max	0.00040	0.0032	0.00070	0.0022	0.00029	0.0022	0.00027	0.00093	0.00055
Std.	0.00011	0.0010	0.00022	0.00065	0.0000072	0.00068	0.000046	0.000088	0.00019
RSD	45	81	73	97	3	89	21	10	82

	^{182}W	^{193}Ir	^{195}Pt	^{197}Au	^{202}Hg	^{205}Tl	^{208}Pb	^{209}Bi	^{232}Th
Resolution	LR	LR	LR	LR	LR	LR	LR	LR	LR
DR	72	0	2	61	33	83	89	50	33
Mean	0.0033	<LOD	0.00063	0.00020	0.0023	0.00054	0.0049	0.0012	0.00091
Median	0.0014	<LOD	<LOD	0.00017	<LOD	0.00033	0.0025	0.00083	<LOD
Min	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Max	0.029	<LOD	0.00063	0.00056	0.0056	0.0030	0.044	0.0060	0.0026
Std.	0.0056	-	-	0.00011	0.0011	0.00054	0.0075	0.0012	0.00066
RSD	169	-	-	53	49	98	155	98	72
	^{238}U	^{23}Na	^{25}Mg	^{27}Al	^{29}Si	^{31}P	^{34}S	^{39}K	^{44}Ca
Resolution	LR	MR	MR	MR	MR	MR	MR	MR	MR
DR	80	80	100	94	80	100	100	100	100
Mean	0.00082	380	222	1.9	38	698	377	2513	522
Median	0.00049	177	103	0.93	19	338	211	1581	100
Min	<LOD	<LOD	19	<LOD	<LOD	68	30	559	17
Max	0.0040	2542	1052	13	248	4298	1562	13122	5161
Std.	0.00080	551	253	2.6	48	998	428	2419	1067
RSD	98	145	114	141	129	143	114	96	205
	^{45}Sc	^{49}Ti	^{51}V	^{53}Cr	^{55}Mn	^{56}Fe	^{59}Co	^{60}Ni	^{63}Cu
Resolution	MR	MR	MR	MR	MR	MR	MR	MR	MR
DR	2	94	70	89	100	100	80	96	100
Mean	0.0019	0.29	0.0085	0.072	3.3	11	0.0084	0.14	0.87
Median	<LOD	0.073	0.0030	0.030	1.7	4.4	0.0055	0.065	0.47
Min	<LOD	<LOD	<LOD	<LOD	0.039	0.58	<LOD	<LOD	0.031
Max	0.0019	5.4	0.075	0.60	20	79	0.040	0.53	3.7
Std.	-	0.85	0.014	0.11	4.7	17	0.0081	0.16	0.96
RSD	-	290	159	157	144	160	96	117	110
	^{66}Zn	^{69}Ga	^{75}As	^{85}Rb	^{88}Sr	^{109}Ag	^{121}Sb	^{137}Ba	^{72}Ge
Resolution	MR	MR	MR	MR	MR	MR	MR	MR	HR
DR	100	0	26	100	100	0	74	100	0
Mean	5.9	<LOD	0.011	1.7	0.70	<LOD	0.55	0.38	<LOD
Median	2.2	<LOD	<LOD	0.90	0.35	<LOD	0.0017	0.20	<LOD
Min	0.23	<LOD	<LOD	0.20	0.017	<LOD	<LOD	0.014	<LOD
Max	43	<LOD	0.033	11	2.6	<LOD	18	2.3	<LOD
Std.	9.8	-	0.0073	2.0	0.70	-	2.8	0.44	-
RSD	167	-	67	118	101	-	514	116	-

Table 18: Descriptive statistics of toxic elements in the 49 infant food products.

ng/g	**Mean**	**Median**	**Min**	**Max**	**DR**	**OL**
Cd	4.9 ± 4.7	3.8	<LOD	27	67	1
Hg	2.3 ± 1.1	<LOD	<LOD	5.6	33	0
Pb	4.9 ± 7.5	2.5	<LOD	44	89	5
U	0.82 ± 0.80	0.49	<LOD	4.0	80	1
Cr	72 ± 114	30	<LOD	596	89	4
As	11 ± 7.3	<LOD	<LOD	33	26	1
Sb	545 ± 2802	1.7	<LOD	17565	74	8

4.3.1 Concentrations in Main Ingredient Groups

The mean concentration of each toxic element in the 49 infant food samples are shown in **table 19**. These average concentrations are also shown graphically in **fig. 7**. As can be seen from **table 19** and **fig. 7** Cr and Sb are found in particularly high concentrations in the infant food products. As Cr(III) is essential it is to be expected that Cr is found at somewhat higher concentrations than other toxic elements. Some of the Cr in the infant food products may be essential and beneficial to human health, but in the case that all detected Cr was in fact Cr(VI) the results were alarming. As previously mentioned, this distinction cannot be made without further investigation. Sb is not an essential element, and was found in high concentrations in *Fruit* and *Grain* products. The three infant food products with Sb concentrations higher than the mean concentrations of 545 ng/g (from **table 18**) were products 1, 14 and 15.

Table 19: Average concentration (ng/g) of toxic elements in food groups.

	Corn	**Dairy**	**Fruit**	**Grain**	**Meat**	**Vegetable**
#	5	5	24	7	6	2
Cd	4.7 ± 3.0	0.91[a]	2.8 ± 2.1	7.9 ± 8.1	4.7 ± 2.5	8.5 ± 4.2
Hg	2.5[a]	1.6 ± 0.88	2.3 ± 1.0	3.5 ± 1.9	1.7 ± 0.56	2.9[a]
Pb	3.6 ± 1.1	3.2[a]	6.6 ± 10	3.5 ± 2.3	2.1 ± 0.71	3.7 ± 0.32
U	0.25 ± 0.050	0.82 ± 0.94	0.79 ± 0.94	0.80 ± 0.61	1.1 ± 0.58	1.4 ± 1.3
Cr	26 ± 9.9	15 ± 1.1	88 ± 126	49 ± 46	93 ± 167	66[a]
As	5.5[a]	8.5[a]	12 ± 9.6	8.4 ± 0.84	8.6 ± 4.3	15[a]
Sb	1.2 ± 0.25	1.4 ± 0.43	1030 ± 4129	449 ± 1136	1.9 ± 1.0	42 ± 55

[a] st.d. not possible, only one detected sample in group.

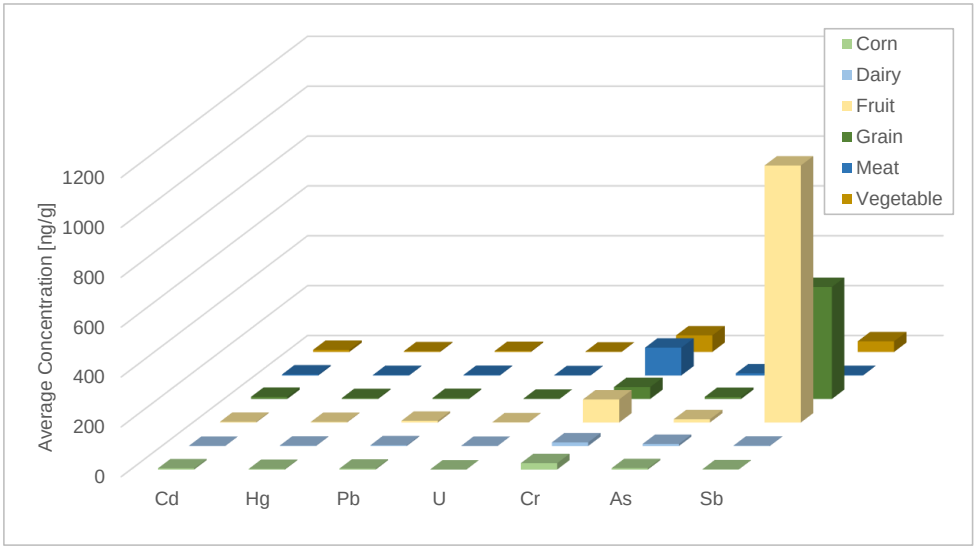

Figure 7: Average amount of each toxic element in the six food groups.

The repeating tests and extra parallels showed a high relative standard deviation of the mean for Sb, indicating analytical inaccuracy in the detection of Sb. Out of all 63 elements analyzed Sb had the highest RSD value in the 49 infant food samples of 514%. On the other hand, no Sb was detected in the blank samples and the certified reference material was detected with a low RSD value for Sb (16%).

In order to regard the relative distribution of the seven toxic elements in the infant food samples the outliers were removed in **fig. 8**. This also to disregard the extremely high concentrations of Sb in some samples that may have resulted from analytical inaccuracy. In general, all food groups showed the same trend considering toxic element concentrations when subtracting outliers in the data - similar concentrations in all food groups, with slightly higher concentrations in *Vegetable* products. Cr was found in high concentrations in almost all food groups. Sb, on the other hand, showed overall low concentrations when subtracting the 8 outliers in the Sb-data set. The remaining five toxic elements were found at average concentrations below 10 ng/g in all food groups, except for As, which was found in high average concentrations in *Vegetable* and *Fruit* products. Cd was also found in moderate average concentrations in *Vegetable* products, while the elements Hg, Pb and especially U were found at low concentrations in the six food groups. However, there is no clear trend of toxic element levels in the main ingredient groups of infant foodstuffs. The infant food product that contained the most outliers was product number 11, a solid *Fruit*-bar intended for infants above 12 months of age. This sample contained the maximum concentrations of Pb, U and As and an outlier concentration of Cr.

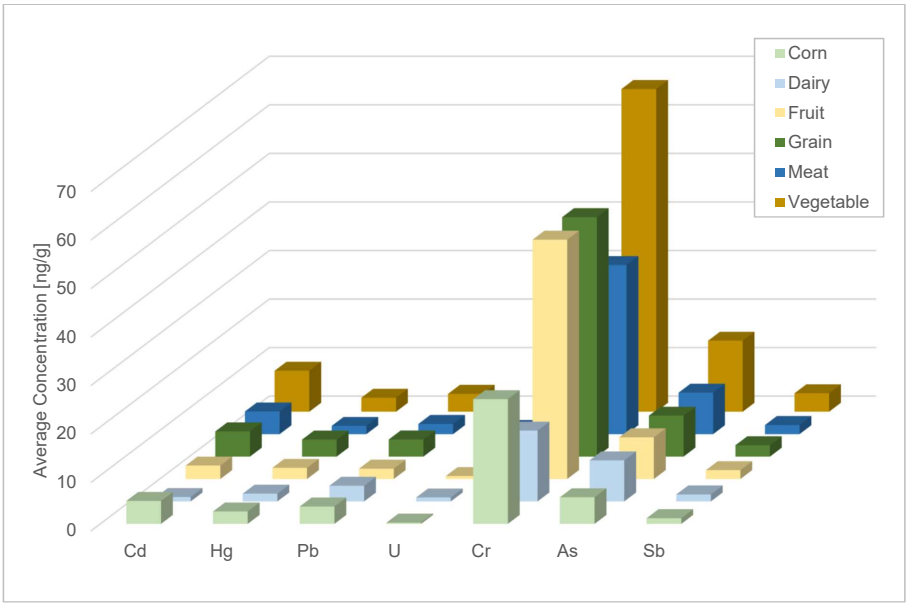

Figure 8: Average amount of each toxic element in the six food groups, outliers subtracted.

