MASTER THESIS IN HUMAN NUTRITION

MAY 2014

Runa Borgund Barnung

Seafood Intake and Serum Levels of Polychlorinated Biphenyls in Mother and Child

INSTITUTE OF MEDICINE, UNIVERSITY OF BERGEN (UIB)

NATIONAL INSTITUTE OF NUTRITION AND SEAFOOD RESEARCH (NIFES)

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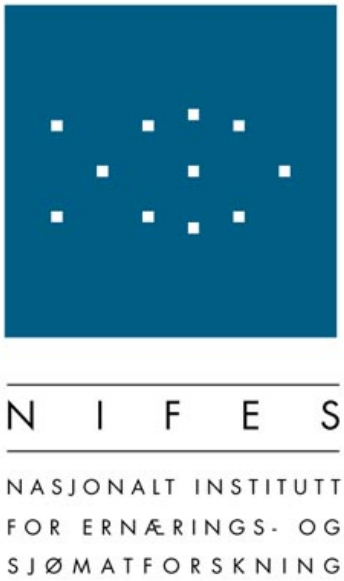

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Bergen 15.05.2014

Runa Borgund Barnung

Abstract

Background and objectives

Seafood is considered a natural part of a healthy and balanced diet. Seafood is an excellent source of high quality protein, and contains many essential nutrients like omega-3 fatty acids, vitamin D, B_{12}, iodine, and selenium. At the same time it contains some environmental contaminants, such as polychlorinated biphenyls (PCBs), which may have undesirable health effects. The risk-benefit of seafood is therefore under debate, with focus on oily fish in particular. Based on this public debate the Norwegian Directorate of Health recently advised young, as well as pregnant women, to consume no more than two meals of oily fish per week over longer periods of time. This might reduce the foetal exposure to PCBs that can have a negative effect on development. The National Institute of Nutrition and Seafood Research (NIFES) did not support this advice, as it was based on contaminant data from 2004, and contaminant levels have decreased since then. In this project mothers' seafood intake during pregnancy and postpartum was assessed, and serum concentrations of PCBs from both mother and child have been determined. The relationship between PCB-concentrations in mother and child and reported seafood intake was then evaluated. Confounding factors for body burden of PCBs, like BMI, age, breastfeeding, and parity, were also examined to see if they had an influence on the PCB-concentrations.

Method

This project was part of the prospective longitudinal population-based study "Nutrition, Mental Health and Infant Development" that took place in the municipality Fjell in Western-Norway, 2010-2012. Blood was sampled from mothers in the 28th gestation week and at 3-, 6- and 12 months after birth from both mother and child. Dietary habits of mothers were assessed by a seafood-food frequency questionnaire (seafood-FFQ), and dietary habits of the children were assessed by a 24-hour recall interview and an interviewer administrated FFQ. The following PCBs: PCB-118, PCB-138, PCB-153 and PCB-180 were extracted from 100 µl serum and analysed by High Resolution Gas Chromatography – High Resolution Mass Spectrometry (HRGC-HRMS).

Results and conclusion

The women in this study population consumed less seafood than the amount recommend by the Norwegian Directorate of Health. All four PCBs were detected in serum from both mother and child, at levels considerably lower than other European countries. Whereas breastfeeding in itself had little impact on the serum PCB-levels in mothers, breastfed children had increased

serum PCB-levels compered to not breastfed children. The serum PCB-levels increased with age and parity in non-breastfeeding and breastfeeding women respectively. Fish liver consumption was the only seafood that decisively had an impact on serum PCB-levels. When excluding fish liver consumers there were no relationship between maternal fish intake and serum PCB-levels in mother or child. Pregnant and young women should therefore increase their seafood intake to achieve the overall beneficial health effects, but avoid consumption of fish liver.

Sammendrag

Bakgrunn og mål
Sjømat er en naturlig del av et balansert og sunt kosthold. Sjømat er en meget god kilde til proteiner av høy kvalitet, og inneholder også mange essensielle næringsstoffer som omega-3 fettsyrer, vitamin D, B_{12}, jod og selen. Samtidig vet man at sjømat inneholder kontaminanter, som polyklorerte bifenyler (PCB), og disse kan ha en negativ helseeffekt. Det er derfor mye debatt rundt risiko-nytte analyser av sjømat, med spesielt fokus på fet fisk. Fet fisk spises regelmessig av mange, men inneholder noe PCB. Basert på denne debatten anmodet Helsedirektoratet nylig gravide og unge kvinner om å ikke spise mer enn to måltider fet fisk i uken over tid. Dette kan bidra til å redusere fosterets eksponering for PCBer som kan ha en negativ effekt på utviklingen. Nasjonalt Institutt for ernærings- og sjømatforskning (NIFES) støttet ikke denne anmodningen siden den er basert på data fra 2004, og nyere data viser lavere kontaminantnivå i dag. Dette prosjektet tar for seg mødres sjømatinntak i svangerskapet, og etter fødselen, og undersøker PCB-konsentrasjoner i serum hos både mor og barn. Konsentrasjoner av PCBer i mor og barn ble så sett på i sammenheng med fiskeinntaket. Mulige faktorer som kan påvirke PCB-konsentrasjonen, som BMI, alder, amming og tidligere barn, ble også sett nærmere på.

Metode
Denne studien er en del av den prospektive longitudinelle populasjonsbaserte studien ”Kosthold, Mental Helse og Spedbarns Utvikling” som foregikk i Fjell-kommune i Vest-Norge, 2010-2012. Blodprøver ble samlet inn fra mor i uke 28 i svangerskapet, og ved 3-, 6- og 12 måneder etter fødselen for både mor og barn. Spisevanene til mødrene ble undersøkt ved hjelp av sjømat-frekvensskjema, og spisevanene til barna ble undersøkt med 24-timers intervju metoden og et intervjuer administrert matfrekvensskjema. De følgende PCBer: PCB-118, PCB-138, PCB-153 og PCB-180 ble ekstrahert fra 100 µl serum og analysert med High Resolution Gas Chromatography – High Resolution Mass Spectrometry (HRGC-HRMS).

Resultat og konklusjon
Sjømatinntaket i denne studiepopulasjonen var lavere enn det som er anbefalt av Norske helsemyndigheter. Alle de fire PCBene ble funnet i serum hos mor og barn, men nivåene var betraktelig lavere enn nivåer i andre europeiske land. Ammefrekvens hadde liten effekt i seg selv på serum PCB-nivåer i mor, mens ammede barn fikk en økning i PCB-nivåer sammenlignet med barn som ikke var ammet. Serum PCB-nivåene i mor økte med alderen og ble redusert hos

de som hadde biologiske barn fra før i henholdsvis ikke-ammende og ammende mødre. Fiskelever var eneste sjømatkilden som påvirket serum PCB-konsentrasjonene. Ekskluderer man de som spiser fiskelever var det ingen sammenheng mellom sjømatinntak og serum PCB-konsentrasjoner hos mor eller barn. Gravide og unge kvinner bør derfor øke sjømatinntaket for å oppnå de gunstige helsefordelene, men samtidig holde seg unna fiskelever.

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Abbreviations

Ah:	Aryl hydrocarbon
ALSPAC:	Avon Longitudinal Study of Parents and Children
BMI:	Body mass index
b.w.:	body weight
CHD:	Cardiovascular heart disease
CRM:	Certified reference material
DHA:	Docosahexaenoic acid
dl-PCB:	dioxin-like Polychlorinated Biphenyls
DPA:	Docosapentaenoic acid
EEA:	European Environment Agency
EFSA	European Food Safety Authority
EPA:	Eicosapentaenoic acid
EU:	European Union
FFQ:	Food Frequency Questionnaire
HRGC-HRMS:	High Resolution Gas Chromatography/High Resolution Mass Spectrometer
IQ:	Intelligence Quotient
ISTD:	Internal standard
LOD:	Limit of Detection
LOQ:	Limit of Quantification
MoBa:	the Norwegian Mother and Child Cohort; den Norske Mor-Barn Undersøkelsen
n.dl-PCB:	non dioxin-like Polychlorinated Biphenyls
NIFES:	National Institute for Nutrition and Seafood Research; Nasjonalt Institutt for Ernæring og Sjømat
NMID:	"Nutrition, Mental Health and Infant Development"
NSD:	Norwegian Social Science Data Service
OH-PCB:	Hydroxylated Polychlorinated Biphenyls
PCB:	Polychlorinated Biphenyls
PCB-118:	2.3'.4.4'.5-PCB
PCB-138:	2.2'.3.4.4'.5'-PCB
PCB-153:	2.2'.4.4'.5.5'-PCB
PCB-180:	2.2'.3.4.4'.5.5'-PCB
PCDD:	Polychlorinated dibenzo-p-dioxin
PUFA:	Polyunsaturated fatty acids
REC West:	Regional Committees for Medical Health Research Ethics
RKBU West:	Regional Centre for Child and Youth Mental Health and Child Welfare
rpm:	revolutions per minute
RSTD:	Recovery standard
Rv95:	95th percentile reference values
SEF:	The Government's advice for nutrition and physical activity; Statens råd for ernæring og fysisk aktivitet
Seafood-FFQ:	Seafood Food Frequency Questionnaire
T3:	Triiodothyronine
T4:	Thyroxine
TCDD:	2.3.7.8-tetrachlorodibenzo-p-dioxin
TEF:	Toxic Equivalent Factor
TEQ:	Toxic Equivalent
THS:	Thyroid stimulating hormones
TWI:	Tolerable Weekly Intake
VKM:	Norwegian Scientific Committee for Food safety; Vitenskapskomiteen for Matrygghet
WHO:	World Health Organisation
WMA:	The World Medical Association

1. Introduction

1.1. Background

In the summer of 2013 several Norwegian newspaper articles concerning environmental contaminants in fish and their impact on human health were published (Aftenposten, 2013; VG, 2013). The focus was especially on consumption of oily fish among young or pregnant women, as well as children. Since it is believed that it is in the foetus and children these contaminants have the potential to do most harm (Lanting et al., 1998c; Patandin et al., 1999; Schantz et al., 2003). The Norwegian Health Authority clarified after this that young, as well as pregnant women should limit their consumption of oily fish to two meals per week over time (The Norwegian Directorate of Health, 2013). This statement is based on a report from 2006, that use data from 2004 (VKM, 2006). Since this report came out some of these environmental contaminants have declined in nature. The introduction of more vegetable based feed to Norwegian farmed salmon has also contributed to lowering the content of PCBs in this fish (NIFES, 2013a). Based on this the National Institute of Nutrition and Seafood Research (NIFES) stated that "The present content of environmental pollutants in fatty fish does not support a limit of two meals of fatty fish a week for pregnant women" (NIFES, 2013c).

It is important to know what level of contaminants actually exist in the Norwegian population. Only when these levels are established is it possible to say something about the potential harm from these contaminants in seafood. In this project mothers' seafood consumption during pregnancy and postpartum was assessed, and serum concentrations of polychlorinated biphenyls (PCBs) from mothers' and their children have been determined. The relationship between these PCB-concentrations and reported seafood consumption was then evaluated. Potential confounding factors to the serum PCB-concentrations were also explored.

This project followed mothers and children from pregnancy to 12 months after birth and focused mainly on seafood and serum PCB-levels, and their respective positive and potential negative effect on mother and child in this period.

1.2 Seafood

Seafood, including fish, contains important nutritional compounds that are beneficial to human health. However, it can also contain undesirables such as environmental contaminants and other substances with a potential negative impact on health. The level and types of nutrients and contaminants in the seafood depends on many factors, such as age, life stage (developmental, reproductive), species, feed, season and location (VKM, 2006).

1.2.1 Positive nutritional contributions from seafood

Fish is an excellent source of high quality protein, containing all the essential amino acids, with an average of 15-20 g protein/100 g fish (VKM, 2006). Oily fish (>8g fat/100g filet), is the most important dietary source of vitamin D and the marine omega-3 polyunsaturated fatty acids (PUFA) in the Norwegian diet (VKM, 2006). Other important nutrients in fish are especially iodine, but also B12, iron, and selenium (Nasjonalt Råd for Ernæring, 2011). Cod liver oil also contain a substantial amount of vitamin A. However, the content of vitamin A Norwegians get from fish alone is not significant (VKM, 2006).

Vitamin D is fundamental for maintaining normal blood levels of calcium and phosphate. This is important for bone mineralization, muscle contraction and nerve function (FAO/WHO, 2001a; Pludowski et al., 2013).

The marine omega-3 polyunsaturated fatty acids (PUFAs) are eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). These are important for foetal growth and neurological development. About 50% of the human brains dry weight consists of lipids, and about 50% of these lipids are phospholipids with high proportions of DHA (Innis, 2003). In the third trimester of pregnancy the foetus alone consumes 67 mg/day of omega-3 PUFA (Innis, 2003). It is therefore important that the mother have an intake that covers both her and the foetus's need for omega-3 PUFAs. DHA is also important for proper eye sight as it is the main PUFA in the outer parts of the rods and cones in the eye's retina, it is therefore vital for transmission of visual signals (Innis, 2003). High intake of marine omega-3 PUFAs (DHA and EPA) during pregnancy has also been linked to a lower risk of postpartum depression (Markhus et al., 2013b; Vaz et al., 2013).

Iodine is a component of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). It is essential for brain development, growth and metabolism, not only for the foetus, but throughout life (FAO/WHO, 2001b). The foetus is entirely dependent on the mother's thyroid hormone supply for the first trimester, and it is therefore important that the mother has an adequate level of iodine in this period (Zimmermann, 2009). Bath et al. (2013) found that mothers with low iodine-to-creatinine ratio were more likely to have children in the lowest quartile of the verbal intelligence quotient (IQ), reading accuracy, and reading comprehension. This study included omega-3 PUFA as a confounder, and still found the same results.

There are some studies that focus on fish consumption solely and find positive effects from this in mothers and children. Neurodevelopment in children is positively associated with mothers' seafood intake, even when corrected for negative effects from mercury in the same study (Suzuki et al., 2010). Different results were found in an American cohort, where visual cognition in infants was better among children of mothers' with a high fish consume only when the fish was low in mercury (Oken et al., 2005). Williams et al. (2001) found that children of mothers that consumed oily fish at least once every 2 weeks during pregnancy had better visual stereoacuity at 3.5 years than children from mothers that did not consume oily fish. In the same cohort (Avon Longitudinal Study of Parents and Children (ALSPAC)), it was found that a higher fish intake by mothers during pregnancy was associated with higher mean developmental scores. Children that consumed fish once a week at 6 and 12 months also had a slightly better mean developmental score than those that did not consume fish (Daniels et al., 2004). Mothers who consumed fish less than 3 times per week were found to have increased risk of their child being in the lowest quartile of verbal IQ (Hibbeln et al., 2007). This risk increased with sinking amount of fish consumed. Fish consumption (1-3 times per week) has also been associated with less anxiety during pregnancy (Vaz et al. 2013). High consumption of especially lean fish can also seem to dercrease the risk of giving birth to a small baby (<2500g) (Brantsaeter et al., 2012). However intake of, especially, oily fish have been linked with prevention of cardiovascular heart diseases (CHD) and decreased deaths caused by CHD (Simopoulos, 2002; Whelton et al., 2004; Raatz et al., 2013).

1.2.2 Negative contaminant contributions from seafood

Fish may also contain a certain degree of environmental contaminants that the fish accumulate throughout life. Types of undesirables normally found in fish are methyl mercury, insecticides,

and organic contaminants like polychlorinated biphenyls (PCBs) (VKM, 2006). This project focuses solely on PCBs, which are one of the most prominent contaminants in fish because of their known persistence in nature and their lipid accumulating abilities.

1.3 Polychlorinated Biphenyls

PCBs are man-made industrial chemicals that were first synthesized around 1920 (Crinnion, 2011). PCBs occurs in mixtures and have some chemical qualities that make them useful for industrial purposes. They are known to be resistant to breakdown by acids, bases, and oxidation, and are non-flammable (Broadhurst, 1972). PCBs also have excellent isolating and cooling capabilities and were therefore actively used as heat transfers and cooling agents in electric transformers (Crinnion, 2011). Furthermore, they have been used in electrical appliances, windows, copy paper, pesticides, paints, newsprints, as plasticizers, and as lubricants in hydraulic systems (Ross, 2004).

PCBs consist of two phenyl rings connected to each other (the biphenyl molecule) with 1-10 chlorine (Cl) atoms connected to the phenyl rings (Crinnion, 2011). The chemical formula is $C_{12}H_{10-n}Cl_n$. There are 209 different structures, of these 130 are normally found in different, formerly industrially produced, PCB-mixtures (WHO, 2000). PCBs are split into two groups; the 12 “dioxin-like” (dl-PCBs) and the 197 “non-dioxin-like” (n.dl-PCBs) (Crinnion, 2011). Dl-PCBs share a similar structure and toxicity with polychlorinated dibenzo-p-dioxins (PCDD) (Crinnion, 2011). As illustrated in **Figure 1.1** most PCBs have their Cl-atoms distributed evenly between the two phenyl molecules. Cl-atom in position 2 and 6 makes the PCB an *ortho*-PCB (EEA, 2001). With more than one Cl-atom in these positions the biphenyl is forced into a twisted position. *Non-ortho*-PCBs also exist, but are quite rare (EEA, 2001). PCB-138, -153 and -180 are *ortho*-substituted non-planar PCBs that are also known as phenobarbital-like PCBs (EEA, 2001). It is the Cl-atoms and the carbon-chloride (C-Cl) bond that makes PCBs so environmentally persistent, and gives the molecule its stability against hydrolysis (Matthews et al., 1980; Chu et al., 2006). The more Cl- or other substitutions connected to the C-atoms in the phenyl ring, the greater resistance against biological and photolytic degradation of the molecule. Intrinsic human elimination half-life approximations, that account for effects of ongoing exposure and fluctuating body-weight, for the PCBs studied in this project (**Figure 1.1**) are 9.3 years for PCB-118, 10.8 years for PCB-138, 14.4 years for PCB-153 and 11.5 years for PCB-180 (Ritter et al., 2011).

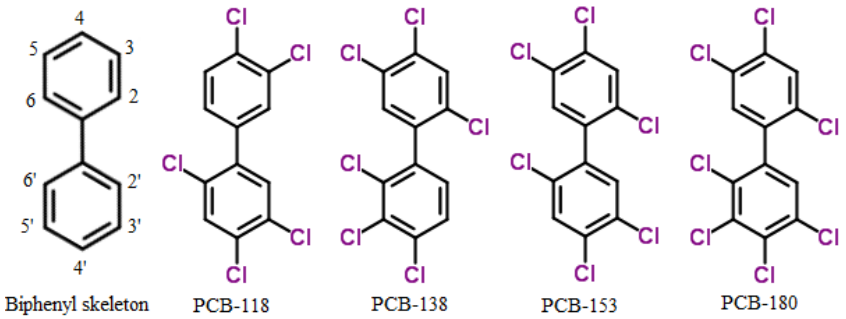

Figure 1.1 Biphenyl skeleton and the PCBs examined in this study (PCB-118, -138, -153, -180)

Source: adapted from ChemSpider

The European Food Safety Authority (EFSA) normally looks at six indicator n.dl-PCBs (PCB-28, - 52, -101, -138, - 153 and – 180) (PCB_6), that normally constitutes 50% of the total n.dl-PCBs found in food (EFSA, 2010). From the analysis of these 6 PCBs they found that PCB-153 and PCB-138 constituted about 50% of these, followed by PCB-180, -28, -101 and -52 in that order. Sometimes PCB_7 is used instead of PCB_6, which include PCB-118. Other studies have also found that the main congeners are PCB-138, -153 and -180, these are therefore suitable to use as indicators on PCB-status, and are often summed to represent half the body burden of PCBs, or doubled after summation to represent the total body burden (Grandjean et al., 1995; Nakamura et al., 2008).

It took about 50 years before the health dangers of using PCBs became apparent. Hence, between 1972 and 1980 a production ban on PCBs was implemented in many countries (EEA, 2001). In 2001 the "Stockholm Convention on Persistent Organic Pollutants" launched an international production ban on PCBs (Crinnion, 2011). It is estimated that 1.3 million tons of PCBs were produced between 1930 and 1993, but the true number is likely even higher (Breivik et al., 2002). To this day there are persistent PCBs in nature because of their long half-life, and also from leakage of PCBs from old appliances that have not been discarded properly (Lu et al., 2012).

PCBs are part of a bigger group of pollutants called persistent organic pollutants (POPs). These all have long half-life's that allows them to persist in the environment and migrate through air, soil, water and sediments, and accumulate in the food chain (Jones et al., 1999). When exposed to higher environmental temperatures POPs tend to transfer to a gaseous phase and can emigrate from the waterbodies and land they have been introduced to into the atmosphere (Jones et al., 1999). When PCBs are anaerobicly degraded in nature, one of the outcomes are declorination,

which might lead to a higher degree of volatility (Chiarenzelli et al., 1996). They can then follow the atmospheric air currents for long, or short, periods of time before they are adsorbed to atmorpherical particles and are deposited (Jones et al., 1999). Deposition can occur when the PCBs get to a higher latitude with colder temperature, or when entering colder climates (Jones et al., 1999). Rain showers can also deposit PCBs back to nature (Jones et al., 1999). This atmospheric transport is the reason behind the widespread distribution and why PCBs can be found in polar regions like the Arctic and Antarctica (Wania et al., 1996). The vaporisation of PCBs is highest when it evaporates toghether with water (Chiarenzelli et al., 1997). In colder climates this water evaporation is lower than in warmer climates, and PCBs are therefore more easily accumulated in these climates.

PCBs are hydrophobic and fat soluble and therefore accumulate in adipose tissue, especially in storage lipids like triglycerides and cholesterol esters which they have a higher affinity for than phospholipids and cholesterol (Lanting et al., 1998b). Generally PCBs are very lipophilic compounds, with increasing lipophilicity following the degree of chlorination (Safe, 1990). PCBs will therefore accumulate in fish and animals, and biomagnify up the food chain when predators eat their prey (El-Shahawi et al., 2010). Some of the highest concentrations of PCBs are found in livers of lean fish and in fillet of oily fish like mackerel, salmon and trout (**Table 1.1**). Seafood is the biggest source of PCB exposure for Norwegians, accounting for 41% of all dioxin-like compound, and 49% of PCB-153 intake (Caspersen et al., 2013). Other sources can be cereals, milk products, and eggs, but PCB exposure from these sources are low among pregnant Norwegians (Caspersen et al., 2013). PCBs are taken up in the human body by enterocytes in the gastrointestinal tract by co-absorption together with dietary lipids in mixed micelles (Dulfer et al., 1996). They are then packed in chylomicrons with resynthesized lipids and transported via the lymphatic system to the blood veins (Kelly et al., 2004; Iqbal et al., 2009). In human infants over 90% of ingested PCBs get absorbed (McLachlan, 1993; Dahl et al., 1995).

Table 1.1 Declining mean concentrations for PCB_7 in Norwegian seafood

	PCB_7 (µg/kg)				
	1994/1995	2000/2001	2006/2007	2008/2009	2010/2011
Atlantic Salmon (farmed)	17	15*	8.9	6.7	5.1
Atlantic Cod, filet (wild)	1	0.6	0.38	0.1	
Atlantic Cod, liver (wild)			113	92	
Greenland Halibut			32	37	23.8
Rainbow Trout (farmed)			12	6.4	(3.1-4.5)[a]
Prawn (without shell)	1	0.4	0.9*	0.8	0.3*

**1994, 2001, 2007, 2009, 2010* *Source:(NIFES, 2013b)*

[a]range of concentrations

Several exposure assessments now point toward declining levels of both dioxins and PCBs in nature and humans (Axmon et al., 2008; Kvalem et al., 2009; EFSA, 2012b). EFSA states that the dietary exposure to dioxins and dl-PCBs has decreased between 16 and 79%, while n.dl-PCBs have decreased between 2 and 75%, depending on the population group, from 2002 to 2010 (EFSA, 2012b). This decrease can also be seen in human breast milk. The concentration of PCB_6 in breast milk from Norwegian mothers is now less than one third of what it was in 1986 (**Figure 1.2**) (FHI, 2009).

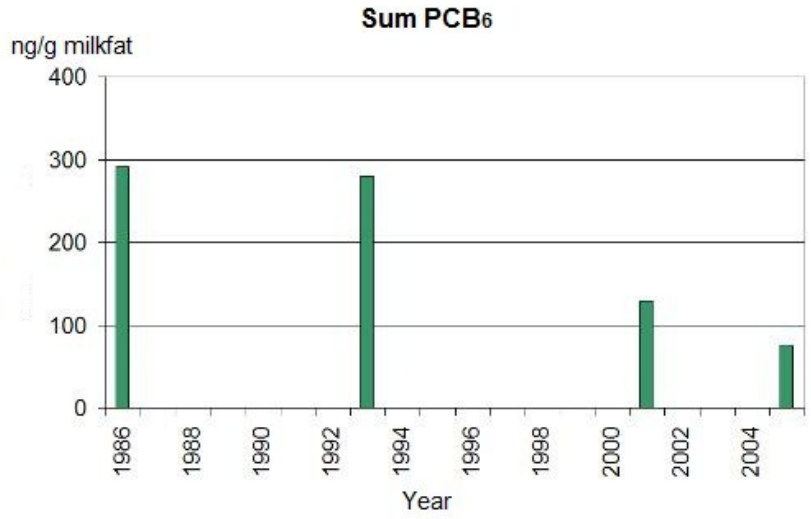

Figure 1.2 Declining PCB_6-concentration in breast milk: Time trend for PCB_6 in Norwegian breast milk from 1986-2005. Before 2005 these are based on a pooled sample, 2005 is median from about 50 individual samples.

Source: (FHI, 2009)

Dioxin and dl-PCBs bind to the same receptor protein, the aryl hydrocarbon (Ah) receptor. This is thought to be the main mediator for the toxic effects from dioxin and dl-PCBs. The Ah-receptor is, after binding to the compounds, transported in to the nucleus where it can affect gene expression that again affects several biological processes (Mimura et al., 2003). The 2.3.7.8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic of the dioxins and is the dioxin that the other dioxin-like compounds are measured against (WHO, 2000). The toxic potency of the compound correlates to its affinity for the Ah-receptor (Ahlborg et al., 1994). This toxicity is expressed in toxic equivalency factors (TEF) in relation to TCDD which has a TEF of 1(Ahlborg et al., 1994). PCB-118, which is examined in this project, has a TEF of 0.0001 (Ahlborg et al., 1994). The TEF of a congener is multiplied with the amount of the congener in a sample, and then added together with the other congeners TEFs to get the total toxic equivalence (TEQ) in the sample.

The most prominent effects from n.dl-PCBs are most likely from disruption of endocrine systems, or components in endocrine systems. These components can be hormones, carrier proteins, metabolic enzymes, receptors, endocrine glands, and feedback regulators (Brouwer et al., 1999). During the metabolizing of PCBs by the microsomal cytochrome P-450 system they are hydroxylated (OH) (Safe, 1993). Some OH-PCBs have shown affinity to endocrine receptors and proteins, and have been shown to compete with estrogen for their receptor (Korach et al., 1988; Connor et al., 1997; Gierthy et al., 1997) and thyroxin for their plasma binding protein (Brouwer et al., 1990; Lans et al., 1993). Endocrine systems that can be disrupted by PCBs are neurodevelopment, reproductive systems, the immune system and induction of endocrine-sensitive tumours (Brouwer et al., 1999). Some PCBs, and intermediate products from the formation of OH-PCB, have been found to covalently bind DNA, making DNA-adducts. These are therefore suspected of being carcinogenic or mutagenic agents (Buff et al., 1989; Dubois et al., 1995; Oakley et al., 1996). Studies on rats also indicate that some PCBs can inhibit calcium uptake in microsomes and mitochondria (Kodavanti et al., 1996).

A tolerably weekly intake (TWI) has been set to 14 pg WHO-toxic equivalence factor/kg bodyweight/week (TEQ/kg b.w./week) for dioxins and dioxin-like compounds (SCF, 2001). In the Norwegian Fish and Game study the median intake of dioxins and dl-PCBs from fish and seafood was as low as 4.7 pg TEQ/kg b.w./week (VKM, 2007). The Norwegian Scientific Committee for Food Safety has concluded that this TWI for dioxins and dl-PCBs will also protect against adverse effects from PCBs including n.dl-PCBs, given the composition and concentrations that were found in Norwegian food in 2008 (VKM, 2008). Epidemiologic studies suggests, that some damage can occur to the neurological and immune systems to foetus and children if the mother has 1 µg or more PCB/g body fat (VKM, 2008). This concentration can be obtained if the mother consumes 40 ng PCB/kg body weight per day for her whole life, or about 20 ng PCB_6/kg b.w. of PCB_6 (VKM, 2008). The median intake per day from the Norwegian Fish and Game study has been calculated to be about 4.3 ng PCB_6/kg b.w. for those with average fish consumption, or about 6.4 ng PCB_6/kg body weight for those that are high consumers of fish (Kvalem et al., 2009). This is inside the estimated weekly average in Europe that ranges between 26.6-80.5 ng PCB_6/kg bodyweight per week for n.dl-PCBs, and 3.5-11.9 pg TEQ/kg bodyweight per week for dioxins and dl-PCBs (EFSA, 2012b).

1.3.1. Health implications from PCBs

In humans the PCBs get transferred prenatally to the foetus through the placenta, and are also secreted postnatally with fat globules in breast milk. Breastfeeding infants can therefore have a daily exposure up to two orders of magnitude higher than adults on a b.w. basis (EFSA, 2005). Postnatal body burden of PCBs will increase with the duration of breast-feeding (Sioen et al., 2013). Most negative health effects are observed from prenatal contamination, likely due to higher neurodevelopmental sensitivity in this period (VKM, 2013). For adults the long term effects can possibly affect reproduction systems, as well as give a possible increased cancer-risk (Brouwer et al., 1999).

Neurologic effects

Several studies have concluded that early exposure to PCBs can be linked to neurological dysfunction and impaired cognitive behaviour in the first years of life (Lanting et al., 1998c; Patandin et al., 1999; Schantz et al., 2003). Some of the neurological outcomes reported from studies considering PCBs are verbal-, numerical-, and short-term memory deficits, visual fixation-time problems, and psychomotor delay (only until 2 years) (Schantz et al., 2003). Jacobsen et al. (1996) found that children of mothers with the highest concentrations of PCBs (at least 9.7 ng total PCBs/ml maternal serum) were more easily distracted, had poorer verbal-, word-, and reading comprehension, and also lower IQ at 11 years of age than those with lower in-utero PCB exposure. These observations were supported by a Dutch study, which documented poorer cognitive skills at 42 months from children of mothers with the highest concentrations of PCBs (Patandin et al., 1999). The perseverance of these negative neurological effects remain unclear as Lanting et al. found neurological effects linked to PCBs at 18 months, but not at 42 months (Lanting et al., 1998c). Patandin et al. (1999) also looked at prenatal dl-PCBs and dioxin effect at 42 months and found no link to cognitive behavioural problems associated with these. A more favourable home environment can seem to combat these outcomes. This can include a higher degree of breastfeeding, as well as parents with a higher education (Patandin et al., 1999; Walkowiak et al., 2001; Schantz et al., 2003).