Previously analyzed infant food shown in **table 3** in **section 2.4** showed significantly higher concentrations of Hg and Sb than analyzed infant foods in this thesis, and similar concentrations of Cd, Pb, U, Cr and As. The Hg concentrations did not contain any outliers indicating that the 49 infant food samples generally contained higher concentrations of Hg than previously reported products. Melø et al. (2008) previously reported a Hg concentration of 1.6 ng/g in cereal infant foodstuffs, while *Grain* foods reported here contained an average Hg concentration of 3.5 $\pm$ 1.9 ng/g [34]. The analytical inaccuracy of Hg was high as the relative standard deviation in the extra parallel samples were 63% and 118%. This may explain why the analyzed samples contained more Hg than previously reported for this type of product, but the inaccuracy in detection may also indicate higher levels in Hg. The high Sb concentrations in some infant food products may be explained by analytical error, as Sb had a large relative standard deviation in the mean of 514%. When subtracting the 8 outliers Sb had an average concentration of 1.9 ng/g in the infant food products, which is still higher than the previously reported average of 0.58 ng/g.

4.4 Correlations

In order to assess the correlation of PFAS and toxic element concentrations in infant food products a bivariate Pearson correlation analysis was performed using IBM SPSS statistics 25. The correlation coefficients of all PFAS and toxic elements are shown in **fig. 9**. PFDA is not included in **fig. 9** as there was not enough data on PFDA concentrations to perform correlation analysis. Correlation coefficients below $\pm$ 0.1 were removed from the data. DecaS did not correlate significantly with any of the PFAS. However, DecaS correlated significantly at the 0.01 level with

U and 0.05 level with As with correlation coefficients of 0.445 and 0.719, respectively. The three longest PFAS, PFDoDA, PFTrDA and PFTeDA had high positive correlations with many of the other target analytes. They also correlated positively with each other with correlation coefficients of over 0.94 at a significance level of 0.01. PFTeDA correlated significantly at the 0.01 level with all PFAS except PFPA, PFHpA and PFNA, with varying coefficients. It correlated strongest with PFUnDA (1.000), PFDoDA (0.999), PFTrDA (0.949), PFBS (0.901) and PFHxS (0.828). PFDoDA and PFTrDA both correlated with PFBS, PFHxA and PFHxS by over 0.8 at a significance level of 0.01. Additionally, PFDoDA correlated with PFOSA, PFOS, Sulf and PFUnDA with correlation coefficients above 0.8. PFPA correlated significantly with PFHxS, PFOA and PFNA, as well as the toxic elements Hg and Sb. Out of the PFAS PFPA had the strongest correlation with PFOA and PFNA, indicating a stronger correlation with other perfluoroalkyl carboxyl acids. In addition to the three longest chained PFAS PFBS also correlated significantly with PFHxS, PFOA, PFOSA, PFOS and Sulf at the 0.01 significance level. Out of the five PFAS PFBS correlated with, PFOA had the lowest correlation coefficient, of 0.508, indicating that PFBS correlated stronger with other PFSAs and PASFs. PFBS also correlated with Hg at the 0.05 significance level. PFHxA correlated significantly with PFHxS, PFOS and Sb in addition to the long chained PFAS PFDoDA, PFTrDA and PFTeDA. PFHpA did not correlate significantly with any of the other PFAS or toxic elements. PFHxS correlated with the highest number of other target analytes, as it correlated significantly and positively with ten PFAS and one toxic element, namely Hg. PFHxS correlated especially strongly with PFBS, PFDoDA, PFTrDA and PFTeDA (coefficients above 0.8). PFOA correlated significantly with seven other PFAS and with the toxic element Hg. It had the highest correlation coefficient with PFPA, of 0.811 at the 0.01 significance level. As PFOA and PFPA have a very similar chemical structure it is natural that they should correlate strongly. PFNA significantly correlated with PFPA, PFOSA and PFUnDA. It correlated with PFUnDA with a coefficient of 1.000 at the 0.01 significance level. PFOSA correlated significantly with eight other PFAS. PFOSA and Sulf correlate especially strong with a coefficient of 0.956 at the 0.01 significance level. These two PFAS are the only two classified as PASFs, indicating that PASFs will correlate in this type of sample matrix. PFOSA also correlated significantly at the 0.01 level with all PFSAs indicating that PASFs and PFSAs occur simultaneously in infant foodstuffs. PFOSA also correlated significantly with the PFCAs of chain lengths 8, 9, 13 and 14. PFOS correlates significantly with eight other PFAS, the strongest correlations being to the three longest PFAS, PFDoDA (0.985), PFTrDA (0.667) and PFTeDA (0.683). The remaining five correlations at the 0.01 level are all sulfonic acids or sulfonyl fluorides, again indicating that PFSAs and PASFs correlate with each other. This is expected as PASFs may be precursors of PFSAs. PFUnDA correlated significantly with PFNA (1.000), PFDoDA (-1.000) and PFTeDA (1.000) and with the toxic element Sb (0.765), all at the 0.01 significance level. This result points at the previously mentioned presumption that the PFAS correlates with other PFAS of similar chain lengths. The negative correlation between PFUnDA and PFDoDA may indicate that these PFAS diminish each others detected signals, as they both

have moderate negative matrix effects. As PFUnDA had a stronger negative matrix effects than PFDoDA its detection may have been diminished by interference of the PFDoDA signal. Cd only correlated significantly with Hg. As previously mentioned Hg significantly correlated with several PFAS, but among the toxic elements, only with Cd. Pb, U and As positively correlated with each other at the 0.01 significance level. Cr only significantly correlated with Pb (0.340). Sb did not correlate with any of the other toxic elements, but correlated strongly with PFPA (0.705) and PFHxA (0.577) at the significance level of 0.05 and PFUnDA (0.765) at a significance level of 0.01.

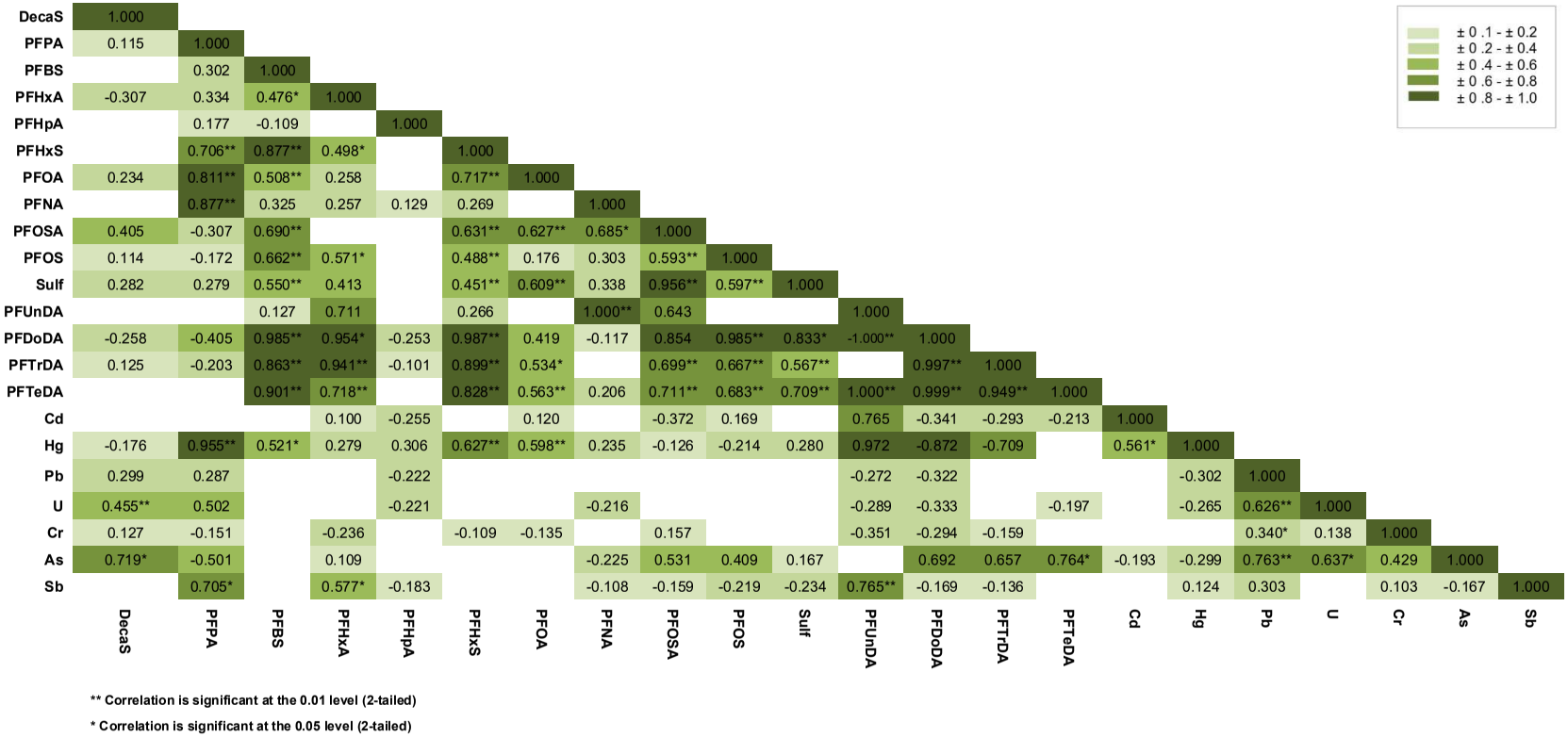

Figure 9: Correlation coefficients of the PFAS and toxic elements.

4.5 Estimated Daily Intakes

To evaluate the risk associated with consumption of the infant food products analyzed estimated daily intakes (EDIs) of the target analytes were calculated, as described in **section 2.5**. The calculation of estimated daily intake of PFAS and toxic elements from consumption of the 49 infant food products were based on the average weight of a meal. Ten products were used to calculate an average meal weight (m_{if}) of 143.5 g. As shown in **eq. (1)**, this average meal weight was multiplied by the average amount of meals pr day, which according to WHO (2002) is 3 [41], and the average toxic substance concentration. The EDIs of PFAS and toxic elements for exclusively breastfed infants are listed as a comparison, and the full list of toxic element and PFAS concentrations in breast milk previously studied is shown in **section 2.4**, in **table 6** and **table 7**, respectively. The minimum risk levels (MRLs) of PFAS and the tolerable daily intakes (TDIs) of toxic elements are also shown in the table. As many PFAS are not restricted in their use or characterized as toxic to human health MRLs for all the PFAS are not yet established.