Maternal OH-PCB-concentrations have been found to be significantly positively associated with thyroid stimulating hormones (TSH) in new-borns (Salay et al., 2009). TSH is important for the production of thyroid hormones (thyroxin and triiodothyronine). These are essential for the neurodevelopment in new-borns. Thyroid hormones are needed for stimulating the

development of neuronal and glial cell proliferation and differentiation (Porterfield et al., 1993). A high TSH indicates low levels of thyroid hormones because the thyroid hormones send a negative feedback to lower the production of THS, and this might suggest underdeveloped thyroid functions (Salay et al., 2009; Hisada et al., 2013).

Birth weight

There are conflicting studies on whether PCBs can be associated with birth weight. In the Norwegian mother and child study (MoBa) a high total PCB concentration in mothers was negatively associated with foetal weight and length (Papadopoulou et al., 2013). However, this negative association decreases with increased seafood intake (Papadopoulou et al., 2013). Similar negative associations were found by a large meta-analysis of 12 European birth cohorts (Govarts et al., 2012). They found that birth weight decreased by 150 g per 1 μg/L increase of PCB-153 in cord-serum blood (Govarts et al., 2012). Contrariwise a smaller Swedish cohort study found that the di-*ortho* PCBs (PCB-138, PCB-153, and PCB-180) were significantly associated with increased birth weight (Lignell et al., 2013). The MoBa study (Papadopoulou et al., 2013) had a much higher number of participants than the Swedish cohort (Lignell et al., 2013) (50 651 to 413). MoBa (Papadopoulou et al., 2013) also analysed a greater number of PCBs (6 n.dl-PCBs and 12 dl-PCBs).

1.4 Risk-benefit assessment

Risk assessment is a systematic method that aims at quantifying the risk of a known hazard. Risk assessment of contaminants in food is dependent on both how humans get exposed for the contaminants, via food and other routes, and the contaminants potential for causing adverse health effects (Alexander J et al., 2012). The risk is then the probability for adverse health effects to occur at a set exposure (Alexander J et al., 2012).

Risk-benefit assessments study the beneficial health effects of some substances in fish and seafood and confronts the observed benefits with the negative outcome from other substances. This is difficult, and depends not only on the different nutrients and contaminants in fish as mentioned above, but also on how a person absorbs nutrients and contaminants, as well as genetics, and overall diet. Amount and type of fish eaten over time is what will determine if the fish has a positive or negative effect on human health. The toxicity is a consequence of the body burden that is built up over time, more than recent dietary exposure (VKM, 2006).

Risk-benefit assessments can be carried out on nutrients and contaminants in fish and seafood by comparing tolerable upper levels (UL) and nutrient recommendations for the nutrients and tolerable weekly intakes (TWI) for contaminants with national intake estimates (VKM, 2006). The UL states the nutrient level that a person can ingest without risking a negative health effect. The TWI is set to give good protection against toxic effects and is based on the lowest dose that does not cause a toxicological effect in animal studies. Sensitive groups may have different assessment than the general population, and it is important to separate out these groups.

Several risk-benefit assessments have been undertaken for seafood by different countries (Fødevaredirektoratet, 2003; Scientific Advisatory Committee on Nutrition, 2004; EFSA, 2005; VKM, 2006; Institute of Medicine, 2007; Livsmedelsverket, 2007). The various assessments concluded that fish consumption inside the recommendations are beneficial for human health, and especially some sensitive groups, like pregnant women, are advised to include fish in their diet. However, pregnant women are recommended to take special precautions for some seafood products.

1.5 Dietary advice for pregnant and breastfeeding women concerning seafood intake

To ensure the recommended daily dosage of DHA and EPA, pregnant and breastfeeding women should consume seafood (The Norwegian Directorate of Health, 2001; 2009). In 2013 the Norwegian Directorate of Health specified that young and pregnant women should stay within 2 meals of oily fish per week over time (The Norwegian Directorate of Health, 2013). Based on newer research NIFES have made a statement saying that it is safe to consume more than two meals of oily fish per week, even for pregnant women (NIFES, 2013c).

The Norwegian Food Safety Authority provides dietary warnings for the public based on health risk assessments conducted by the Norwegian Scientific Committee for Food Safety (VKM) (VKM, 2006). Seafood with higher concentrations of environmental contaminants exist. The general public and especially vulnerable groups should limit their intake of these. For pregnant women this includes all pike and perch over 25 kg, trout over 1 kg, arctic char over 1 kg, Greenland halibut over 3 kg, brown crabmeat, shark, swordfish, fresh tuna, whale meat, seal meat, fish liver, bread spreads made from fish liver, and seagull eggs (The Norwegian Directorate of Health, 2009; The Norwegian Food Safety Authority, 2010; 2011a;, 2011b).

Fish liver is traditionally eaten in many coastal communities, and is the main ingredient in roe liver pate products such as Svolværpostei and Lofotpostei. Fish liver and seagull eggs are especially high in PCBs. From the MoBa study it was found that pregnant women who consumed seagull eggs and fish liver products had a much higher level of PCB-153 compared to those that did not consume them (Caspersen et al., 2013). Oily fish alone did not cause a disturbingly high intake of PCBs in the MoBa study (Caspersen et al., 2013).

1.6 Dietary advice for infants

Infants should be exclusively breastfed for the first 6 months, after 6 months other foods can be introduced to keep up with the child's nutritional needs (The Norwegian Directorate of Health, 2001). It is recommended to continue breastfeeding throughout the first year. Through breast milk the child will acquire all the nutrients needed, including omega-3 PUFAs, however contaminants will also be transferred from mother to child through breastfeeding (VKM, 2013). VKMs recent risk-benefit assessment for breast milk states that the benefits from breast milk clearly outweigh any risk of impaired neurodevelopment that might come from contaminants in breast milk (VKM, 2013).

Infant formula is an alternative available for mothers that are not able to breastfeed, or if the child needs more milk than the mother is able to supply (VKM, 2013). Tests on infant formula do find some PCBs there, but the concentrations are low, and the estimated dietary exposure is lower for formula fed infants than breastfed infants (Weijs et al., 2006; Loran et al., 2009; Pandelova et al., 2011; EFSA, 2012a). When other foods get introduce these will also contribute to the intake. For PCB it is particularly the consumption of fish and some food jars that contribute, but still the intake estimate is lower than for those that are exclusively breastfed (Weijs et al., 2006; Pandelova et al., 2011; EFSA, 2012a).

It is also recommended to give D-vitamin drops or cod liver oil (2.5 ml) to infants from 4 weeks of age to ensure a sufficient vitamin D status (The Norwegian Directorate of Health, 2001). By choosing cod liver oil the infant would also acquire marine omega-3 PUFAs. The amount of cod liver oil should be increased from 2.5 to 5 ml from 6 months. Norwegian cod liver oil is purified for PCBs. In 2010-2011 the concentrations in 14 fish oil samples were measured at NIFES and these were between 0.1 -83 µg PCB_6/kg oil (NIFES, 2011b). These concentrations are therefore well below the 200 µg PCB_6/kg oil limit set by the European Commission (EU, 2006, 01.09.2012; NIFES, 2011b).

1.7 Fish intake in Norway

The Norwegian Directorate of Health recommend eating fish for dinner 2-3 times per week (150-200g/portion), and about 200 g should be oily fish (salmon, mackerel or herring) (Nasjonalt Råd for Ernæring, 2011).

In the national dietary study Norkost 2010-2011 the average consumption of pure fish was 308 g for women and 448 g for men per week (The Norwegian Directorate of Health, 2012). Less than ¼ of the questioned population consumed the recommended 200 g of oily fish per week, with women consuming on average 154 g, and men 203 g (The Norwegian Directorate of Health, 2012). In MoBa the average intake was 252 g/week of total seafood, 140 g/week of lean fish, 84 g/week of fatty fish and 28 g/week of shellfish (Brantsaeter et al., 2012).

Norwegians also use fish as a spread on bread. The normal choices are mackerel in tomato sauce, smoked salmon, shrimps or fishcake. One portion of fish as bread spread is approximately 25 g, thus 6 portions of fish spread equals one dinner portion.

1.8 Dietary assessment

There are different ways to assess food intake among a study population. The most commonly used methods are dietary records, recall interviews and FFQ (Block, 1982; Stumbo, 2013). Dietary records, with weighed or measured intake, are often considered the most accurate approach for the intake of the period recorded. Recall interviews can be for 24-hours or a certain number of days. Here the exact intake over the questioned period is recorded by an interviewer. 24-hour recall is easy to recall with greater accuracy, whereas longer periods of recall give greater uncertainty (Block, 1982). However dietary records and recall interviews are not always representative for the typical long term intake. FFQs were developed to get a better overview of the long term intake in a study population (Stumbo, 2013). These can be based on frequency of food consumption in a year or for shorter periods. It is important to get the FFQ validated to be sure that the reported intake is in correlation with the actual intake. Different biomarkers measured in blood are often used for this purpose.

1.9 Aims of the study

This project is part of a study that focuses on the importance of seafood consumption during pregnancy and the nursing period. There are few studies on PCB-levels in infants. Serum samples from both mother and child were used in this project to determine the concentration levels of PCBs in a Norwegian coastal community.

Main hypothesis of this project:

- Maternal fish consumption increase serum PCB-concentrations in mother and child.

Specific aims of the project are:

- How is the seafood consumption in this study population during pregnancy and postpartum compared to the recommendations?
- Is it possible to detect PCB-118, -138, -153 and -180 in very small volumes of serum from mother and child?
- What are the current levels of PCB-118, -138, -153 and -180 in serum for a selected group of Norwegian mothers and their children?
- Do the maternal serum concentration of PCB-118, -138, -153 and -180 correlate with the serum concentration in the child?
- Does BMI, age, parity and breastfeeding have an impact on serum levels of PCB-118, -138, -153 and -180?
- Do mothers consuming fish liver or roe liver pate, elevated in POPs, have a higher serum concentration of PCB-118, -138, -153 and -180?

2. Materials and Methods

2.1 Design

This project was part of a prospective longitudinal population-based study called "Nutrition, Mental Health and Infant Development" (NMID). The aim of the study was to assess associations between seafood intake, maternal mental health, and infant development. The study took place in the municipality Fjell outside Bergen in Norway, and was a collaboration between the Regional Centre for Child and Youth Mental Health and Child Welfare (RKBU West), Uni Health and the National Institute of Nutrition and Seafood Research (NIFES).

The project fulfilled the Declaration of Helsinki ethical principles for medical research involving human subjects as presented by The World Medical Association (WMA) in 1965, and revised in 2008 (WMA, 2013). The project was reported to and approved by the Regional Committees for Medical Health Research Ethics (REC West, ref.nr: 2009/570/REC. project nr: 083.09) and the Norwegian Social Science Data Service (NSD, ref.nr: 21904). The master project was financed by NIFES and the University of Bergen.

2.2 Participants and recruitment

Pregnant women who were to give birth between 2010 and 2011 in Fjell were the target population for this study. They were recruited by their doctor or midwife between 24th and 28th gestation week at their paediatric clinics, or by their public health nurse at the local health care centre within three months postpartum in the period September 2009 to June 2011 (Markhus et al., 2013a).

No exclusion criteria were given. Every participant gave written consent before inclusion and was free to withdraw from the study at all times without giving any reason. They were also informed that all biological material obtained in the study would be stored in a biobank at NIFES until destruction in 2015, and that all registered data would be retained until 2028.

2.3 Data collection

Dietary data (seafood-FFQ, general FFQ) and biological samples (blood, hair and urine) were collected from the mothers during the 28th gestation week, then at 3-, 6- and 12 months after birth. Blood and hair were sampled from the children at 3-, 6- and 12 months of age. Dietary

recall was done by a 24 hour recall interview at 3-, 6- and 12 months. An interviewer administrated FFQ was also completed at 6- and 12 months (Appendix 1).

2.3.1 Assessment of seafood intake

Self-reported dietary data was reported by the mothers through a validated short seafood-FFQ (Dahl et al., 2011; Markhus et al., 2013a). This is a short questionnaire about habitual fish intake, as well as some general information about the dietary practice. This seafood-FFQ has shown strong correlations between reported intake of seafood and omega-3 supplements to the level of marine omega-3 PUFAs in erythrocytes (Dahl et al., 2011).

The seafood-FFQ was sent out electronically by e-mail and answered through "Questback.com" around the 28th gestation week and at 3 months postpartum. At 6 and 12 months postpartum the seafood-FFQ was posted to the participants as a paper version. The first two seafood-FFQs from 28th week in pregnancy and 3 months after birth were automatically generated as a Statistical Package for the Social Sciences (SPSS), but the last two from 6 and 12 months had to be implemented by hand.

The seafood-FFQ (Appendix-1) included questions about normal intake and frequency of seafood through dinner, snacks, salads and sandwich spread for the last three months. The questionnaire was split into two parts. The first part was about average frequency of seafood consumption. The frequency intervals were from never to over 5 times per week for seafood as dinner and for spreads (used on sandwiches, in salads or as a snack). Five different portion sizes were available to choose from, ranging from half a portion up to three portions. One portion was defined as 150 g of fish. There were two summarized questions on frequency of seafood as dinner and as spread. The type of seafood and spread were then specified in further questions. Open spaces for items not included made it easier for the participants to describe their diet. There was also one question concerning consumption of liver and roe. The second part included questions regarding other dietary sources of PUFAs and vitamin D (butter, margarine, cooking fat). Other questions focused on use of supplements, amount of fruit and vegetables, breastfeeding, medicines, lifestyle (interest in healthy eating, exercising), anthropometric and demographic factors as well as height, weight and previous births. Fish oil intake were separated into liquid or capsules and defined as teaspoon (3ml), child's spoon (5ml) and tablespoon (10ml) or 1-2, 3-4 or 5 or more capsules. The most common Norwegian brand names were also listed.

A seafood index has been established and validated (Markhus et al., 2013a) for the seafood-FFQ used in this study. In this seafood index the ordinal data with reported frequency from the seafood-FFQ was converted to numerical data. The basis for conversion of frequency of seafood consumption into a seafood index can be seen in **Table 2.1**. This made it possible to combine different types of seafood and quantity estimations of seafood consumptions into a numerical index.

Seafood index was established for 5 dinner items: oily fish, lean fish, processed fish, shellfish and freshwater fish, as well as for total seafood spread, and total seafood supplements (including all omega-3 supplements). The 5 dinner indexes were summed to a total seafood dinner index. A total seafood index was then computed as a sum of total seafood dinner index, seafood spread index and supplement index.

Table 2.1 Seafood index: The seafood index - conversion of frequencies to numerical values (Markhus et al., 2013a)

Reported frequency	Numerical interval per week[a]	Seafood index (summary question)[b]	Seafood index (detailed item question)[c]
Never	0	0	0
<1 time/month	>0-0.25	0.15	0.1[d]
1-3 times/month	0.25-0.75	0.5	0.25
1-2 times/week	1.0-2.0	1.5	1
≥3 times/week	≥3	3	3

[a] *Numerical interval based on the average weekly frequency of seafood intake for dinner and seafood intake as spread.*
[b] *Seafood index assigned the average weekly frequency of seafood intake for dinner and seafood intake as spread.*
[c] *Seafood index based on the lowest possible weekly intake of seafood items eaten as dinner and items eaten as spread.*
[d] *Numerical value set to 0.1 to enable distinction between the two lowest frequencies.*

2.3.2 Infant diet

To assess the food intake for infants, a combination of 24-hour recall interviews (3-, 6- and 12 months) and a short interviewer administrated FFQs (6- and 12 months) were given. The same interviewer conducted all the interviews to get the best contingency in the dietary data.

During the 24-hour recall interviews the mother was asked to list everything the infant had consumed for the last 24-hours including supplements (vitamin D, omega-3), and if breast milk, formula or both were given.

The short FFQ had questions about milk (breast milk, formula, and cow's milk), exclusive breastfeeding/formula, introduction to solids, frequency of cereal, dinner, bread, fruit, and seafood.

2.3.3 Blood sampling

Non-fasting venous blood was collected from the elbow cavity of both mother and child at 3-, 6- and 12 months postpartum as well as in 28th gestation week for the mother (Markhus et al., 2013b). The blood was then put into a 3.5 ml BD VacutainerH SSTTM vials II Advanced for preparation of serum that coagulated for 30-60 minutes before centrifugation (1000×g) at 3000 rpm for 10 minutes. The serum was transferred to plastic tubes and stored at -20°C for up to 4 weeks before it was transported to NIFES where it was stored at - 80°C until analysis.

2.3.4 Anthropometric data

Age, height and weight, both before pregnancy and at current date, were included in the seafood-FFQ for the mothers.

Children were weighed naked on an infant scale after birth and at health checks at 3, 6 and 12 months (after the blood sample was taken). Head circumference and length were measured at birth and provided at the 3 month time point. These were also measured at 6 and 12 months.

2.4 PCB-analysis

The determination of PCB-concentrations in human serum is based on a liquid-liquid extraction method developed by Lu et al. (2012) and further quantifications by High Resolution Gas Chromatography-High Resolution Mass Spectroscopy (HRGC-HRMS). The low amount (20-100 µl) of serum sample required for the PCB-analysis was considered the most attractive feature when selecting this particular method. The limited amount of serum from new-borns could be a limitation in PCB biomonitoring, hence the importance of the selected method.

All serum samples were analysed between October 2013 and March 2014, total number of analysed serum samples that passed evaluation can be seen in **Table 2.1**. The specific analytical PCB-congeners assessed in this project were PCB-118 (2.3'.4.4'.5-PCB), PCB-138 (2.2'.3.4.4'.5'-PCB), PCB-153 (2.2'.4.4'.5.5'-PCB) and PCB-180 (2.2'.3.4.4'.5.5'-PCB).

2.4.1. Internal and recovery standards

The internal standard (ISTD) consists of known concentrations of isotopically labelled analytes that is added to the serum samples to find the unknown concentrations through isotope dilution-method (see calculation 2 and 3 in Section 2.6). A recovery standard (RSTD) is added at the

end of sample preparation in order to qualify the extraction process. Preparation of ISTD and RSTD with a chemical-list can be seen in the Appendix-2 **Table A.1.**

2.4.2 Preparation of serum sample

Randomized serum samples were thawed at room temperature before analysis, and kept at -4ºC in between analysis. The thawed serum samples were vortex-mixed for a few seconds before pipetting 100 µl of serum into to a polypropylene plastic container. Aliquots of 20 µl ISTD and 10 µl formic acid were added and the mixture was vortex-mixed for 1 min. An aliquot of 100 µl distilled water and 50 µl acetone was added, and the mixture was vortex-mixed for 1 min. A mixture of dichloromethane/isohexane 1:4 (1 ml) was added and the mixture was vortex-mixed for 1 min before it was centrifuged (1720×g) at 3200 rpm for 2 minutes. The upper-phase was collected and transferred to a plastic tube while the lower-phase was treated with 1ml isohexane, vortex-mixed for 1 min and centrifuged again. This lower-phase with isohexane was then placed in a freezer (-4ºC) for a minimum of 20 minutes. After freezing the sample was once again centrifuged (1720×g) at 3200 rpm for 2 minutes before the upper phase was transferred to the plastic tube together with the previously collected upper-phase. The tubes were placed on a block heater at 35ºC and nitrogen gas was used to vaporize the solution until 1 ml was left. The solution was then transferred to a tapered vial and vaporized to 0.5 ml. Aliquots of 10 µl RSTD and 30 µl of sulphuric acid were added to each sample before they were vortex-mixed for 1 min. The sample was left to stand for 10 minutes before it was vortex-mixed for 30 sec. and then centrifuged (284×g) at 1300 rpm for 2 minutes. The upper phase was then transferred over to a tapered vial containing 20µl nonane and vaporized down to 20 µl in a ventilated hood. List of chemicals is presented in Appendix-2 **Table A.2.**

2.4.3 High Resolution Gas Chromatography/High Resolution Mass Spectrometer

The instrument used to determine the concentration of the various PCB-congeners was the Trace GC Ultra/DFS model from Thermo Fischer Scientific (Waltham, Massachusetts, USA). This is a High Resolution Gas Chromatography/High Resolution Mass Spectrometer (HRGC/HRMS). This instrument combines the separating properties of gas chromatography with the detection and identifying properties of the mass spectrometer (McMaster, 2007).

The gas chromatography process starts with the injection of 1 µl of the sample through the injection port. The sample is heated to 300° C which turns it into a gas before it goes into the

30 m long capillary column ("TR-DIOXIN-5MS" Thermo) with an internal diameter of 0.25 mm. The chromatographic column is coated with a 0.1 µm inert polymer which is the stationary phase. The mobile phase is the carrier gas and consists of helium. Separation of the analytes in the gas depends on the volatility and affinity of the analyte. The higher the volatility the easier the analyte moves with the carrier gas, but with greater affinity to the liquid phase the analyte will be retained and use longer time through the column. From the HRGC the analytes are sent into the HRMS for detection and identification. In the interface module before the analytes reach the HRMS some of the carrier gas is removed by differences in diffusion coefficients between the carrier gas and the analytes (McMaster, 2007). The analytes then go through the ionization chamber where they are pummelled with electrons to turn them into ions. These ions then enter the mass-analyser which contains a double focusing magnetic mass spectrometer (DFS). There are two major parts to the DFS. First the ions get magnetically deflected in the magnetic section before they enter the electrostatic sector where they are focused before they enter the detector (Holcapek, 2014). In the detector the ions get amplified when they hit the first wall. When the ions hit the wall they cause a cascade of electrons that again will start a new cascade as they hit the next wall in the same detector (McMaster, 2007). When this current of electrons gets big enough it can be measured, and the information is sent to a computer where a mass spectrum chromatogram is created. The size of the current is dependent on the amount of ions that hits the detector wall in the first place (McMaster, 2007).The instrument generates a mass chromatogram and traditional chromatograms. The former is a representation of the signal strength against the mass/charge ratio and it is generally known as total ion chromatogram (TIC, **Figure 2.1**), whereas the latter shows the classical signal against time. The chromatograms are created by the Xcalibur / TargetQuan software Version 2.0, 1998-2005.

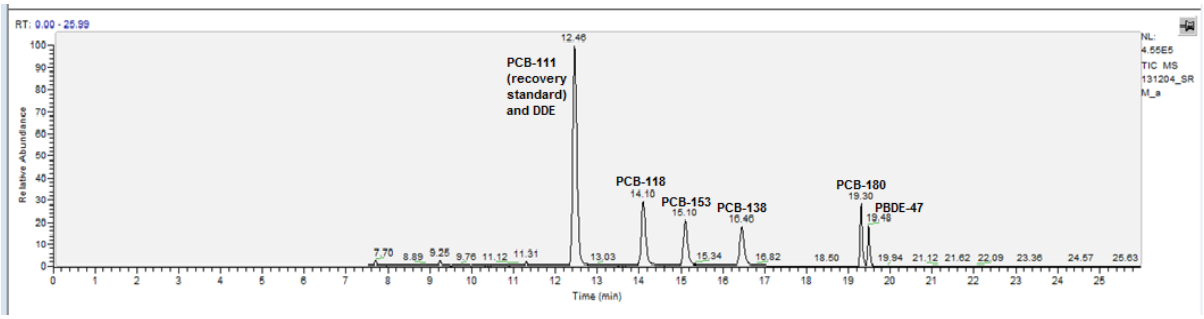

Figure 2.1 Total ion chromatogram of the certified reference material: with PCB-111 (recovery standard), PCB-118, PCB-138 and PCB-153 marked at their chromatogram peak

2.5 Practice in the laboratory

Due to risk of exposure of pathogens, special precaution has to be taken when dealing with human samples. All human samples that are collected by NIFES are risk evaluated by the infection/disease control group before analysis. The people working with human samples undergo theoretical and practical training given by the infection/disease group before they are authorized to work with human samples, as well as offered vaccination against hepatitis A and B.

2.6 Calculations

Body mass index (BMI) was calculated on the basis of height and weight information self-reported in the seafood-FFQ (1).

$$BMI = \frac{weight\ (kg)}{height^2\ (m^2)} \quad (1)$$

Concentrations of PCBs (3) in each serum sample was assessed by isotope dilution and calculated by using the relative response factor (RR) (2) which is the ratio of the slope and were determined from the calibration curve. RR compensates for differences in instrument response and analytical gain. ISTD and PCB are denoted by the subscripted ISTD and PCB.

$$RR = \frac{A_{\mathrm{PCB}}\ x\ C_{ISTD}}{A_{ISTD}\ x\ C_{\mathrm{PCB}}} \quad (2)$$

$$C_{\mathrm{PCB}} = (\frac{(A_{PCB}\ x\ A_{ISTD}\ x\ M_{ISTD})}{RR})/V(ml) \quad (3)$$

K = slopes, A= area under peak, C= concentration, M= mass of ISTD, V= volume of sample

To find the effect size (r) from the Mann-Whitney test, formula (4) is used.

$$r = \frac{z}{\sqrt{\mathrm{n}}} \quad (4)$$

r= effect size, z= standard score, n= number of observations

Cohen's rule of thumb was used to determine the effect size: r=0.10 equals a small effect, r=0.30 is a medium effect and r=0.50 is considered a large effect (Fiels, 2013).

2.7 Method evaluation

Since this is a new method different validation parameters were considered to evaluate the correct application of the analytical method. The parameters evaluated in this project were: selectivity, accuracy, precision, recovery, sensitivity and limit of quantification (LOQ).

Selectivity defines the ability of an analytical method to quantify and separate the analyte from the other sample components (Huber, 2010). This can be evaluated by analysing blank samples of an appropriate matrix and testing it for interference with the analyte. In this project distilled water samples were analysed to ensure that the 100 µl of added distilled water did not interfere with the PCB-concentrations in the serum samples (Appendix 4: **Figure A.8-A.11**). The blank water samples was not retracted from the PCB-concentrations in the serum samples. This was done to give an indication of the uncertainty in the method, and will probably be a part of the uncertainty-calculations when the method is validated.

Accuracy describes how close the mean test results are to the true concentration of the analyte. Precision describes the closeness between individual measurements of an analyte (degree of scatter). The precision of a method also includes reproducibility of the analysis, which can involve different analysts and precision over time (Huber, 2010). Serum Certified Reference Material (SRM 1957, National Institute of Standards and Technology, Gaithersburg, US) was used to check the accuracy and precision of the method (Appendix 4: **Figure A.4-A.7**).

Recovery of an analyte is the detector response from the internal standard and recovery standard compared to the extraction of the analyte in the sample. Although the recovery of a particular method need not be 100%, its range of variation should be specified in advance. For example, in the present project the recovery is set between 30-130%. The selected recovery range is based on previous results from contaminant analyses which were relatively robust inside this range.

Sensitivity is the lowest analytical value that a particular method is able to detect. To establish the sensitivity of a method a calibration standard curve between instrument response and known concentrations of the analytes should be made. The lowest standard on this curve should be accepted as the lower limit of quantification (LOQ). The calibration standards are solutions with known concentrations of the different analytes. Considering that the analytical method used in the present project has not been validated yet, preliminary LOQ-values (**Table 2.2**) were established based on previous results during method development. It is important to mention

that values below the LOQ levels are referred in the present project as limit of detection values (LOD). A LOD value suggests that the analyte was detected, but its associated uncertainty is too high to be considered quantitatively reliable. Therefore the values under LOQ were set to the LOQ-value. This is an example of "upper bound" limit, used to not underestimate the PCB-concentration (FAO/WHO, 2012). Most values under LOQ were just below the LOQ, which suggests that the value was detectable, but the uncertainty around this value is too high to be considered quantitative.

Table 2.2 Limit of quantification (LOQ) values for PCB-118, -138, -153 and -180.

Analyt	PCB-118	PCB- 138	PCB-153	PCB-180
LOQ (ng/ml)	0.01	0.009	0.02	0.01

Mean values are reported for serum samples analysed in duplicate. A particular standard (Standard 3) with a known concentration (Appendix 2: **Table A.3**) was consistently used throughout the HRGC-HRMS analysis to confirm the validity of the calibration curve and consequently the reliability of the computed concentrations at specific days (control card in Appendix 4, **Figure A.12 to A.15**). Standard 3 is the third standard used in the calibration standard curve of the instrument.

The analysed samples were also excluded if the chromatogram showed abnormal elution of compounds of interest. The areal of the peak was then not possible to calculate.

Table 2.3 shows the dietary data collected for mothers and children, as well as the number of serum samples for mothers and children that passed evaluation, and are used in this project.

Table 2.3 Dietary data and analysed serum samples: Listed are number of serum samples analysed for PCB-118, -138, -153, -180 that passed evaluation in this project for mothers at 28th week in pregnancy and for mothers and children at 3-, 6-, and 12 months postpartum. As well as number of Seafood-FFQs collected from mothers, and 24 hour recall interview (3 months), and short FFQs completed for the children in this study population at 3-, 6- and 12 months postpartum

	28th week in pregnancy	3 months postpartum	6 months postpartum	12 months postpartum
Serum samples mothers	61	82	85	73
Seafood-FFQ mothers	55	92	87	82
Serum samples children	-	47	61	52
24h or FFQ children	-	97	94	83

2.8 Statistical analysis

All statistical analyses were carried out using IBM Statistical Package for the Social Sciences (SPSS) Statistics 21 or GraphPad Prism 6.

Figures and tables were made using IBM SPSS Statistics 21, GraphPad Prism 6 or Microsoft Office Excel 2013.

Outliers were examined with "Robust regression and Outlier removal"-test (ROUT-test) and scatterplots. There was little difference in mean and median with and without ROUT-test (Appendix 3: **Figure A.2 and Table A.5**). It is normal to have some biological variations in a population, with some subjects at higher or lower values than others; therefore all the values were used in this project.

Normality was tested with the parametric Shapiro-Wilk test and the non-parametric Kolmogorov-Smirnov test. Distribution was examined with scatterplots and histograms. Dietary seafood intake and PCB-concentrations were not normally distributed, except for total seafood consumption and CRM. These two were therefore analysed with one-way ANOVA (total seafood over time) and t-test (CRM). Non-parametric tests were used for all other statistical analysis and median is therefore used, instead of mean, when non-parametric tests are used.

The results are presented as mean ± standard deviation (SD) and median ± interquartile range (IR). Where appropriate, minimum and maximum values are also provided. Number of significant digits in mean and median values varies according to the SD or IR.

Differences between groups were examined using either the two-sided non-parametric Mann-Whitney U test to look at difference in distribution, or the Kruskal-Wallis test to look at differences in median. Spearman Rank Correlation was used to examine bivariate and partial associations. Friedman two-way test and Wilcoxon Signed Rank test was used to detect differences across several sample concentrations. Pairwise comparison followed Friedman two-way test in those cases where there was a significant difference.

A p-value below 0.05 was used as statistical value of significance in all the tests, whereas a p-value around 0.1 indicates a trend.