As the only two target analytes, PFOS and PFUnDA concentrations resulted in higher EDI from breast milk than from infant food products. The consumption of infant food would therefore not cause any added risk of PFOS and PFUnDA exposure compared to breastfeeding. The same holds for PFOA, as EDIs of PFOA from infant food and breast milk were the same. The target analytes exceeding the minimum risk level/tolerable daily intake by infant food consumption were the PFAS PFHxS, PFOA, PFNA and PFOS and the toxic element Sb, when including outlying values. Consequently, all the risk assessed PFAS exceeded the MRLs in infant food, and it is not possible to presume the risk of the remaining 12 PFAS. Sb however, did not exceed the TDI when subtracting the outliyng concentrations in the dataset. The Sb results showed high analytical uncertainty, and it is reasonable to conclude that the few outlying concentrations of Sb were detection errors. PFHxS, PFNA and Sb EDIs did not exceed the MRL/TDI in breast milk. Consequently, breastfeeding would not cause any risk in regard to these three target analytes, and would therefore be a safer approach than introducing complementary feeding into an infant's diet. The PFAS PFPA, PFHxA, PFDA and PFTeDA had estimated daily intakes above 0.55 µg/day, and these are therefore the PFAS of highest concern in the infant foodstuffs. Especially PFPA and PFHxA resulted in a high EDI of 2.6 and 1.6 µg/day, respectively, and should be thoroughly analyzed and risk assessed. However, as long-chained PFAS are assumed to pose more danger to human health than short-chained PFAS it can be argued that the risk assessment of PFDA and PFTeDA is more pressing. The four PFAS PFPA, PFHxA, PFDA and PFTeDA were found in highest concentrations in *Corn* and *Grain* products intended as snack foods. As snack foods are usually not fed to infants as meals (at least not every meal of the day) the EDI may be estimated too high compared to the infants' actual exposure. On the other hand, it is important to have knowledge and inform about the risk of endocrine diseases from consumption of these snack foods as well.

The infant food products introduced a higher concentration of all toxic elements than previously reported breast milk samples, except for Sb (when subtracting outliers). However, all EDIs were lower than the TDIs of the toxic elements.

Table 20: EDIs, MRLs and TDIs of PFAS and toxic elements in infant food and breast milk.

$\mu g/day$	**EDI**		**MRL/TDI**
	Infant Food	**breast milk**	
DecaS	0.52	0.069[a]	-
PFPA	2.6	0.0044	-
PFBS	0.31	0.0018	-
PFHxA	1.6	0.026	-
PFHpA	0.21	0.047	-
PFHxS	0.30	0.027	0.17
PFOA	0.14	0.14	0.026
PFNA	0.28	0.023	0.026
PFOSA	0.040	0.017	-
PFOS	0.19	0.27	0.017
PFDA	0.55	0.10	-
Sulf	0.19	-[b]	-
PFUnA	0.028	0.040	-
PFDoDA	0.27	0.015	-
PFTrDA	0.30	0.072	-
PFTeDA	0.58	0.00049	-
Cd	2.1	0.14	7.1
Hg	1.0	0.32	31
Pb	2.1	1.4	60
U	0.35	0.039	17
Cr	31	0.24	20-60
As	4.5	2.1	18
Sb	0.80 (235)	0.83	8.6

[a] Fluorinated DecaS was previously analyzed in breast milk [39]

[b] Sulf was not previously analyzed in breast milk samples

5 CONCLUSIONS

The non-fluorinated analyte DecaS recovered poorly and was strongly effected by interferring compounds in the infant food matrix, indicating that the analysis procedure did not fit similar non-fluorinated substances. The 15 PFAS analyzed generally recovered well from the extraction procedure. PFPA, PFTrDA and PFTeDA had slightly lower relative recoveries, and the detection of these PFAS may have been affected by sample preparation. Matrix effects of the PFAS were generally low, with the exceptions of PFPA and PFUnDA with highly negative matrix effects and PFOS with highly positive matrix effects. PFPA and PFUnDA detection signals may therefore have been diminished by interferring analytes, while PFOS may have been enhanced. The elemental analysis resulted in good recovery for the seven chosen toxic elements, as accuracy was between 72-114 % recovered certified reference material. However, the repeating tests and extra parallels of samples showed poor results for Hg and Sb. The relative standard deviations in the mean concentrations of repeating tests and extra parallels ranged from 30 % to 118 % for Hg and Sb, and there was therefore a great deal of uncertainty in the detection of these elements.

All PFAS and toxic elements analyzed were detected in infant foodstuffs. PFBS, PFHxS and Sulf were detected in all the 49 products. Target analytes of special concern were PFPA, PFHxA, PFTeDA and Sb as they were found in high concentrations in the infant foodstuffs. The elevated PFAS concentrations were mainly found in *Corn* and *Grain* foods. However PFHxA was found in high concentrations in all food groups except *Dairy*. The 2009 restriction of PFOS and its precursors appeared to have decreased the levels of PFOS in the environment, as PFOS was found in much lower concentrations in this analysis than previously reported. A restriction of other or all PFAS may therefore be wise. Sb was found in elevated concentrations in *Fruit* products, more specifically concentrated fruit bars. However, the high concentrations of Sb contained a lot of uncertainty, and these samples need to be examined again in order to determine the Sb concentration in infant foodstuffs with certainty.

As PFPA had the strongest correlation with PFOA and PFNA it can be concluded that perfluorinated carboxyl acids correlate strongly with each other. The same can be said for perfluoroalkyl sulfonic acids and sulfonyl fluorides as PFBS had the strongest correlation to PFHxS, PFOSA, PFOS and Sulf. Consequently, it can be concluded that different PFCAs occur together in infant foodstuffs, and as does PFSAs. Additionally, the non fluorinated sulfonic acid DecaS did not correlate with the PFAS analyzed in infant foodstuffs.

The EDI calculations showed exposure exceeding the MRL/TDI of all risk assessed PFAS from an infant food diet. However, the levels of the not risk-assessed PFAS PFPA, PFHxA, PFDA and PFTeDA were of concern, as they were significantly higher than the other PFAS in infant foodstuffs. PFPA and PFHxA introduced very high EDIs, especially from *Corn* and *Grain* foods. PFDA and PFTeDA, however, pose a higher adverse health risk at lower concentrations, as they are not

excreted in urine at the same rate as shorter chained PFAS, and need to be further analyzed in infant foodstuffs as well. The EDI of Sb exceeded the TDI when including the outlying concentrations of Sb. However, as there were some uncertainty in the detection of Sb further analysis of Sb in infant foodstuffs is necessary. More generally, the toxic elements were found in high concentrations in *Vegetable* products, but many outlying concentrations were found in *Fruit* products.

The infant food products of special concern were the products 1, 3 (powdered grain porridge products), 7, 8, 10 (corn puff products), 11, 14 and 15 (concentrated fruit snack bars). Consequently, consumption of infant snack foods such as corn puffs, granola bars and fruit bars appears to pose the highest risk of PFAS toxicity and toxic element intake. Snack foods are usually not fed as a meal, and especially not as all three meals of the day, and the estimated daily intake of PFAS and toxic elements from these snack foods may not be relevant. Consuming one or two such products a day may not pose a risk of adverse health effects in the infant. However, the higher levels of long-chained PFAS in *Corn* products are of especially great concern and should be analyzed further. The PFAS in most urgent need to be risk assessed are PFPA and PFHxA, as they were the most present in infant foodstuffs.

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Appendices

A Chemical Structure of Perfluorinated Substances Analysed

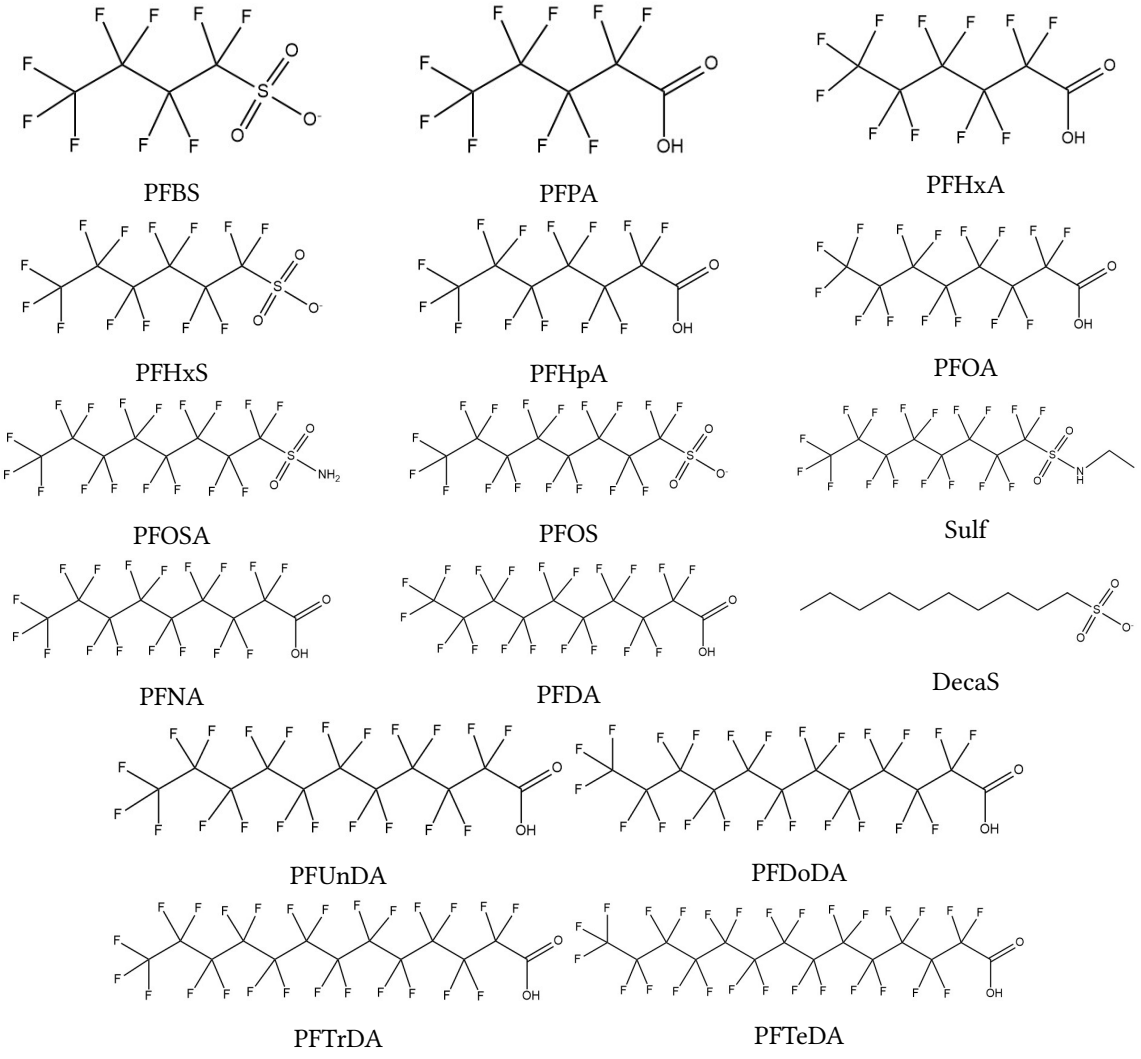


Figure A.1: Chemical structure of perfluorinated target analytes.

B List and Characteristics of Infant Food Products

Table B.1: Characteristics of infant food products analyzed.