3. Results

3.1 Descriptive characteristics of the study population

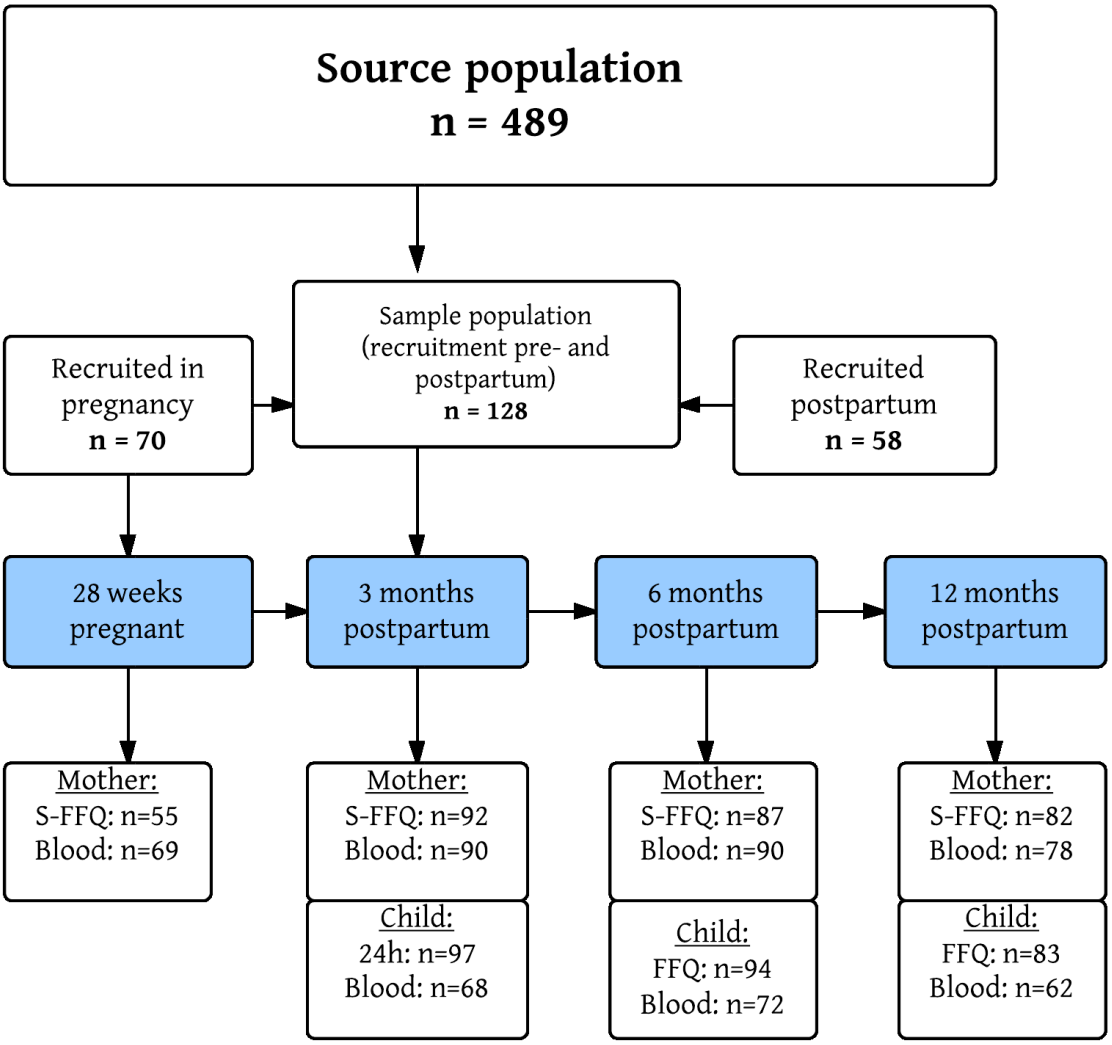

Figure 3.1 Flowchart of study population and collected data: Number (n) of blood samples and dietary data from mothers and children at 28th week in gestation, 3-, 6- and 12 months after birth. Dietary data is reported in seafood-FFQ (S-FFQ) for the mothers, and as 24hour (24h) recall interview (3 months) and short FFQ (6- and 12 months).

Of the 128 women recruited in this study, 70 were enrolled in pregnancy and 58 after birth, but before 3 months postpartum (**Figure 3.1**). There were 27 women and 20 children that had a complete longitudinal dataset with seafood-FFQ and serum samples analysed for PCBs at all the time points.

The characteristics of all the mothers that answered seafood-FFQs at the different time-points are summarised in **Table 3.1**.

The mothers had a significantly higher body mass index (BMI) at 3- and 6 months after birth than before pregnancy (3 months: $p<0.001$, $\chi^2(1)=-1.294$, $n=34$, 6 months: $p=0.039$, $\chi^2(1)=-0.853$, $n=34$). However, there was no difference at 12 months compared to pre-pregnancy weight. The focus on eating healthy in this study population was reflected in 62% reporting a high or very high focus level on having a healthy diet during the pregnancy. After birth (3 months) this high focus on a healthy diet had declined to 53%.

In this study population, between 61- and 71% were educated at a university or university college. This degree of higher education was also evident in the income of this group with more than 70% of the expecting mothers earning over 300 000 Norwegian kroner/year, and over 20% earning more than 400 000 Norwegian kroner/year. Among the partners/husbands over 70% earned more than 400 000 Norwegian kroner/year.

The children had the same mean weight and length at birth at each time-point (**Table 3.2**). The distribution of sexes were also fairly constant with a slightly higher number of boys (54-57%).

Table 3.1 Mothers characteristics: Characteristics for mothers' that answered the Seafood-FFQ at 28th gestation week and 3-, 6- and 12 months postpartum with mean, standard deviation (SD), minimum (min), maximum (max) and number of mothers (n).

Characteristics	28th gestation week	3 months	6 months	12 months
n	55	92	87	82
	Mean ± SD (min, max)	Mean ± SD (min, max)	Mean ± SD (min, max)	Mean ± SD (min, max)
Age (years)	30 ± 5 (19, 41)	30 ± 5 (19,42)	31 ± 5 (19,42)	32 ± 5 (20,42)
Current weight (kg)	76 ± 11 (55, 102)	71 ± 13 (51,109)	70 ± 13 (49,117)	69 ± 13 (48,110)
Pre-pregnancy weight (kg)	67 ± 11 (47,95)			
Height (cm)	168 ± 7 (152,180)	169 ± 6 (152,180)	168 ± 7 (152,180)	168 ± 7 (153,181)
BMI (kg/m2)	24 ± 4 (17,35) [a]	25 ± 5 (17,43)*	25± 4 (17,40)*	24 ± 4 (16,37)
	Count n (%)	Count n (%)	Count n (%)	Count n (%)
BMI category [a]				
Severely underweight (<16)	0 (0)	0 (0)	0 (0)	0 (0)
Underweight (16-18.49)	3 (6)	2 (2)	2 (2)	3 (4)
Normal (18.5-24.99)	32 (59)	46 (52)	43 (51)	43 (52)
Overweight (25-29.99)	15 (28)	27 (30)	31 (36)	30 (37)
Obese class 1 (30-34.99)	3 (6)	10 (11)	7 (8)	4 (5)
Obese class 2 (35-40)	1 (2)	3 (3)	2 (2)	2 (2)
Obese class 3 (>40)	0 (0)	1 (1)	0 (0)	0 (0)
Education				
Lower secondary school	0 (0)	4 (4)	5 (6)	6 (7)
Higher secondary school	16 (29)	28 (30)	29 (33)	25 (30)
< 4 years of university[b]	24 (44)	34 (37)	37 (43)	39 (48)
> 4 years of university[b]	15 (27)	26 (28)	16 (18)	12 (15)
Self-reported smoking/snuff	1 (2)	7 (8)	7 (8)	11 (13)
Exercise				
Never	1 (2)	2 (2)	4 (5)	2 (2)
<1 time/week	5 (9)	6 (7)	5 (6)	9 (11)
1 time/week	13 (24)	13 (14)	3 (3)	11 (13)
2-3 time/week	26 (47)	27 (29)	33 (38)	30 (37)
4-6 time/week	7 (13)	30 (33)	27 (31)	21 (26)
Every day	3 (5)	14 (15)	15 (17)	9 (11)
Focus on a healthy diet				
Small	3 (5)	3 (3)	4 (5)	3 (4)
Medium	18 (33)	40 (43)	41 (47)	37 (45)
High	32 (58)	44 (48)	36 (41)	35 (43)
Very high	2 (4)	5 (5)	6 (7)	7 (8)
Caucasian descent	52 (95)	89 (97)	83 (95)	76 (93)
Primiparous[c]	51 (49%)			

[a] *BMI and the BMI category for 28 weeks is based on weight before pregnancy*
[b] *University or University College*
[b] *Primiparous: first pregnancy. This was the combined answer from question on previous children from all seafood-FFQs; some did not answer this question when they had answered it in a previous seafood-FFQ*
***** *BMI significantly higher than at 28th week in gestation*

Table 3.2 The children's characteristics: Characteristic for children with answered 24hour recall interview or short FFQ at 3-, 6- and 12 months after birth, with number of children (n), mean, standard deviation (SD) and minimum (min) and maximum (max) values

Characteristics	3 months	6 months	12 months
n	97	94	83
	Count n (%)	**Count n (%)**	**Count n (%)**
Sex			
Girls	44 (46)	42 (45)	36 (43)
Boys	52 (54)	52 (55)	47 (57)
Not reported	1	0	0
Twin pairs, n	2	2	2
	Mean ± SD (min. max)	**Mean ± SD (min. max)**	**Mean ± SD (min. max)**
Birth week	40 ± 2 (34, 42)	40 ± 2 (34, 42)	40 ± 2 (36, 42)
Weight at birth (g)	3558 ± 645 (1610, 5140)	3558 ± 645 (1610, 5140)	3558 ± 626 (1640, 5140)
Length at birth (cm)	51 ± 3 (43, 57)	51 ± 3 (43, 57)	51 ± 3 (43, 57)
Current weight (g)	6353 ± 830 (4470, 9000)	8106 ± 935 (6250, 11500)	100057 ± 1170 (7900, 13500)
Current length (cm)	62 ± 3 (53, 68)	68 ± 3 (57, 75)	77 ± 3 (68, 85)

3.2 Maternal seafood intake

Estimations of seafood consumption in grams per week were based on the seafood index for the different seafood products and the specified portion sizes (**Table 3.3**). There was also a question concerning total seafood as dinner, and the estimated median per week there was 225 g, which was consistent over the four time points.

No difference was found between total seafood (dinner, spread and supplement) consumed at the different time points using the seafood index. There was also no difference in seafood intake as dinner, seafood as spread, supplement intake, shellfish, lean fish, roe liver pate (Svolvær or Lofoten), or processed fish. The only category that was significantly different over the time points was oily fish ($p=0.017$, $n=37$, $\chi^2(3)=10.16$) with a higher consume at 6- and 12 months than at 28th week in gestation.

These was no significant differences in the estimated weekly intake of seafood as dinner, spread, oily, lean, processed or shellfish between those that exclusively breastfed and those that

did not breastfeed, except for a higher lean fish consumption at 3 months for non-breastfeeding mothers ($p=0.013$, $r=0.27$, $n_{total}=84$, $n_{non\text{-}breastfeeding}=15$)

Table 3.3 Seafood consumption: Estimated consumption of seafood as grams (g) per week at 28th week in gestation and at 3-, 6- and 12 months after birth. Estimated from median index intake for each seafood category at the different time-points.

Seafood category	28 weeks	3 months	6 months	12 months
	g/week (%)	g/week (%)	g/week (%)	g/week (%)
Oily	53 (22)	56 (22)	83 (28)	79 (26)
Lean	79 (33)	75 (29)	83 (28)	90 (29)
Processed	79 (33)	94 (36)	90 (30)	90 (29)
Shellfish	32 (13)	34 (13)	45 (15)	49 (16)
Total	243 (100)	259 (100)	301 (100)	308 (100)

All the women in the study population consumed seafood for dinner at least once during the last three months (**Figure 3.2**). The majority of the mothers consumed seafood for dinner 1-2 times per week. During pregnancy 71% of the participants had seafood for dinner 1-2 times per week, but only 4% had it 3 or more times per week. Postpartum only 59-60% of the participants had seafood 1-2 times per week, but 12-15% reported consuming fish 3 or more times per week at these stages.

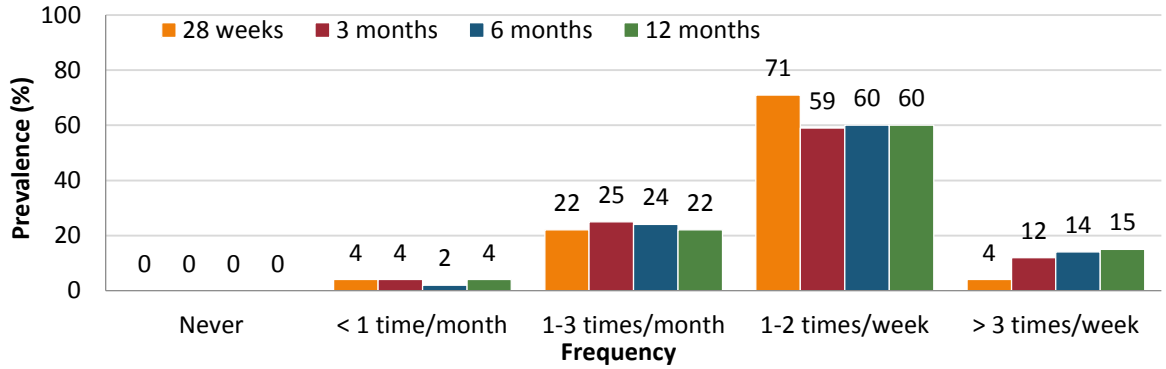

Figure 3.2 Seafood as dinner: Frequency of seafood as dinner for mothers at 28th week in gestation and 3, 6 and 12 months postpartum.

There was little difference in the prevalence of frequencies over time for seafood as spread (**Figure 3.3**). There was a tendency (p= 0.074, $\chi^2(3)=6.94$, n=36) towards increased consumption of spread per week, when looking at the seafood index, from in pregnancy to 12 months postpartum. Over 70% of the participants consistently reported consuming an oily type of seafood as spread (mackerel, herring or salmon) while the remaining 30% only ate the lean type (shrimps, fishcakes).

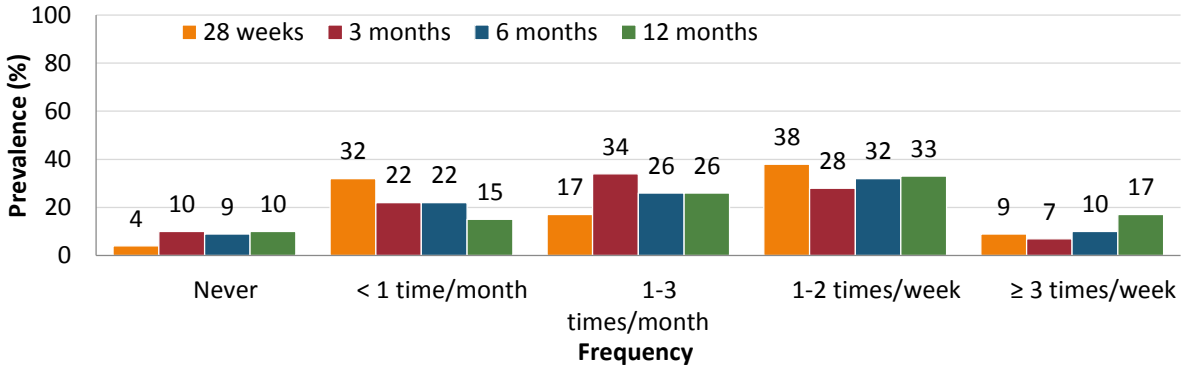

Figure 3.3 Seafood as spread: Frequency of consumption of seafood as spread for mothers at 28th week gestation and at 3, 6 and 12 months postpartum.