Number	Main Ingredients	Texture	Minimum Age	Packaging Material	Producer number
1	Grain	Powder	12	Aluminum	1
2	Grain	Powder	8	Aluminum	2
3	Grain	Powder	8	Aluminum	3
4	Dairy	Powder	0	Aluminum	2
5	Grain	Solid	12	Aluminum	4
6	Corn	Solid	-	Plastic	3
7	Corn	Solid	6	Plastic	5
8	Corn	Solid	6	Plastic	6
9	Corn	Solid	-	Plastic	3
10	Corn	Solid	7	Plastic	7
11	Fruit (conc.)	Solid	12	Plastic	7
12	Fruit (conc.)	Solid	12	Plastic	8
13	Fruit (conc.)	Solid	12	Plastic	8
14	Fruit (conc.)	Solid	12	Plastic	2
15	Fruit and grain	Solid	12	Plastic	2
16	Fruit and grain	Solid	12	Plastic	6
17	Dairy and corn	Semi-solid	6	Paperboard	2
18	Dairy and grain	Semi-solid	6	Paperboard	2
19	Fruit	Semi-solid	6	Stand-up Pouch	2
20	Fruit	Semi-solid	6	Stand-up Pouch	2
21	Fruit	Semi-solid	6	Stand-up Pouch	2
22	Fruit	Semi-solid	6	Stand-up Pouch	2
23	Fruit	Semi-solid	6	Stand-up Pouch	2
24	Fruit	Semi-solid	6	Stand-up Pouch	2
25	Fruit	Semi-solid	6	Stand-up Pouch	2
26	Fruit	Semi-solid	6	Stand-up Pouch	2
27	Fruit	Semi-solid	6	Stand-up Pouch	2
28	Fruit	Semi-solid	4	Stand-up Pouch	6
29	Fruit	Semi-solid	6	Stand-up Pouch	6
30	Fruit	Semi-solid	6	Stand-up Pouch	3
31	Fruit	Semi-solid	4	Stand-up Pouch	3

Number	Main Ingredients	Texture	Minimum Age	Packaging Material	Producer number
32	Fruit	Semi-solid	6	Stand-up Pouch	3
33	Fruit	Semi-solid	8	Stand-up Pouch	3
34	Fruit	Semi-solid	6	Stand-up Pouch	4
35	Grain	Semi-solid	6	Stand-up Pouch	1
36	Grain and ham	Semi-solid	6	Stand-up Pouch	1
37	Rice and chicken	Semi-solid	6	Stand-up Pouch	1
38	Vegetable	Semi-solid	6	Stand-up Pouch	3
39	Grain	Semi-solid	6	Stand-up Pouch	3
40	Dairy	Liquid	6	Paperboard	2
41	Dairy	Liquid	6	Paperboard	2
42	Grain and cattle	Semi-solid	15	Glass	2
43	Grain and vegetable	Semi-solid	12	Glass	2
44	Grain and chicken	Semi-solid	12	Glass	2
45	Grain and cattle	Semi-solid	8	Glass	2
46	Vegetable and cattle	Semi-solid	15	Glass	1
47	Fruit	Semi-solid	4	Glass	4
48	Vegetable	Semi-solid	4	Glass	2
49	Fruit	Semi-solid	6	Plastic	2

C Data for Preparation of Target Analyte Mixture

Table C.1: Preparation of target analyte mixture for QA analysis (prepared 16.01.20).

TA name	Concentration	Volume TA	Volume MeOH	Final Conc.
	ppm	*uL*	*uL*	*ppm*
DecaS	980	100	900	98
PFBS	860	120	880	103
PFDA	980	100	900	98
PFHpA	790	130	870	103
PFNA	870	110	890	96
PFOA	1110	90	910	100
PFOSA	100	n.a.	n.a.	100
PFOS	100	n.a.	n.a.	100
PFPA	1470	70	930	103
PFTeDA	920	110	890	101
PFTrDA	960	100	900	96
PFUnA	910	110	890	100
Sulf	830	120	880	100
PFDoDA	930	110	890	102
PFHxS	1030	100	900	103
PFHxA	1240	80	920	99

D Gradient Elution Program and Parameters for UPLC-MS/MS

The separation of PFASs using UPLC-MS/MS was performed using a gradient elution program with MilliQ water with 2 mM ammonium acetate (A) and methanol of gradient grade (99.93 % pure) (B). **Table D.1** shows the gradient elution program used for the separation. Parameters for the ESI-interface in the UPLC-MS/MS instrument are shown in **table D.2**.

Table D.1: Gradient elution program for UPLC analysis.

Time [min]	**A** %	**B** %	**Step**
Initial	90	10	Initial
0.2	90	10	6
3.0	0	100	5
3.5	0	100	6
3.6	90	10	6
4.0	90	10	6

Table D.2: Electronspray ionization parameters for UPLC-MS/MS interface.

ESI voltage	1.8 kV
Cone voltage	30 V
Source temp.	150 °C
Desolvation temp.	450 °C
Collision gas flow	0.15 mL/min

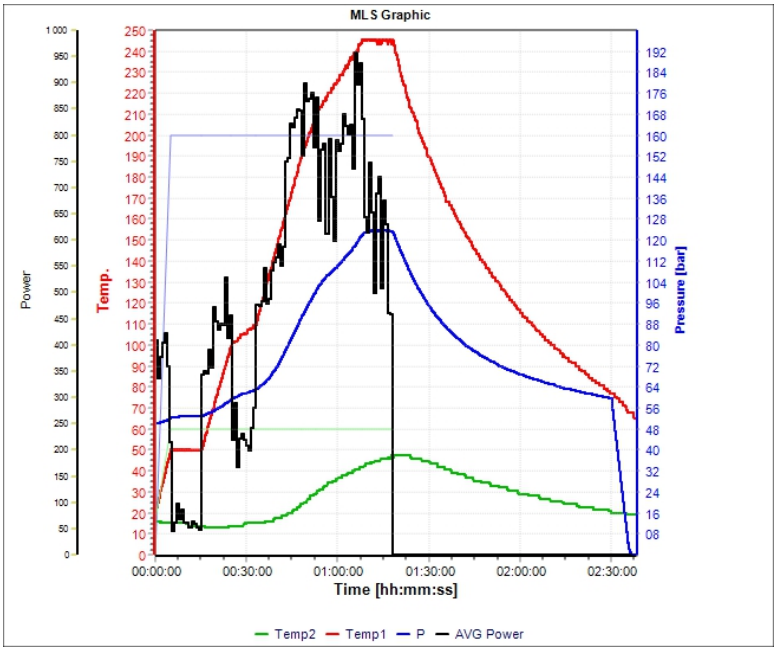
E UltraClave Digestion Temperature Profile

MLS Microwave Report

Systemtest: MWT AG

Application: pressPREP
Report 29.10.2019 16:45:52
Filename: M:\A\2019\PROJECT NOT FINISHED\KJEMI\MASTER\Ingrid Bakke - babymat\UC-nr 1216 221019 vials 81-120 Babymat Ingrid Kaia.dpr

MWT AG



Parameter

```
Signature name  :
Signature date  :
Signature func. :
Operator        :Administrator
Date            :22.10.2019
14:22:46
Method filename
:Stand-profile-245C-10-min-oper
Create run      :Administrator
Microwave Power :pulse
Load pressure   :50.0 bar
Release temp.   :78.0 °C
Release pressure:10.0 bar/min
Cooling         :ON
Auto open       :OFF
Cooling on Temp.:40.0 °C
Ground load     :300 30 2
Ventilation time:01:20:05
Rotor           :40 positions
Vessel-Type     :18ml teflon and
quarts
```

Remark:

Ultraclave run nr 1216, vials used 81-120
Prosedyre, ca 350 mg prove ble tilsatt 8 ml 50% HNO3 v/v, dekomponert i henhold til vis temperaturprofil, fortynnet til ca 85 ml, gir ca 0.6M HNO3, denne losningen benyttes til ICP-MS analysene.
Prosjekt, Masteroppgave for Ingrid og Kaia, grunnstoffer i Babymat 49 prover

MW Program

Step	Time [hh:mm:ss]	Temp 1 [°C]	Temp 2 [°C]	Press [bar]	Engery [Watt]
1	00:05:00	50	60	160	1 000
2	00:10:00	50	60	160	1 000
3	00:10:00	100	60	160	1 000
4	00:08:00	110	60	160	1 000
5	00:15:00	190	60	160	1 000
6	00:05:00	210	60	160	1 000
7	00:15:00	245	60	160	1 000
8	00:10:00	245	60	160	1 000

Figure E.1: Temperature profile and parameters used in UltraClave digestion of infant foodstuffs.

F ICP-HR-MS Sample Uptake, Nebulizer and Plasma Parameters

Table F.1: Key parameters for sample uptake into ICP-HR-MS.

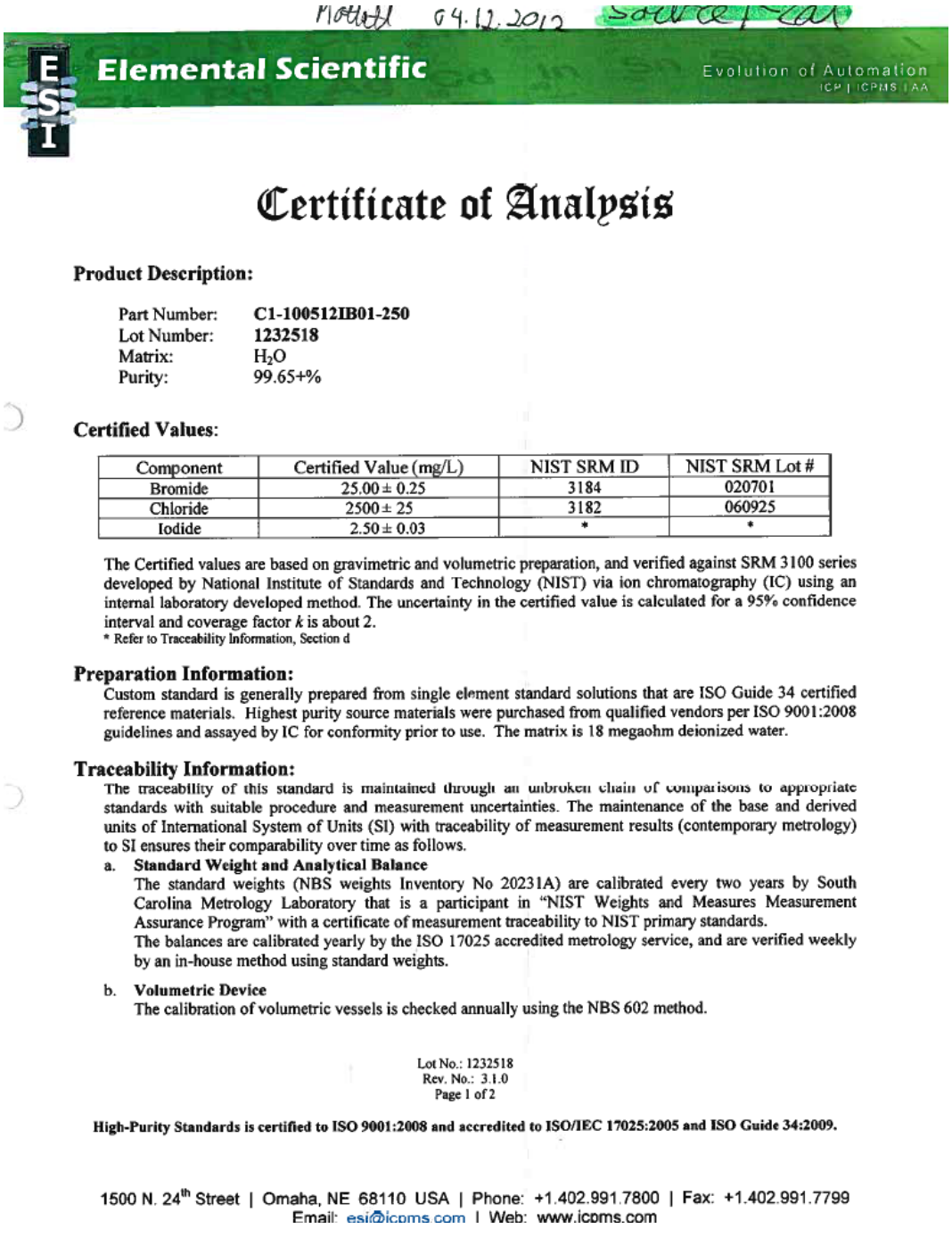
Sample uptake time	3 s
Sample aspirating speed	0.84 mL/s
Sample loop volume	500 µL
Washing loop time	5 s
Flushing sample probe time	2 s
Read delay	20 s

Table F.2: prepFAST syringe system, PFA-ST nebulizer, plasma and interface parameters.