At 28th week in pregnancy, 63% of the women reported consuming omega-3 supplements more than 4 times per week. At 3 months after birth, 74% reported using omega-3 supplements more than 4 times per week. After 12 months the omega-3 supplement intake had declined to 44% taking it more than 4 times per week.

There was a significant correlation ($p < 0.05$) between seafood as dinner, total seafood (dinner, spread and supplement) and intake of salmon/trout for dinner against reported focus on a healthy lifestyle in pregnancy (Appendix 3: **Table A.4**). At 3 months a healthy lifestyle was again correlated with seafood as dinner and total seafood intake, which include omega-3 supplement, but not with salmon/trout for dinner. Instead it was now also correlated with intake of cod for dinner, supplement intake and seafood as spread intake. At 6 months it was only salmon/trout for dinner that was correlated with focus on a healthy lifestyle, and at 12 months there was no correlation between any seafood and focus on a healthy lifestyle.

Fish liver was consumed on a yearly basis by 16 mothers in the study population. Roe liver pate consumption was low, peak consumption occurred 12 months after birth, and was then consumed by 7 mothers.

3.3 Children's dietary habits

At 3- and 6 months the majority of the children were not given omega-3 supplements. However, at 12 months over 50% received omega-3 supplements and 37% received omega-3 supplements more than 4 times per week. Between 40 - 50% of the breastfed children got omega-3 supplements at 3- and 6 months, while among the non-breastfed the prevalence was below 40%.

Breastfeeding prevalence decreased from 64% fully breastfed at 3 months to 7% at 6 months, whereas 70% and 33% were still breastfeeding daily at 6 and 12 months respectively (**Table 3.4**). At 6 months 88% had started introducing other foods than formula or breast milk. Of particular interest, seafood as dinner was consumed by 15% of the children at 6 months and 89% at 12 months.

Table 3.4 The children's eating habits: at 3-, 6- and 12 months after birth with number of children (n)

Nutrition	3 months	6 months	12 months
	Total n =97	Total n =94	Total n =83
	n (%)	n (%)	n (%)
Exclusively breastfed	62 (64)	7 (7)	-
Breastfed daily	79 (81)	66 (70)	27 (33)
Not breastfed	18 (19)	28 (30)	56 (67)
Formula	35 (36)	54 (57)	37 (46)
Porridge	-	83 (88)	74 (90)
Other[a]	-	58 (62)	81 (99)
Seafood[b]	-	14 (15)	74 (89)

[a] *Fruit, bread and dinner*
[b] *Seafood as dinner*

3.4 Serum PCB-concentrations

The mean and median of PCB-118, -138, -153 and -180 in ng/ml serum from mother and child are listed in **Table 3.5**. PCB-118 was at the most modest levels, which is reflected by several observations below LOQ. PCB-153 was found to be the most prominent congener, although several values were also here found to be below LOQ. PCB-138 and -180 appears to accumulate at the same levels, and also have fewer values below LOQ than PCB-118 and PCB-153.

Table 3.5 Descriptive of PCBs: Descriptive serum PCB-118, -138, -153, -180 and sum PCB (138+153+180) (Sum PCB) concentrations in mothers at 28th week in gestation and for mothers and children at 3-, 6-, and 12 months, with number of participants (n), mean value in ng/ml serum, standard deviation (SD), median in ng/ml serum, interquartile range (IR), maximum (Max) and minimum (Min) concentrations

	n	Mean (ng/ml)	SD	Median (ng/ml)	IR	Max	Min
PCB 118							
Mother 28 weeks	61	0.03	0.02	0.03	0.02	0.13	1<LOQ
Mother 3 months	82	0.02	0.02	0.02	0.01	0.13	12<LOQ
Mother 6 months	85	0.02	0.02	0.02	0.02	0.16	14<LOQ
Mother 12 months	73	0.02	0.01	0.02	0.02	0.07	16<LOQ
Child 3 months	47	0.03	0.02	0.03	0.02	0.08	6<LOQ
Child 6 months	61	0.03	0.02	0.03	0.02	0.1	8<LOQ
Child 12 months	52	0.03	0.02	0.03	0.03	0.07	5<LOQ
PCB 138							
Mother 28 weeks	61	0.08	0.06	0.07	0.06	0.41	0.015
Mother 3 months	82	0.06	0.06	0.04	0.04	0.42	0.009
Mother 6 months	85	0.06	0.07	0.05	0.03	0.55	0.009
Mother 12 months	73	0.06	0.04	0.05	0.04	0.21	0.01
Child 3 months	47	0.08	0.06	0.06	0.08	0.29	1<LOQ
Child 6 months	61	0.07	0.06	0.05	0.07	0.34	1<LOQ
Child 12 months	52	0.08	0.09	0.06	0.08	0.55	1<LOQ
PCB 153							
Mother 28 weeks	61	0.14	0.08	0.13	0.07	0.52	0.02
Mother 3 months	82	0.09	0.06	0.08	0.06	0.48	2<LOQ
Mother 6 months	85	0.10	0.09	0.08	0.06	0.7	3<LOQ
Mother 12 months	73	0.10	0.07	0.08	0.06	0.34	2<LOQ
Child 3 months	47	0.12	0.09	0.1	0.1	0.36	2<LOQ
Child 6 months	61	0.12	0.09	0.1	0.1	0.44	5<LOQ
Child 12 months	52	0.1	0.1	0.1	0.1	0.41	4<LOQ
PCB 180							
Mother 28 weeks	61	0.09	0.05	0.08	0.06	0.23	0.01
Mother 3 months	82	0.07	0.05	0.06	0.04	0.33	0.01
Mother 6 months	85	0.07	0.05	0.06	0.05	0.31	0.02
Mother 12 months	73	0.07	0.06	0.06	0.04	0.35	1<LOQ
Child 3 months	47	0.07	0.05	0.06	0.07	0.28	2<LOQ
Child 6 months	61	0.08	0.06	0.07	0.09	0.26	5<LOQ
Child 12 months	52	0.1	0.1	0.1	0.1	0.75	3<LOQ
Sum PCB							
Mother 28 weeks	61	0.3	0.18	0.3	0.2	1.15	0.08
Mother 3 months	82	0.2	0.15	0.2	0.1	1.11	0.06
Mother 6 months	85	0.2	0.19	0.2	0.1	1.56	0.06
Mother 12 months	73	0.2	0.14	0.2	0.1	0.69	0.06
Child 3 months	47	0.3	0.19	0.3	0.3	0.90	0.04
Child 6 months	61	0.3	0.20	0.3	0.3	0.99	0.04
Child 12 months	52	0.3	0.27	0.3	0.3	1.72	0.04

To simplify the comparison between the different PCB-congeners, and between mother and child, the median is visualised in **Figure 3.4**, and **Figure 3.5**.

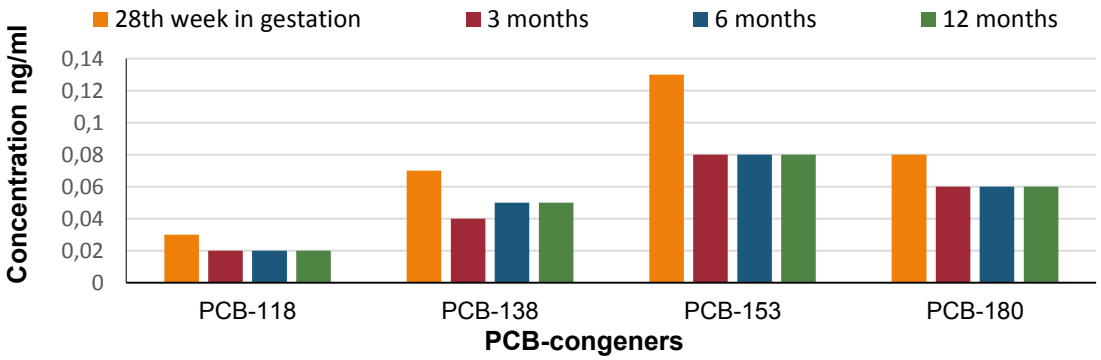

Figure 3.4 Mothers' PCB medians: Mothers median concentration in ng/ml serum for the PCB-118,-138,-153, and -180 at 28th week in gestation and at 3-, 6- and 12 months after birth

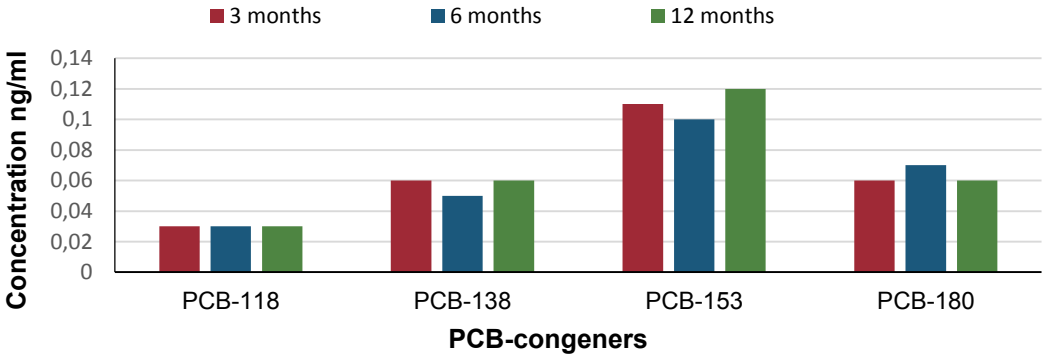

Figure 3.5 Children's PCB medians: Children's median concentration in ng/ml serum for PCB-118, 138, 153, and -180 at 3-, 6- and 12- months after birth

The decrease of PCB-concentrations during pregnancy and postpartum is reflected in the increasing number of samples below LOQ (**Table 3.5**, Appendix 3: **Figure A.2** and **Figure A.3**).

3.4.1 Seafood intake and serum PCB-levels

Mothers (n=16) who reported consuming fish liver had higher serum PCB-levels compared to those not consuming fish liver at any time-point. Transfer of more PCBs from mothers who consumed fish liver to child was evident for PCB-138 at 3 months only (**Table 3.6**).

Table 3.6 Fish liver consumer statistics: Mann-Whitney-test statistics for significant different distributions of PCB-118, -138, -153, and -180 at 28 week (w) in gestation and 3-, 6- and 12 months (m) after birth with probability (p), effect size (r), number (n) of mothers (M), or children (C), and median with interquartile range (IR) in ng/ml serum for mothers consuming liver or not consuming liver.

	p	r	n_{total} ($n_{consumed\ liver}$/ $n_{not\ consumed\ liver}$)	Liver consumers: Median (ng/ml) ± IR	Not liver consumers Median (ng/ml) ± IR
Consumes liver every year					
PCB-118 M28w	0.014	0.3	61 (7/54)	0.03 ± 0.02	0.02 ± 0.01
PCB-138 M28w	0.012	0.3	61 (7/54)	0.1 ± 0.1	0.06 ± 0.06
PCB-153 M28w	0.002	0.4	61 (7/54)	0.2 ± 0.1	0.12 ± 0.08
PCB-180 M28w	0.006	0.3	61 (7/54)	0.1 ± 0.05	0.08 ± 0.06
PCB-118 M3m	0.001	0.4	82 (9/73)	0.03 ± 0.02	0.018 ± 0.009
PCB-138 M3m	0.010	0.3	82 (9/73)	0.07 ± 0.1	0.04 ± 0.04
PCB-153 M3m	0.005	0.3	82 (9/73)	0.13 ± 0.09	0.08 ± 0.05
PCB-180 M3m	0.012	0.3	82 (9/73)	0.09 ± 0.05	0.06 ± 0.04
PCB-118 M6m	0.026	0.2	85 (14/71)	0.03 ± 0.02	0.02 ± 0.01
PCB-138 M6m	0.019	0.3	85 (14/71)	0.07 ± 0.08	0.04 ± 0.03
PCB-153 M6m	0.003	0.3	85 (14/71)	0.1 ± 0.1	0.08 ± 0.06
PCB-180 M6m	0.001	0.4	85 (14/71)	0.1 ± 0.07	0.05 ± 0.04
PCB-118 M12m	0.005	0.3	73 (12/61)	0.03 ± 0.03	0.02 ± 0.01
PCB-138 M12m	<0.001	0.4	73 (12/61)	0.1 ± 0.07	0.04 ± 0.04
PCB-153 M12m	0.003	0.3	73 (12/61)	0.2 ± 0.2	0.07 ± 0.05
PCB-180 M12m	<0.001	0.4	73 (12/61)	0.1 ± 0.1	0.05 ± 0.04
PCB-138 C3m	0.026	0.3	47 (6/41)	0.1 ± 0.1	0.06 ± 0.06

At 12 months there were 7 mothers that reported consuming roe liver pate, but there were no significant differences between these 7 participants and the rest of the mothers at 12 months.

There were no significant differences in serum concentrations of PCBs for those who ate more or less than 300 g of total seafood for dinner per week, nor between those that ate more or less than 450 g of total seafood per week, at any of the time points.

For oily fish there was no significant difference between those who consumed more or less than 200 g. For those who consumed more or less than 300 g there was only a significant difference between PCB-180 for mothers at 6 months ($p = 0.042$, $r = 0.23$, $n_{under300g} = 71$, $n_{over300g} = 7$), with

higher serum concentration among oily fish consumers. When dividing the study population into breastfeeding or non-breastfeeding, this difference for oily fish was found only among those that did not breastfeed (p =0.027, r=0.25, $n_{under300g}$= 22, $n_{over300g}$=3). Of the 7 consuming more than 300 g of oily fish 2 were also fish liver consumers. When fish liver consumers were removed from the dataset the significant difference for PCB-180 at 6 months between mothers who consumed more or less than 300 g of oily fish disappeared.

Shellfish consumption was the only significant correlation found between seafood and serum levels of PCBs in pregnancy, and only for PCB-153 (p=0.040, n=48) and PCB-180 (p=0.044, n=48) (**Table 3.7**). When considering the higher endogenous exposure these women get from losing weight after the pregnancy, as well as the PCBs they might lose through breastfeeding, correlations after birth were considered unreliable and were therefore omitted.

Table 3.7 Seafood correlation: Spearman's correlation coefficient (ρ) for different categories of seafood intake against PCB-118, -138, -138, -180 for week 28 in pregnancy.

	PCB-118	**PCB-138**	**PCB-153**	**PCB-180**
Seafood for dinner				
Total	-.014	-.074	-.177	.018
Oily fish	.246	-.002	-.052	-.019
Lean fish	.053	.255	-.071	-.117
Shellfish	-.002	.217	.297*	.292*
Processed	.228	-.032	-.009	-.118
Lofoten (roepate)	.037	.016	-.089	-.132
Seafood spread	-.021	-.027	.051	-.054
Supplement	-.096	-.044	-.130	-.128
Total seafood	-.125	-.001	-.077	-.033

Total seafood: dinner index +spread index +supplement index **Significant difference: p<0.05*
Supplement: cod liver oil, omega-3 supplement
Lean fish: Lean and intermediate oily fish
Oily fish: fish with more than 5g of fat per 100g of fish

When removing the fish liver consumers from the study population (now n=41) the correlation between shellfish and PCB-153 and -180 disappeared.

No significant difference was found for PCB-levels at 12 months for those children that had started eating seafood, or oily fish, for dinner compared to those who did not.

3.4.2 Longitudinal difference in serum PCB-levels

There was longitudinal data from 27 mothers and 20 children. These 27 mothers and 20 children did not differ from the total group of mothers and children in serum PCB-levels.

In a longitudinal perspective there was a significant difference between the levels of PCB-118, PCB-153 and PCB-180 at the different time points for the mothers (Appendix 3: **Table A.6**). PCB-153 decreased already at 3 months when compared to the level at 28th week in pregnancy, whereas a reduction in PCB-118 and PCB-180 were prominent at 6- and 12 months when compared to levels at 28th week in pregnancy (Appendix 3: **Table A.6**).

There were no differences between the levels for any of the PCBs for the children. When splitting the children into breastfed and not breastfed, the only difference between time-points was for PCB-153 at 12 months for children breastfed for 3 months ($p=0.005$, $\chi^2(2)=6.00$, $n=13$), this difference was not found with daily breastfeeding for 6- or 12 months.

3.4.3 Differences between serum PCB-concentrations in mother and child

The median serum levels of PCB-118, -138 and -153 were higher in children compared to mothers at 3- and 6 months (Appendix 3: **Table A.7**, descriptive summary statistics of the data are presented in **Table 3.5**).

No difference was found for distribution or median for serum concentration of PCB-180 at any time-point, or for any of the PCBs at 12 months after birth between mother and children.

3.4.4 Correlation between mother and child

There was a significant positive correlation (**Figure 3.6** and **3.7**) between all the serum concentrations for PCBs in mother and child at 3 months. PCB-180 had the strongest correlation with a 19.5% shared variance. PCB-180 was also the only PCB that had a significant correlation at 6 months postpartum. At 12 months there were no significant correlations between the serum PCB-concentrations in mother and child.

To control for biased correlation, the dataset was tested without the highest values. Even without these values the correlations were significant.

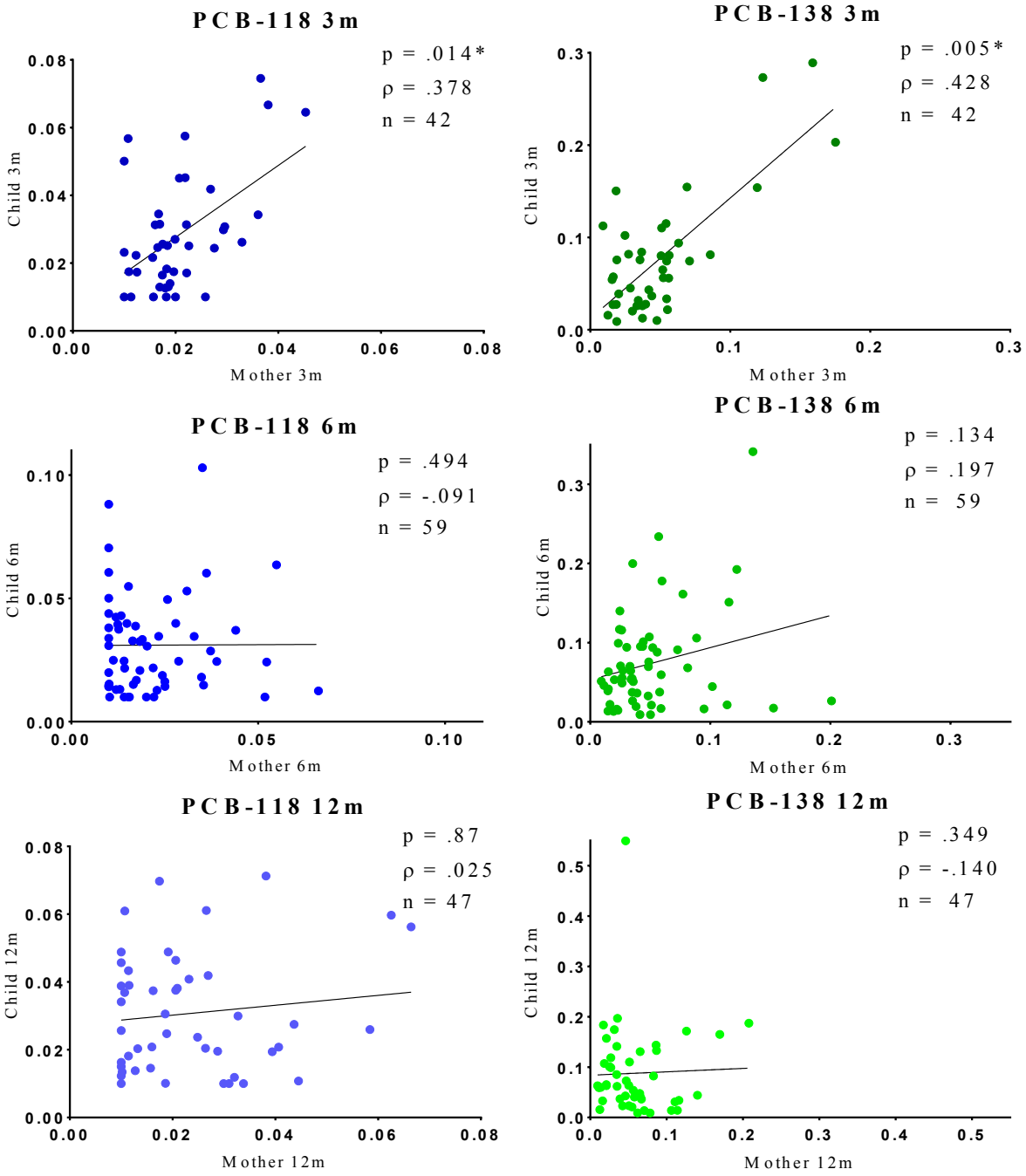

Figure 3.6 Mother correlated against child 1: Correlation between mother and child for serum concentrations of PCB-118 and PCB-138 at 3-, 6- and 12 months (m) with number of mother-child pair (n), Spearman's rho (ρ) and probability (p).

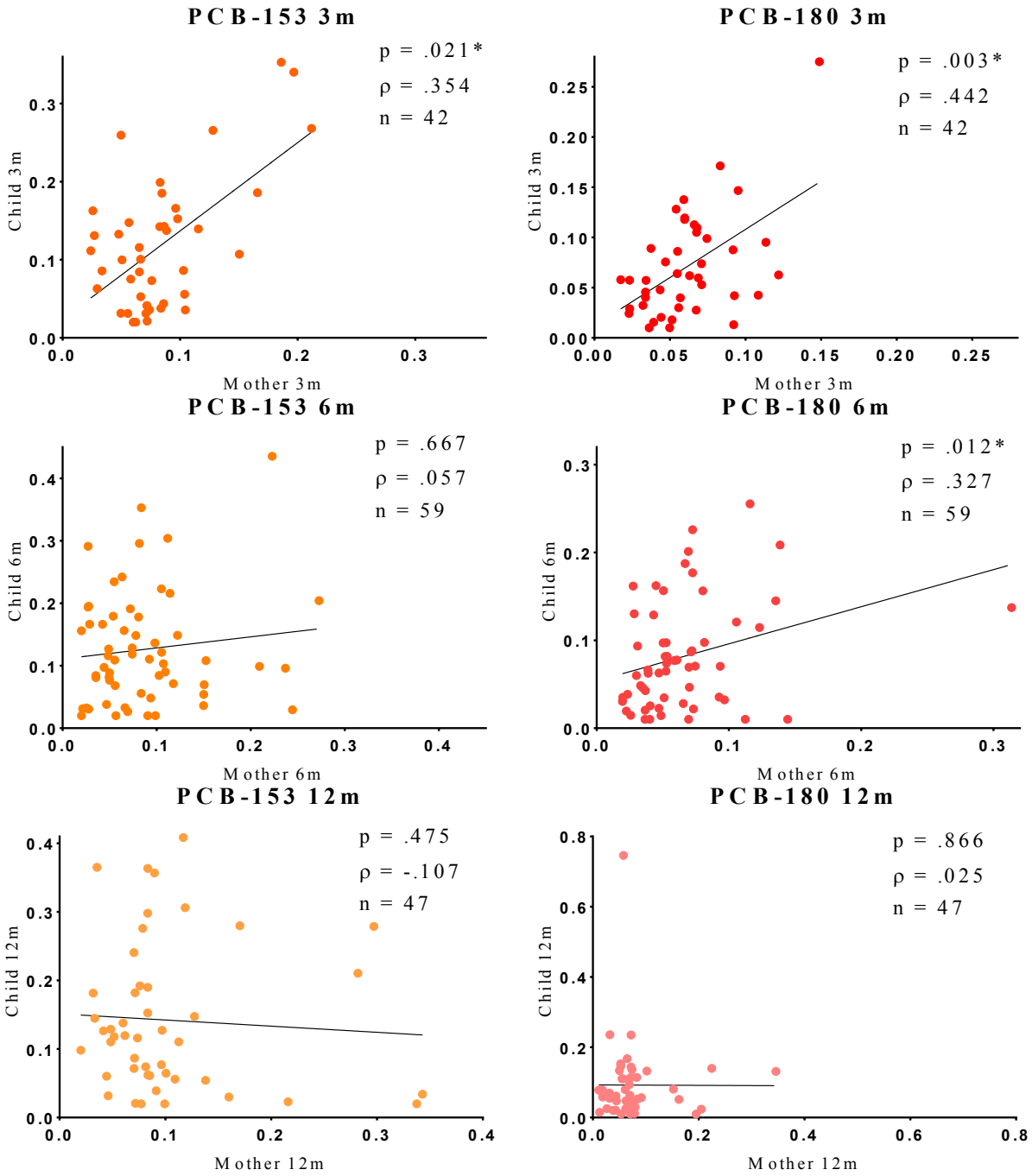

Figure 3.7 Mother correlated against child 2: Correlation between mother and child for serum concentrations of PCB-153 and PCB-180 at 3-, 6- and 12 months (m) with number of mother-child pair (n), Spearman's rho (ρ) and probability (p).

3.4.5 BMI and age

There were no difference between serum PCB-concentrations at 28th week in gestation for mothers that were overweight (BMI ≥ 25) compared to mothers that were not.

There was a significant difference in the distributions of serum concentrations of PCB-118, -153, -180 and sum PCB (138+153+180) between mothers over and under 30 years of age, with mothers over 30 years of age having the highest concentrations at 28th week in gestation (**Table 3.8** and **Table 3.9**). At 3 months PCB-138, -153, -180, and sum PCB (138+153+180) were significantly higher among mothers over 30. Serum concentration of PCB-180 was the only PCB that showed a difference at 6 months. At 12 months there were no differences in PCB-concentrations between the age groups.

Since 11 of the 16 liver consumers were over 30 years of age, and 4 more where 28 or 29, the age differences in PCB-concentrations where examined again without these 16 women. The same significant age differences appeared with or without these mothers.

Table 3.8 Age-difference statistics: Mann-Whitney test statistic for significant different distributions of PCB-118, -138, -153 and -180 at 28th week (w) in gestation, and 3-, and 6 months (m) after birth with probability (p), effect size (r), and number of mothers (n) for mothers over or under 30 years of age.

PCB-congener	p	r	n ($n_{over\ 30}/n_{under\ 30}$)
PCB-118 28w	0.007	0.4	49 (24/25)
PCB-153 28w	0.05	0.3	49 (24/25)
PCB-180 28w	0.012	0.4	49 (24/25)
sum PCB (138+153+180) 28w	0.036	0.3	49 (24/25)
PCB-138 3m	0.031	0.3	72 (43/29)
PCB-153 3m	0.009	0.3	72 (43/29)
PCB-180 3m	0.001	0.4	72 (43/29)
sum PCB (138+153+180) 3m	0.002	0.3	72 (43/29)
PCB-180 6m	0.033	0.2	78 (50/28)

Table 3.9 Serum PCB-medians for mothers over and under 30 years of age: Median concentrations with interquartile range (IR) in ng/ml for PCB-118, -138, -153, -180 and sum PCB (138 + 153 + 180) in serum for mothers divided into under and over 30 years of age at 28th week (w) in pregnancy and 3-, 6-, and 12 months (m) postpartum

Time-point	Age	PCB-118 (ng/ml) Median ± IR	PCB-138 (ng/ml) Median ± IR	PCB-153 (ng/ml) Median ± IR	PCB-180 (ng/ml) Median ± IR	Sum PCB 138+153+180 (ng/ml) Median ± IR
28w	19-29	0.02 ± 0.01	0.07 ± 0.07	0.13 ± 0.07	0.08 ± 0.06	0.3 ± 0.2
	30-41	0.03 ± 0.02	0.07 ± 0.07	0.16 ± 0.07	0.11 ± 0.06	0.3 ± 0.2
3m	19-29	0.02 ± 0.01	0.03 ± 0.04	0.07 ± 0.03	0.05 ± 0.03	0.2 ± 0.1
	30-41	0.02 ± 0.2	0.05 ± 0.05	0.09 ± 0.06	0.07 ± 0.05	0.2 ± 0.2
6m	19-29	0.02 ± 0.01	0.04 ± 0.04	0.08 ± 0.05	0.05 ± 0.04	0.2 ± 0.1
	30-42	0.02 ± 0.02	0.05 ± 0.04	0.10 ± 0.07	0.07 ± 0.05	0.2 ± 0.2
12m	19-29	0.02 ± 0.02	0.05 ± 0.06	0.08 ± 0.06	0.05 ± 0.05	0.2 ± 0.2
	30-42	0.02 ± 0.02	0.05 ± 0.04	0.08 ± 0.05	0.06 ± 0.04	0.2 ± 0.1

When the study population was separated into breastfeeding or not breastfeeding it became evident that the age dependent differences on serum PCB-levels were only present in mothers that did not breastfeed (**Table 3.10**). Mothers that did not breastfeed had significantly higher serum PCB-concentrations at 3- and 6 months for those over 30 years of age, except for PCB-118 at 6 months. At 12 months only serum concentration of PCB-180 was higher among mothers over 30 years that did not breastfeed, but at this time-point many of the non-breastfeeding mothers had stopped breastfeeding only a few months earlier. When testing for mothers that did not breastfeed at 6 months against serum PCB-concentrations at 12 months, there was still a PCB-difference, for all the PCBs, between over and under 30 years of age.

For mothers that breastfed the only exception was serum concentration of PCB-180 at 3 months, which was still higher among those that were over 30.

Table 3.10 Effect of breastfeeding on mothers over and under 30 years of age: Mann-Whitney test statistic for significant different distributions of PCB-118,-138,-153, -180 and sum PCB (138+153+180) at 3-, 6-, and 12 months (m) with probability (p), effect size (r), and number of mothers (n) not breastfeeding, or breastfeeding, over or under 30 years of age.

PCB-congeners	p	r	n ($n_{over\ 30}$/$n_{under\ 30}$)
Not breastfeeding at the time-point			
PCB-118 3m	0.026	0.6	12 (6/6)
PCB-138 3m	0.002	0.8	12 (6/6)
PCB-153 3m	0.004	0.8	12 (6/6)
PCB-180 3m	0.026	0.6	12 (6/6)
sum PCB (138+153+180) 3m	0.004	0.8	12 (6/6)
PCB-138 6m	0.002	0.6	25 (13/12)
PCB-153 6m	0.011	0.5	25 (13/12)
PCB-180 6m	0.005	0.5	25 (13/12)
sum PCB (138+153+180) 6m	0.001	0.6	25 (13/12)
PCB-180 12m	0.032	0.3	50 (31/19)
Not breastfeeding at 6 months compared to 12 month serum concentration			
PCB-118 12m	0.002	0.6	25 (13/12)
PCB-138 12m	0.010	0.5	25 (13/12)
PCB-153 12m	0.016	0.5	25 (13/12)
PCB-180 12m	0.013	0.5	25 (13/12)
sum PCB (138+153+180) 12m	0.002	0.5	25 (13/12)
Breastfeeding at 3 months			
PCB-180 3m	0.008	0.3	59 (36/23)

For the children, the mothers' serum PCB-difference due to age, only had an impact on those that were still breastfed at 12 months. Children of mothers over 30 years of age had higher serum levels of PCB-153 ($p=0.012$, $r= 0.74$, $n=11$, $n_{mothers\text{-}over30years}=8$, $n_{mothers\text{-}under30years}=3$), PCB-138 ($p=0.024$, $r= 0.68$, $n=11$, $n_{mothers\text{-}over30years}=8$, $n_{mothers\text{-}under30years}=3$) and sum PCB (138+153+180) ($p=0.012$, $r= 0.74$, $n=11$, $n_{mothers\text{-}over30years}=8$, $n_{mothers\text{-}under30years}=3$), than children of mothers below 30 years of age at 12 months.