S400V Syringe Pump System	
Flow rate IS	30 µL/min
Flow rate carrier liquid	170 µL/min
PFA-ST Nebulizer	
Flow rate sample	0.75 L/min
Flow rate in T-connection	0.55 L/min
Gas type	Argon with 0.04 % methane
Volume range	50-700 µL/min
Spray chamber	Quartz baffled
Plasma and Interface	
Flow rate cool gas	15.5 L/min
Flow rate auxiliary gas	1.1 L/min
Plasma source	Quartz demountable torch with o-rings
Injector length	2.5 mm
Torch RF-power	1350 W
Skimmer cone type	X-skimmer

G Primary Solutions for ICP-HR-MS

G.1 Primary Solutions from Producer 1



Elemental Scientific

Evolution of Automation
ICP | ICPMS | AA

Certificate of Analysis

Product Description:

Part Number:	**C1-100512IB01-250**
Lot Number:	**1232518**
Matrix:	H_2O
Purity:	99.65+%

Certified Values:

Component	Certified Value (mg/L)	NIST SRM ID	NIST SRM Lot #
Bromide	25.00 ± 0.25	3184	020701
Chloride	2500 ± 25	3182	060925
Iodide	2.50 ± 0.03	*	*

The Certified values are based on gravimetric and volumetric preparation, and verified against SRM 3100 series developed by National Institute of Standards and Technology (NIST) via ion chromatography (IC) using an internal laboratory developed method. The uncertainty in the certified value is calculated for a 95% confidence interval and coverage factor *k* is about 2.
* Refer to Traceability Information, Section d

Preparation Information:

Custom standard is generally prepared from single element standard solutions that are ISO Guide 34 certified reference materials. Highest purity source materials were purchased from qualified vendors per ISO 9001:2008 guidelines and assayed by IC for conformity prior to use. The matrix is 18 megaohm deionized water.

Traceability Information:

The traceability of this standard is maintained through an unbroken chain of comparisons to appropriate standards with suitable procedure and measurement uncertainties. The maintenance of the base and derived units of International System of Units (SI) with traceability of measurement results (contemporary metrology) to SI ensures their comparability over time as follows.

a. **Standard Weight and Analytical Balance**
The standard weights (NBS weights Inventory No 20231A) are calibrated every two years by South Carolina Metrology Laboratory that is a participant in "NIST Weights and Measures Measurement Assurance Program" with a certificate of measurement traceability to NIST primary standards.
The balances are calibrated yearly by the ISO 17025 accredited metrology service, and are verified weekly by an in-house method using standard weights.

b. **Volumetric Device**
The calibration of volumetric vessels is checked annually using the NBS 602 method.

Lot No.: 1232518
Rev. No.: 3.1.0
Page 1 of 2

High-Purity Standards is certified to ISO 9001:2008 and accredited to ISO/IEC 17025:2005 and ISO Guide 34:2009.

1500 N. 24th Street | Omaha, NE 68110 USA | Phone: +1.402.991.7800 | Fax: +1.402.991.7799
Email: esi@icpms.com | Web: www.icpms.com

Figure G.1.1: Primary solution from producer 1 of chlorine, bromine and iodine.

law 3 S 1.035

Elemental Scientific

Evolution of Automation

ICP | ICPMS | AA

Certificate of Analysis

Product Description:

Name:	Custom 70 Element Mix #1
Part Number:	**C1-111219IB01-250**
	Solution A
Lot Number:	**1213643**
Matrix:	2% HNO_3
Purity:	99% - 99.9999%

Certified Values:

Element	(µg/mL)	SRM ID	SRM Lot#	Element	(µg/mL)	SRM ID	SRM Lot#
Al	50.00 ± 0.25	3101a	060502	K	500.0 ± 2.5	3141a	051220
Ca	2000 ± 10	3109a	050825	Si	1000 ± 10	3150	071204
Cu	25.00 ± 0.15	3114	011017	Na	2000 ± 10	3152a	010728
Fe	50.00 ± 0.25	3126a	051031	Sr	25.00 ± 0.15	3153a	990906
Mg	500.0 ± 2.5	3131a	050302	S	1000 ± 10	3154	892205
Mn	25.00 ± 0.25	3132	050429	Zn	100.0 ± 0.5	3168a	080123

The Certified values are based on gravimetric and volumetric preparation, and verified against SRM 3100 series developed by National Institute of Standards and Technology (NIST) via inductively coupled plasma optical emission spectrometry (ICP-OES) using an internal laboratory developed method. The uncertainty in the certified value is calculated for a 95% confidence interval and coverage factor *k* is about 2.

Preparation Information:

Custom standard is generally prepared from single element standard solutions that are ISO Guide 34 certified reference materials. Highest purity source materials were purchased from qualified vendors per ISO 9001:2008 guidelines and assayed by ICP-OES for conformity prior to use. Sub-boiling distilled high-purity acid has been used to place the materials in solution and to stabilize the standard. The matrix is as noted above in 18 megaohm deionized water.

Traceability Information:

The traceability of this standard is maintained through an unbroken chain of comparisons to appropriate standards with suitable procedure and measurement uncertainties.

a. **Analytical Balance Calibration**: All balances are calibrated weekly by an in-house method using NBS weights Inventory No 20231A. The balances are calibrated yearly and the calibration weights are checked biennially by a qualified metrology company with weights traceable to the primary standards developed by NIST.

High-Purity Standards is certified to ISO 9001:2008 and accredited to ISO/IEC 17025:2005 and ISO Guide 34:2009.
1500 N. 24th Street | Omaha, NE 68110 USA | Phone: +1.402.991.7800 | Fax: +1.402.991.7799
Email: esi@icpms.com | Web: www.icpms.com

Figure G.1.2: Primary solution of 70 elements, producer 1 (delivered in 3 containers).

Lot S 51.035

Elemental Scientific

Evolution of Automation

ICP | ICP-MS | AA

Certificate of Analysis

Product Description:

Name:	Custom 70 Element Mix #1
Part Number:	**C1-111219IB01-250**
	Solution B
Lot Number:	**1213645**
Matrix:	5% HNO_3
Purity:	99.964% - 99.9999%

Certified Values:

Element	(µg/mL)	SRM ID	SRM Lot#	Element	(µg/mL)	SRM ID	SRM Lot#
As	10.00 ± 0.05	3103a	100818	Lu	0.100 ± 0.001	3130a	100503
Ba	10.00 ± 0.06	3104a	070222	Nd	0.100 ± 0.001	3135a	992803
Be	1.00 ± 0.01	3105a	090514	Ni	5.00 ± 0.03	3136	000612
Bi	0.200 ± 0.002	3106	991212	P	50.0 ± 0.3	3139a	060717
B	10.00 ± 0.05	3107	070514	Pr	1.00 ± 0.01	3142a	990501
Cd	2.00 ± 0.01	3108	060531	Re	0.100 ± 0.001	3143	010816
Ce	1.00 ± 0.01	3110	890602	Rb	10.0 ± 0.1	3145a	891203
Cs	5.00 ± 0.03	3111a	050614	Sm	0.100 ± 0.001	3147a	892911
Cr	5.00 ± 0.03	3112a	030730	Sc	0.100 ± 0.001	3148a	100701
Co	5.00 ± 0.03	3113	000630	Se	10.0 ± 0.1	3149	100901
Dy	0.100 ± 0.001	3115a	990504	Ag	0.500 ± 0.005	3151	992212
Er	0.100 ± 0.001	3116a	000831	Tb	0.100 ± 0.001	3157a	891603
Eu	0.500 ± 0.005	3117a	991307	Tl	0.200 ± 0.002	3158	993012
Gd	0.500 ± 0.005	3118a	992004	Th	0.200 ± 0.002	*	
Ga	2.00 ± 0.02	3119a	890709	Tm	0.100 ± 0.001	3160a	790912
Ho	0.100 ± 0.001	3123a	790812	U	1.00 ± 0.01	3164	080521
In	0.200 ± 0.002	3124a	110516	V	5.00 ± 0.03	3165	992706
La	2.00 ± 0.01	3127a	890402	Yb	0.100 ± 0.001	3166a	790512
Pb	5.00 ± 0.03	3128	101026	Y	1.00 ± 0.01	3167a	790412
Li	5.00 ± 0.03	3129a	000505				

The Certified values are based on gravimetric and volumetric preparation, and verified against SRM 3100 series developed by National Institute of Standards and Technology (NIST) via inductively coupled plasma optical emission spectrometry (ICP-OES) using an internal laboratory developed method. The uncertainty in the certified value is calculated for a 95% confidence interval and coverage factor *k* is about 2.

* Refer to Traceability Information, Section C.

Preparation Information:

High-Purity Standards is certified to ISO 9001:2008 and accredited to ISO/IEC 17025:2005 and ISO Guide 34:2009.
1500 N. 24th Street | Omaha, NE 68110 USA | Phone: +1.402.991.7800 | Fax: +1.402.991.7799
Email: esi@icpms.com | Web: www.icpms.com

Primary solution of 70 elements, producer 1, continued.

Elemental Scientific

Evolution of Automation

Certificate of Analysis

Product Description:

Name:	Custom 70 Element Mix #1
Part Number:	**C1-111219IB01-250**
	Solution C
Lot Number:	**1213203**
Matrix:	5% HCl + Tr HNO_3 + Tr HF
Purity:	99.9% - 99.9999%

Certified Values:

Element	(µg/mL)	SRM ID	SRM Lot#	Element	(µg/mL)	SRM ID	SRM Lot#
Sb	0.500 ± 0.005	3102a	061229	Pt	0.200 ± 0.002	3140	000615
Ge	0.500 ± 0.005	3120a	080429	Rh	0.200 ± 0.002	3144	070619
Au	0.200 ± 0.002	3121	991806	Ru	0.200 ± 0.002	*	
Hf	0.200 ± 0.002	3122	080430	Ta	0.100 ± 0.001	3155	080502
Ir	0.200 ± 0.002	*		Te	0.100 ± 0.001	3156	892901
Hg	0.250 ± 0.003	3133	061204	Sn	2.00 ± 0.01	3161a	070330
Mo	2.50 ± 0.03	3134	891307	Ti	5.00 ± 0.03	3162a	060808
Nb	0.200 ± 0.002	3137	080502	W	0.200 ± 0.002	3163	080331
Os	0.100 ± 0.001	*		Zr	0.200 ± 0.002	3169	071226
Pd	0.500 ± 0.005	3138	090629				

The Certified values are based on gravimetric and volumetric preparation, and verified against SRM 3100 series developed by National Institute of Standards and Technology (NIST) via inductively coupled plasma optical emission spectrometry (ICP-OES) using an internal laboratory developed method. The uncertainty in the certified value is calculated for a 95% confidence interval and coverage factor *k* is about 2.

* Refer to Traceability Information, Section C

Preparation Information:

Custom standard is generally prepared from single element standard solutions that are ISO Guide 34 certified reference materials. Highest purity source materials were purchased from qualified vendors per ISO 9001:2008 guidelines and assayed by ICP-OES for conformity prior to use. Sub-boiling distilled high-purity acid has been used to place the materials in solution and to stabilize the standard. The matrix is as noted above in 18 megaohm deionized water.

Traceability Information:

The traceability of this standard is maintained through an unbroken chain of comparisons to appropriate standards with suitable procedure and measurement uncertainties.

High-Purity Standards is certified to ISO 9001:2008 and accredited to ISO/IEC 17025:2005 and ISO Guide 34:2009.
1500 N. 24th Street | Omaha, NE 68110 | USA | Phone: +1 402 991 7800 | Fax: +1 402 991 7799

Primary solution of 70 elements, producer 1, continued.

G.2 Primary Solutions from Producer 2

Mettler 22.11.2012

Source-2

QC

Elemental Scientific, Inc
1500 North 24th Street
Omaha, NE 68110
Phone: 1.402.991.7800
Fax: 1.402.991.7799
esi@icpms.com

Elemental Scientific

Certificate of Analysis

Rev 0

Catalog No:	Lot No:	Storage:	Matrix:	Exp. Date:	Description:
2-100512IB01-250	1041166	Ambient	H2O	12/1/2013	3 Element Mix # 1938, 250 ml mg/L ± 2% in H20

Element	Symbol	Purity (%)	CAS No	Concentration, mg/L
Chloride	Cl	99	7647-14-5	2500.0
Bromide	Br	99.999	7647-15-6	25.0
Iodide	I	99.999	20461-54-5	2.5

Amber Woods

Certified By: _____

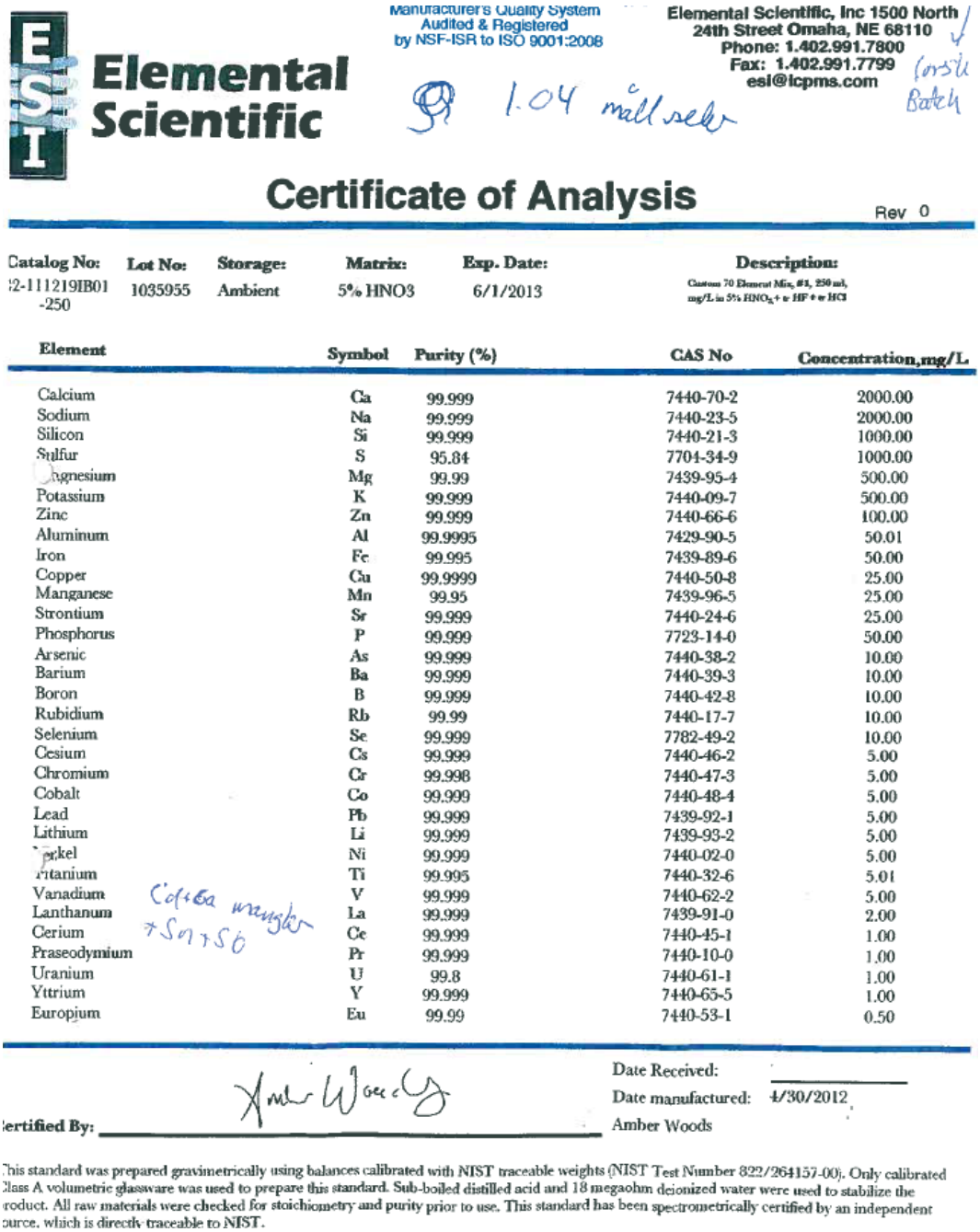
Date Received: _____

Date manufactured: 11/16/2012

standard has been prepared by o2si smart solutions gravimetrically using balances calibrated with NIST traceable weights. Only Class-A volumetric ware was used to prepare this product. Sub-boiled distilled acid and 18 megaohm deionized water were used to stabilize the product. All raw rials were checked for stoichiometry and purity prior to use. This standard has been certified by ICP against an independent source which is directly able to NIST.

o2si smart solutions is Accredited to ISO 9001:2008 by NSF and ISO/IEC 17025:2005 (Certification No. 3031.01) and

Figure G.2.1: Primary solution from producer 2 of chlorine, bromine and iodine.



Elemental Scientific

Manufacturer's Quality System Audited & Registered by NSF-ISR to ISO 9001:2008

Elemental Scientific, Inc 1500 North 24th Street Omaha, NE 68110
Phone: 1.402.991.7800
Fax: 1.402.991.7799
esi@icpms.com

1.04 mill sele

Batch

Certificate of Analysis

Rev 0

Catalog No:	Lot No:	Storage:	Matrix:	Exp. Date:	Description:
2-111219IB01 -250	1035955	Ambient	5% HNO3	6/1/2013	Custom 70 Element Mix, #1, 250 ml, mg/L in 5% HNO_3 + tr HF + tr HCl

Element	Symbol	Purity (%)	CAS No	Concentration,mg/L
Calcium	Ca	99.999	7440-70-2	2000.00
Sodium	Na	99.999	7440-23-5	2000.00
Silicon	Si	99.999	7440-21-3	1000.00
Sulfur	S	95.84	7704-34-9	1000.00
Magnesium	Mg	99.99	7439-95-4	500.00
Potassium	K	99.999	7440-09-7	500.00
Zinc	Zn	99.999	7440-66-6	100.00
Aluminum	Al	99.9995	7429-90-5	50.01
Iron	Fe	99.995	7439-89-6	50.00
Copper	Cu	99.9999	7440-50-8	25.00
Manganese	Mn	99.95	7439-96-5	25.00
Strontium	Sr	99.999	7440-24-6	25.00
Phosphorus	P	99.999	7723-14-0	50.00
Arsenic	As	99.999	7440-38-2	10.00
Barium	Ba	99.999	7440-39-3	10.00
Boron	B	99.999	7440-42-8	10.00
Rubidium	Rb	99.99	7440-17-7	10.00
Selenium	Se	99.999	7782-49-2	10.00
Cesium	Cs	99.999	7440-46-2	5.00
Chromium	Cr	99.998	7440-47-3	5.00
Cobalt	Co	99.999	7440-48-4	5.00
Lead	Pb	99.999	7439-92-1	5.00
Lithium	Li	99.999	7439-93-2	5.00
Nickel	Ni	99.999	7440-02-0	5.00
Titanium	Ti	99.995	7440-32-6	5.01
Vanadium	V	99.999	7440-62-2	5.00
Lanthanum	La	99.999	7439-91-0	2.00
Cerium	Ce	99.999	7440-45-1	1.00
Praseodymium	Pr	99.999	7440-10-0	1.00
Uranium	U	99.8	7440-61-1	1.00
Yttrium	Y	99.999	7440-65-5	1.00
Europium	Eu	99.99	7440-53-1	0.50

Ca+Ba mixture +Sn+Sb

Certified By: Amber Woods

Date Received:

Date manufactured: 4/30/2012

This standard was prepared gravimetrically using balances calibrated with NIST traceable weights (NIST Test Number 822/264157-00). Only calibrated Class A volumetric glassware was used to prepare this standard. Sub-boiled distilled acid and 18 megaohm deionized water were used to stabilize the product. All raw materials were checked for stoichiometry and purity prior to use. This standard has been spectrometrically certified by an independent source, which is directly traceable to NIST.

Figure G.2.2: Primary solution of 70 elements, producer 2 (delivered in 2 containers).

Elemental Scientific

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Elemental Scientific, Inc 1500 North
24th Street Omaha, NE 68110
Phone: 1.402.991.7800
Fax: 1.402.991.7799
esi@icpms.com

Certificate of Analysis

Rev 0

Catalog No:	Lot No:	Storage:	Matrix:	Exp. Date:	Description:
2-111219IB01 -250	1035955	Ambient	5% HNO3	6/1/2013	Custom 70 Element Mix, #3, 250 ml, mg/L in 5% HNO_3 + tr HF + tr HCl

Element	Symbol	Purity (%)	CAS No	Concentration, mg/L
Dysprosium	Dy	99.99	7429-91-6	0.10
Erbium	Er	99.999	7440-52-0	0.10
Holmium	Ho	99.99	7440-60-0	0.10
Lutetium	Lu	99.999	7439-94-3	0.10
Neodymium	Nd	99.999	7440-00-8	0.10
Samarium	Sm	99.999	7440-19-9	0.10
Scandium	Sc	99.99	7440-20-2	0.10
Terbium	Tb	99.999	7440-27-9	0.10
Thulium	Tm	99.99	7440-30-4	0.10
Ytterbium	Yb	99.999	7440-64-4	0.10
Gadolinium	Gd	99.999	7440-52-2	0.50
Palladium	Pd	99.999	7440-05-3	0.50
Gold	Au	99.999	7440-58-5	0.20
Iridium	Ir	99.995	7439-88-5	0.20
Platinum	Pt	99.999	7440-06-4	0.20
Rhodium	Rh	99.995	7440-16-6	0.20
Ruthenium	Ru	99.99	7440-18-8	0.20
Osmium	Os	99.99	7440-04-2	0.10
Tellurium	Te	99.999	10605-80-9	0.10
Gallium	Ga	99.999	7440-55-3	2.00
Germanium	Ge	99.999	7440-56-4	0.50
Niobium	Nb	99.999	7440-03-1	0.20
Tungsten	W	99.999	7440-33-7	0.20
Rhenium	Re	99.997	7440-15-5	0.10
Tantalum	Ta	99.99	7440-25-7	0.10
Hafnium	Hf	99.9	7440-58-6	0.20
Cadmium	Cd	99.999	7440-43-9	2.00
Beryllium	Be	99.999	7440-41-7	1.00
Bismuth	Bi	99.999	7440-69-9	0.20
Indium	In	99.999	7440-74-6	0.20
Thallium	Tl	99.999	7440-28-0	0.20
Tin	Sn	99.999	7440-31-5	2.00
Antimony	Sb	99.999	7440-36-0	0.50

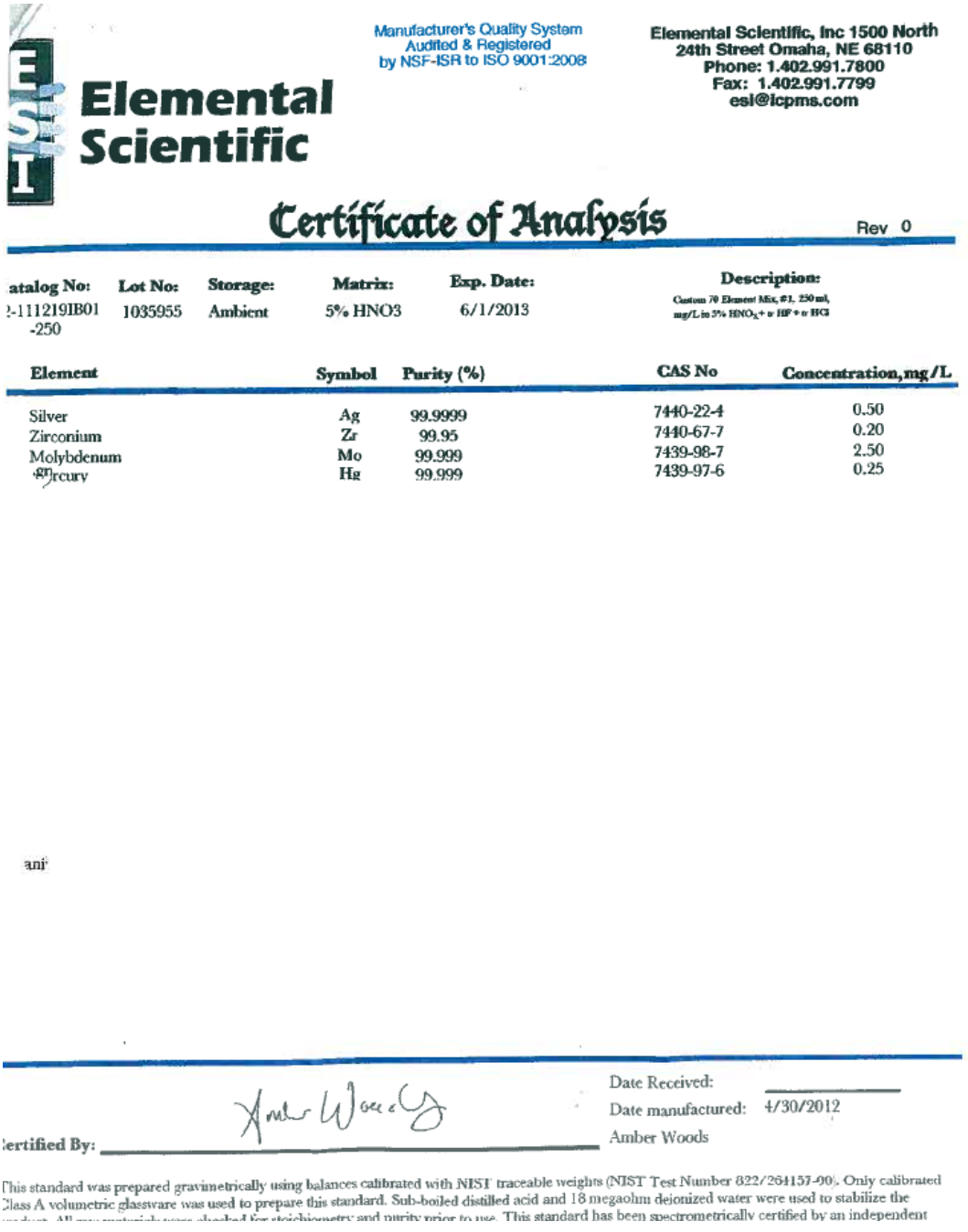
Date Received: _____

Date manufactured: 4/30/2012

Certified By: Amber Woods

This standard was prepared gravimetrically using balances calibrated with NIST traceable weights (NIST Test Number 822/264157-00). Only calibrated class A volumetric glassware was used to prepare this standard. Sub-boiled distilled acid and 18 megaohm deionized water were used to stabilize the product. All raw materials were checked for stoichiometry and purity prior to use. This standard has been spectrometrically certified by an independent source, which is directly traceable to NIST.

Primary solution of 70 elements, producer 2, continued.



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Elemental Scientific, Inc 1500 North
24th Street Omaha, NE 68110
Phone: 1.402.991.7800
Fax: 1.402.991.7799
esi@icpms.com

Certificate of Analysis

Rev 0

atalog No:	Lot No:	Storage:	Matrix:	Exp. Date:	Description:
2-111219IB01 -250	1035955	Ambient	5% HNO3	6/1/2013	Custom 70 Element Mix, #1, 250 ml, mg/L in 5% HNO_3 + tr HF + tr HCl

Element	Symbol	Purity (%)	CAS No	Concentration, mg/L
Silver	Ag	99.9999	7440-22-4	0.50
Zirconium	Zr	99.95	7440-67-7	0.20
Molybdenum	Mo	99.999	7439-98-7	2.50
Mercury	Hg	99.999	7439-97-6	0.25

ani

Certified By: Amber Woods

Date Received:

Date manufactured: 4/30/2012

This standard was prepared gravimetrically using balances calibrated with NIST traceable weights (NIST Test Number 822/264157-90). Only calibrated Class A volumetric glassware was used to prepare this standard. Sub-boiled distilled acid and 18 megaohm deionized water were used to stabilize the

Primary solution of 70 elements, producer 2, continued.

H Total Ion Chromatography of PFASs analyzed

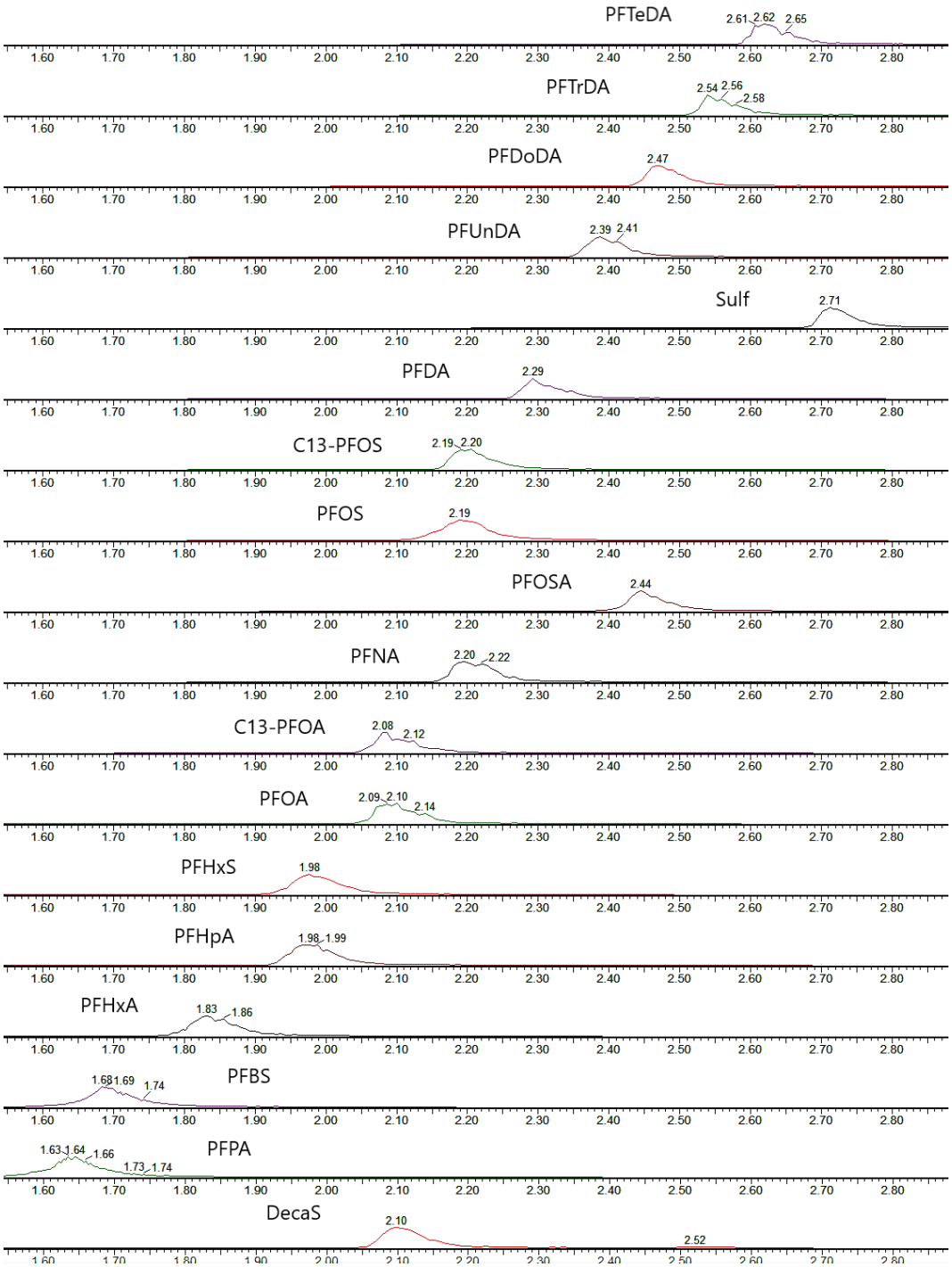


Figure H.1: TIC of the 16 target analytes as well as the two internal standards.

I TIC of DecaS at Descending Concentrations

Calibration concentrations decrease from the top from 50 ppb to 20, 10, 5, 2, 1, 0.5, 0.2 and 0.1 ppb.

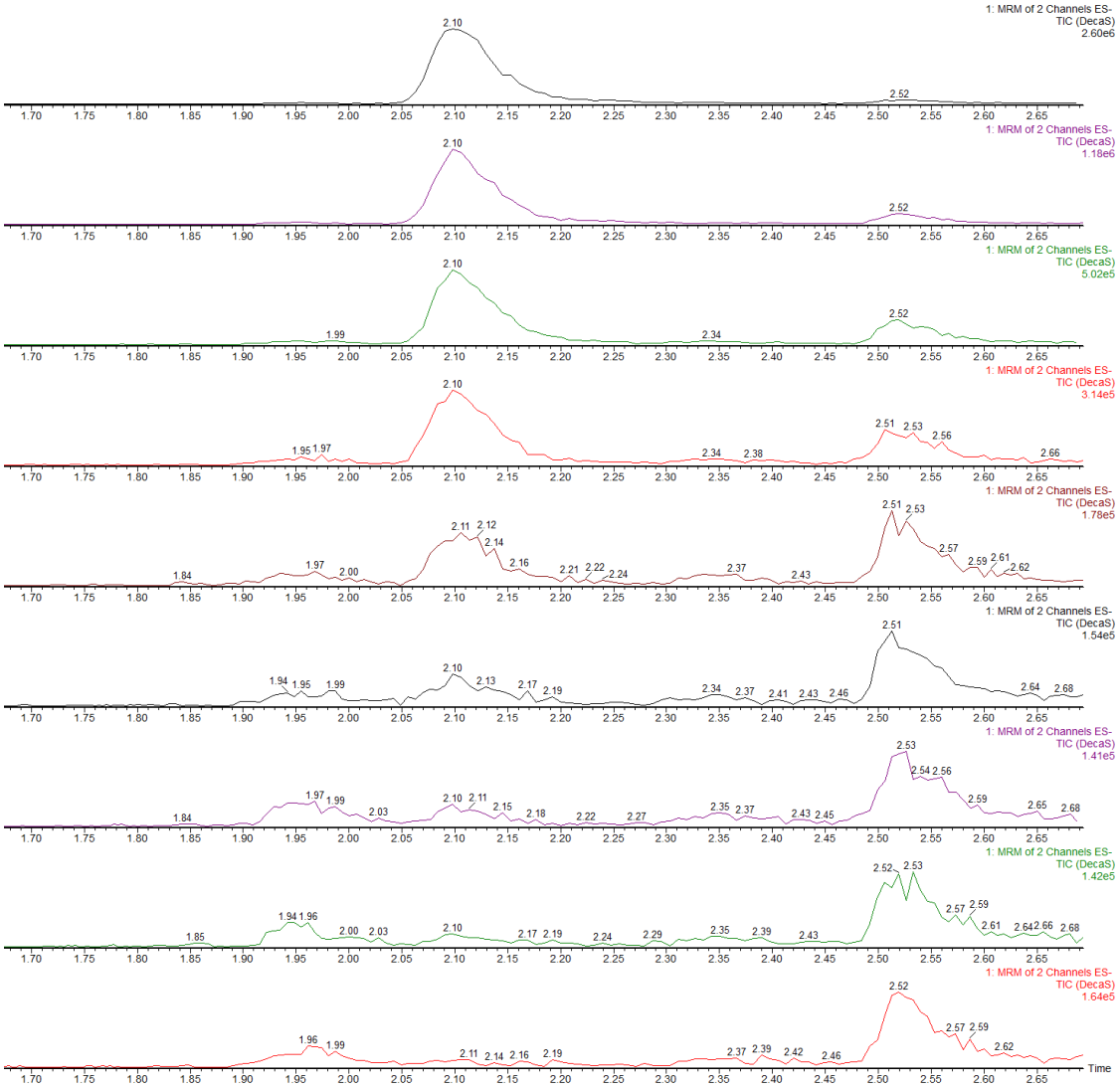


Figure I.1: Nine chromatograms of DecaS at decreasing spike concentrations.

J Calibration Curves of all Target Analytes

Concentrations of calibration solutions (ppb) are on the first axis and area ratios (area TA/area IS) of chromatograms of calibration solutions are on second axis.

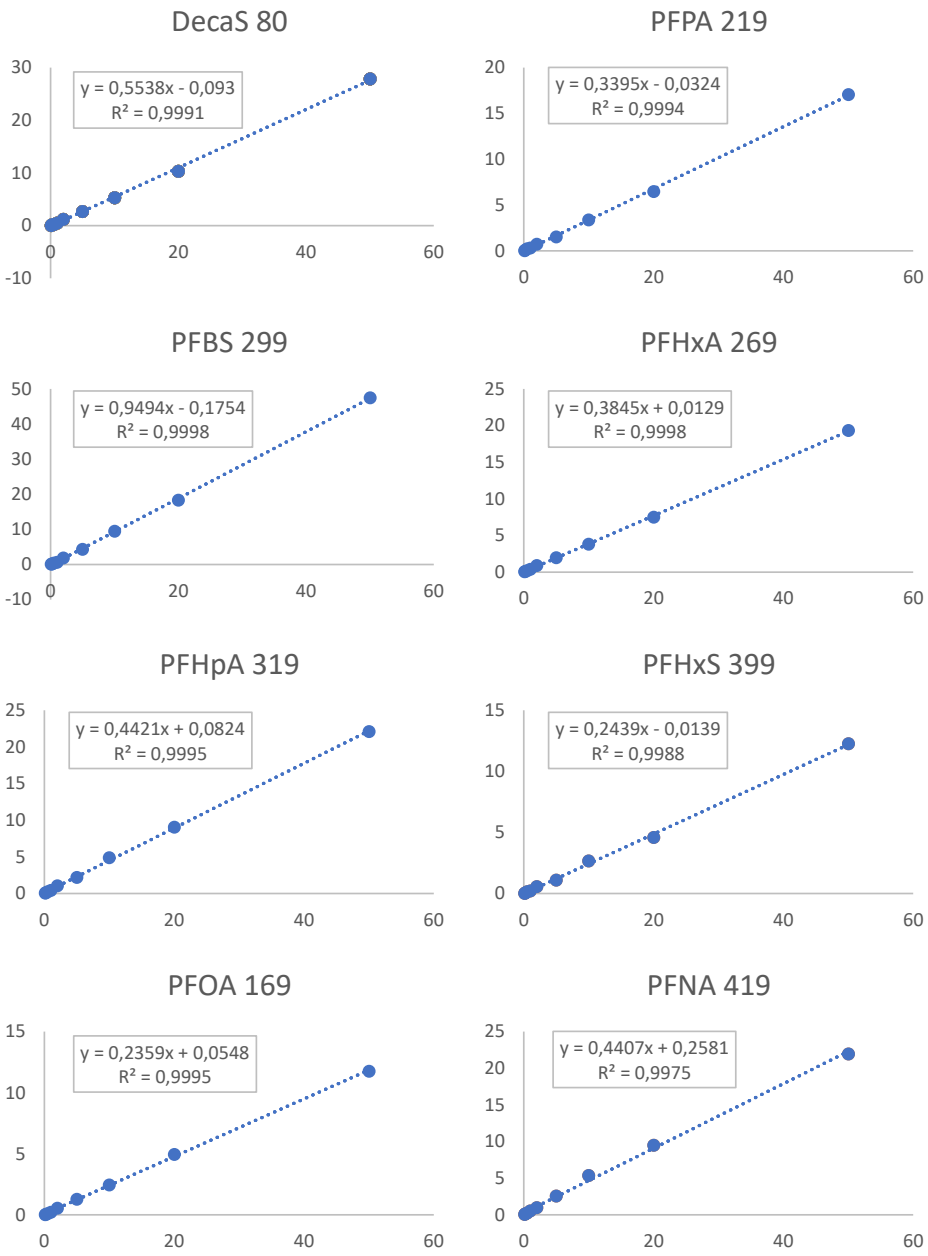
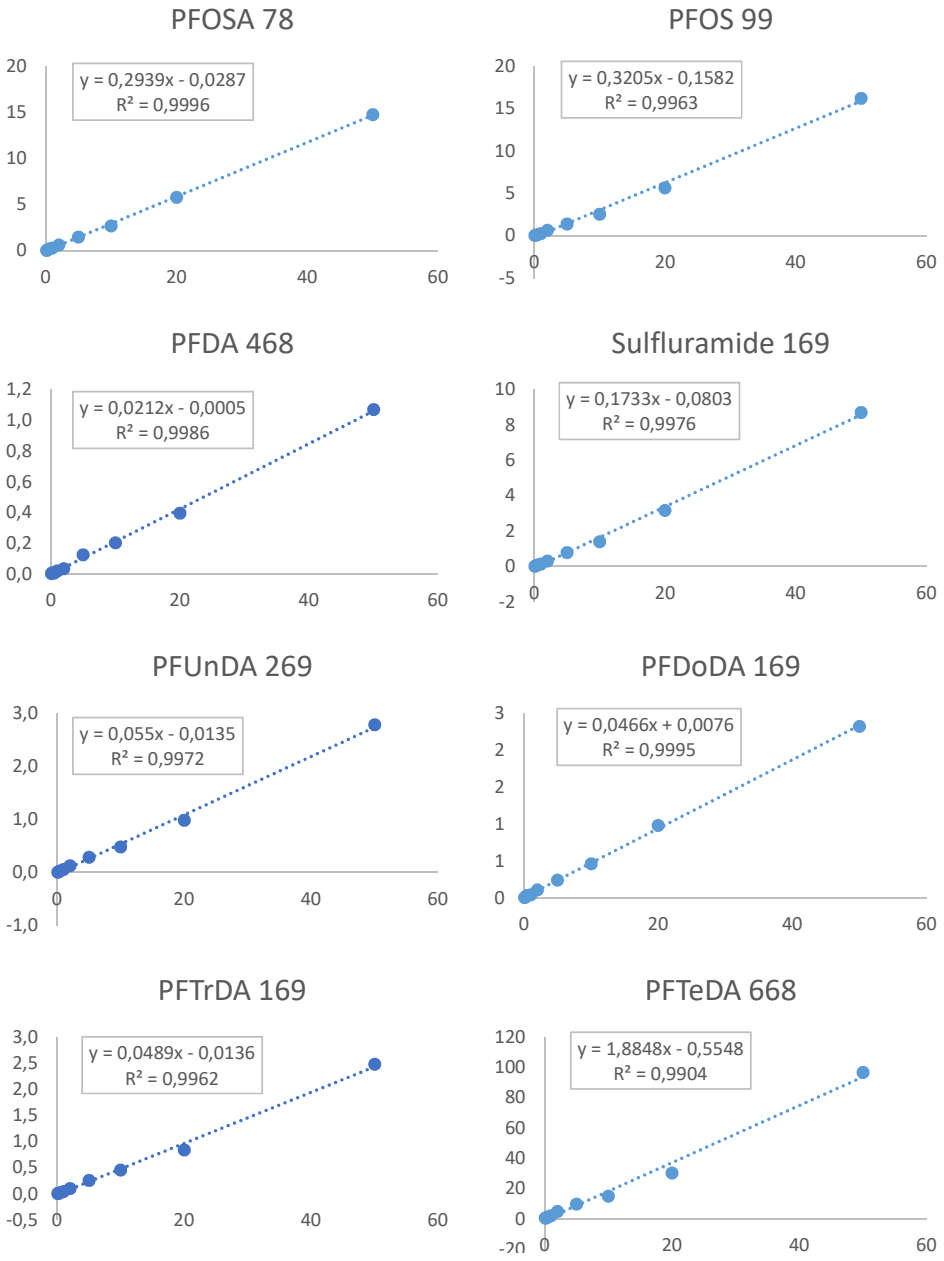


Figure J.1: Calibration curves of first 8 TAs.



Calibration curves of last 8 TAs.