3.4.6 Parity

The only significant difference between serum PCB-concentrations in mothers with previous children and mothers with no previous children, was for PCB-153 at 6 months, where there was a significantly higher serum level among mothers with no previous children ($p= 0.03$, $r= -0.26$, $n= 71$).

When including breastfeeding at 6 months it was evident that this difference in PCB-153 at 6 months was only among the breastfeeding mothers ($p= 0.041$, $r= -0.29$, $n_{first\text{-}child}= 24$, $n_{with\text{-}previous\text{-}children}= 26$). Mothers without previous children that breastfed daily at 12 months had also higher serum concentrations for both PCB-138 ($p= 0.009$, $r= -0.55$, $n_{first\text{-}child}= 12$, $n_{with\text{-}previous\text{-}children}= 10$) and PCB-153 ($p= 0.043$, $r= -0.44$, $n_{first\text{-}child}= 12$, $n_{with\text{-}previous\text{-}children}= 10$), even though this did not show up when testing only for parity.

There were no significant differences in the serum concentrations of PCBs among children with or without siblings at the different time points.

3.4.7 Gender, Birth weight and Length

There was little difference in the PCB-concentrations between girls and boys. The only difference occurred at 3 months for PCB-138 where boys had more PCB-138 than girls did ($p=0.031$, $r=0.3$, $n_{boys}= 27$, $n_{girls}= 19$). There was also a tendency for boys to have a higher concentration than girls for PCB-153 at 3 months ($p= 0.055$, $r=0.3$, $n_{boys}= 27$, $n_{girls}= 19$).

A Spearman's Rho correlation showed no significant correlations between weight at birth and serum PCB-levels at 28th week in gestation for the mother. There was neither any significant difference between children below or above 2.8 kg (15th percentile girls WHO growth for age). There was however, a weak trend for all the PCBs towards a higher median PCB (among mothers at 28th week gestation) among the children below 2.8 kg (PCB-118: $p= 0.092$, $n_{over}= 37$, $n_{under}= 7$, PCB-138: $p= 0.120$, $n_{over}= 37$, $n_{under}= 7$, PCB-153: $p=0.144$, $n_{over}= 37$, $n_{under}= 7$, PCB-180: $p= 0.153$, $n_{over}= 37$, $n_{under}= 7$).

There was no difference in serum PCB-concentrations among children over or under WHOs length-for-age at 0 weeks (49.1 cm) (WHO, 2014).

3.4.8 Breastfeeding

Children exclusively breastfed at 3 months had significantly higher serum PCB-levels at 3 months compared to those that had not been exclusively breastfed (**Table 3.11**).

There was also a significant difference in PCB-138 for mothers at 12 months, where those that exclusively breastfed their child for 3 months had lower serum concentrations than those that had not exclusively breastfed for 3 months ($p=0.045$, $r=-0.2$, $n_{breastfeeding}=43$, $n_{not\text{-}breastfeeding}=24$). For PCB-118, -153, and -180 there were no significant differences among mothers after fully breastfeeding for 3 months.

At 6 months there were only 7 children that had breast milk as the only nutritional source. The parameter "breastfeeding daily" was therefore used instead of "exclusively breastfeeding". Children that were breastfed daily for 6 or 12 months had significantly higher serum PCB-concentrations for all the PCB-congeners compared to the children that were not breastfed daily (**Table 3.11**).

No significant differences were found between the PCB-concentrations for the mothers that breastfed daily for 6 or 12 months.

Table 3.11 Effect of breastfeeding on PCBs: Breastfeeding significance (Mann-Whitney-test) on PCB-118, -138, -153 and -180 concentrations for 3-, 6-, and 12 months (m) with probability (p), number (n) of children (C), effect size (r) and median with interquartile range (IR) in ng/ml serum for breastfed and not breastfed children.

	p	r	n total ($n_{breastfed}/n_{not\ breastfed}$)	Median±IR (ng/ml) Breastfed	Median±IR (ng/ml) Not breastfed
3 months breastfed exclusively					
PCB-118 C3m	0.035	0.3	46 (29/17)	0.03 ± 0.02	0.02 ± 0.02
PCB-138 C3m	0.004	0.4	46 (29/17)	0.08 ± 0.06	0.03 ± 0.08
PCB-153 C3m	0.001	0.5	46 (29/17)	0.1 ± 0.1	0.04 ± 0.09
PCB-180 C3m	0.001	0.5	46 (29/17)	0.09 ± 0.06	0.04 ± 0.04
Sum PCB (138+153+180) C3m	0.001	0.5	46 (29/17)	0.3 ± 0.02	0.1 ± 0.2
6 months breastfed					
PCB-118 C6m	p<0.001	0.6	61 (42/19)	0.03 ± 0.02	0.01 ± 0.01
PCB-138 C6m	p<0.001	0.6	61 (42/19)	0.07 ± 0.06	0.02 ± 0.02
PCB-153 C6m	p<0.001	0.6	61 (42/19)	0.1 ± 0.1	0.03 ± 0.04
PCB-180 C6m	p<0.001	0.6	61 (42/19)	0.08 ± 0.07	0.02 ± 0.02
Sum PCB (138+153+180) C6m	p<0.001	0.6	61 (42/19)	0.3 ± 0.2	0.07 ± 0.08
12 months breastfed					
PCB-118 C12m	p<0.001	0.5	52 (17/35)	0.04 ± 0.02	0.02 ± 0.02
PCB-138 C12m	p<0.001	0.5	52 (17/35)	0.1 ± 0.1	0.04 ±0.06
PCB-153 C12m	0.001	0.5	52 (17/35)	0.1 ± 0.1	0.1 ± 0.1
PCB-180 C12m	0.003	0.4	52 (17/35)	0.11 ± 0.07	0.05 ± 0.09
Sum PCB (138+153+180) C12m	0.001	0.5	52(17/35)	0.4 ± 0.3	0.2 ± 0.2

4. Discussion

Seafood contains important nutritional components like high quality proteins, omega-3 PUFAs, and iodine. However, it will also contain some contaminants, which can have a potential negative effect on human health. The goal of this project was to evaluate the seafood intake and the serum levels of PCB-118, -138, -153, and -180 in mothers at 28th week in gestation and for mother and child at 3-, 6- and 12 months postpartum in a coastal study population.

4.1 Participants

The study population is very similar to the MoBa cohort in mean age (Present: 30 ± 5, MoBa: 29.6 ±4.6) (Brantsaeter et al., 2008). This is also consistent with the average age of all childbirths in Norway, which was 30.4 in 2011 (SSB, 2013). BMI is also equal to the MoBa cohort (Present: 24 ± 4, MoBa: 24.2 ± 4.4) (Brantsaeter et al., 2008). However, the percentage of participants with normal weight before pregnancy was larger in this study population than the MoBa (Present: 59%, MoBa: 53.4%) (Brantsaeter et al., 2008). There is also a slightly higher degree of university/college educated women (Present: 61-71%, MoBa: 58.3%) in the present study population (Brantsaeter et al., 2008). This degree of higher education was considerably higher than the national average in 2010 (SSB: 30.6%) (SSB, 2014). There were also more first time mothers in this study population than in the MoBa cohort (Present: 49%, MoBa: 40.3%) (Brantsaeter et al., 2008).

The children's length and weight at birth were also very similar to what was observed for the children in the MoBa cohort (Brantsaeter et al., 2012). They were also inside 2 SD of the median length and weight for the WHO Child Growth Standards (WHO, 2014). When it comes to breastfeeding, 70% of the children were breastfed daily at 6 months and 33% at 12 months. This is 10 and 13% below the average found in the Spekost 2006-2007 study (Øverby, 2008; Øverby, 2009). In the MoBa cohort there was also a 10% higher degree of partially breastfeeding at 6 months (Haggkvist et al., 2010) Parity is one factor associated with longer duration of breastfeeding (Lande et al., 2003), and could explain why the women in this study population stopped breastfeeding earlier than other studies.

This study population lived by the coast, and had a majority of highly educated, as well as pregnant/formerly pregnant women, the results from this project are therefore not necessarily representative for the whole Norwegian population. It has been reported that mothers with

higher education tend to have different eating habits than those with lower education, such as a higher consumption of healthy food and cod liver oil (Johansson et al., 1999; Brustad et al., 2004). The education level of the participants in this study should therefore be taken into consideration when interpreting the results.

4.2 Seafood intake

This study population consumed a smaller amount of seafood than what is recommended by the Norwegian Health Authorities (Nasjonalt Råd for Ernæring, 2011). Most of the women in this study reported eating seafood for dinner 1-2 times per week. Looking at the estimated summarised seafood intake per week the total was between 243 g at 28 weeks and 308 g at 12 months. At 6- and 12 months the majority of the women ate more than 2 portions (150 g) of seafood, based on this estimate, which would be within the recommended quantity. At 28th week in gestation, and at 3 months, the estimated consumption per week was below 300 g. Thus the majority of the women would be below the recommendations of 2-3 portions per week at these time-points. On the question of total seafood for dinner the estimate was 225 g/week for all the time-points. Based on this the majority would be below the recommended seafood intake. The Norwegian Health Authorities also recommend eating about 200 g of oily fish per week (Nasjonalt Råd for Ernæring, 2011); whereas the estimated quantities consumed in this study population were between 53 and 83 g per week, which mean that most of the women were below the recommended intake. The low fish consumption in this study population was comparable to the fish intake in the MoBa study, where they consumed on average 252 g/week of total seafood and 84 g/week of oily fish (Brantsaeter et al., 2012). The present study was comparable to the Norkost 2010-2011 studies average of 308 g/week in total fish consume, but below the Norkost studies 154 g/week of oily fish (The Norwegian Directorate of Health, 2012). At the same time the Norkost study also found that only about 1/3 of the participants consumed the recommended amount of fish. Only 7-17% of the women in this study population reported eating seafood as spread 3 or more times per week, and around 70% of the women that consumed seafood as spread reported that they used an oily spread. These women with high intake of oily spread might get an additional 75g (+) of oily fish, and this can get them closer to the recommendations. Still, this is a very small part of the study population, and the overall seafood consumption is low.

Considering the consistent low intake of oily fish reported in the 4 questionnaires in this study population, it is likely that the majority of these women also had a moderate to low oily fish intake before pregnancy.

Since the total seafood for dinner estimate was lower than the combined seafood categories for dinner estimate this might imply a possible over-reporting on the subcategories of fish consumed, which is a usual occurrence with an increased amount of questions (Cade et al., 2002).

The fact that these women do not eat the recommended amount of seafood might mean that they do not get enough of the nutrients from fish, like omega-3 PUFAs, vitamin D, and iodine. However, they might still get the amount of omega-3 PUFAs, and vitamin D, they need by taking supplements. At 3- and 6 months the majority of the women reported taking omega-3 supplements more than 4 times a week, but at 12 months this intake had decreased to under 50%. Pregnant women are recommended to take vitamin D supplements in form of cod liver oil during the pregnancy, which also include omega-3 PUFAs (The Norwegian Directorate of Health, 2009).

Most omega-3 supplements produced in Norway have been purified to remove dioxins and dl-PCBs (FHI, 2008). In 2010, when this study started, NIFES found no omega-3 products above 5ng TE/kg oil, which is the upper limit for sum PCDD/F + dl-PCB in oils for human consumption (NIFES, 2011b). For 7 out of 17 omega-3 oils the PCB_7 –concentration was above 10 µg/kg oil, and two seal oil products above 50 µg/kg oil (NIFES, 2011b). The EU has an upper limit for PCB_6 in oils for human consumption at 200 µg/kg oil and all the omega-3 oils measured by NIFES in 2010 were well below that limit (EU, 2006, 01.09.2012). The contribution of PCBs from omega-3 supplements are therefore worth considering, especially among those that consume seal oil every day. In this study there were only 3 mothers that used seal oil. The contribution of PCBs from seal oil was therefore negligible. Breastfeeding infants had a higher intake of omega-3 products than the not breastfeeding infants, the additional PCBs from omega-3 supplements might separate the breastfeeding infants even further from non-breastfeeding infants in serum PCB-concentrations.

The fact that there was a correlation between seafood for dinner and total seafood against reported focus on a healthy lifestyle at 28th week in gestation and at 3 months, but not later, might imply that mothers were more concerned about healthy nutritional intake while

breastfeeding. At 6- and 12 months most children had also started receiving other sources of nutrition than breast milk. A British study of 57 pregnant women found that they received most of their information about pregnancy through books and magazines (Cade et al., 2002). Norwegian Health Authorities have a great deal of information on healthy eating during pregnancy, and postnatally, available both as articles online (https://helsenorge.no/gravid), and as pamphlets available online and at health stations (The Norwegian Directorate of Health, 2009; 2011). The high majority of educated women in this study also makes it more likely that the women would educate them self about pregnancy.

4.3 Serum PCB-concentrations

PCB-118, -138, - 153 and -180 were detected in mothers in the 28th pregnancy week and for mothers and children at 3-, 6-, and 12 months postpartum.

4.3.1 Mothers

The PCB-levels in the present study population were at least 6 times lower than the 95th percentile reference values (RV_{95}) from the German Environmental Survey (1998) (Schulz et al., 2011) (Appendix 5: **Table A.10**). On the other hand, PCB-levels in this study, 28th week in gestation, were comparable to those reported in a Danish study (Morck et al., 2014). The Danish study reported a median serum PCB-138 level of 0.121 ng/ml (Morck et al., 2014), while in this project the median serum level for all the mothers were 0.07 ng/ml in the 28th gestation week. The PCB-153 median serum level in this project was 0.13 ng/ml, which was below the Danish PCB-153 serum level of 0.181 ng/ml (Morck et al., 2014). The serum level of PCB-180 was lower in the Danish study, with 0.024 ng/ml (Morck et al., 2014), compared to the serum median level measured in the present project of 0.08 ng/ml. PCB-118 was not measured in the Danish study, but the median serum level in the present project is below Spanish 4 year olds that had an average serum concentration of 0.1 ng/ml (Grimalt et al., 2010) compared to 0.03 ng/ml in this project. The Spanish study (Grimalt et al., 2010) also had higher serum levels for the three other PCBs than what was found in the present project.

The reduction in PCB-concentrations for PCB-118, -153 and -180 between 28th week in pregnancy and postpartum time-points, but not between postpartum time-points, indicates that the majority of the PCB-transfer happened before birth. Redistribution of PCBs during pregnancy could be explained by the higher hormonal activity, compared to non-pregnant

individuals, as well as an increase in blood volume by 40-50% (Lockitch, 1997; Costantine, 2014). The metabolic systems are also affected, and there might be more cholesterol and other fatty lipids in the blood than normal (Costantine, 2014). The changes in serum-PCB from pregnancy to postpartum may come out differently if compared to pre-pregnancy serum samples. After birth the blood values can also be influenced by weight loss and breastfeeding. The total impacts from these, as well as exogenous PCB-intake from seafood, were therefore what was measured in serum after birth.

PCB-concentrations can be influenced by confounding factors like BMI, age, living area (urban/not urban), previous children, high alcohol consumption, high fish consumption and consumption of seagull eggs and roe liver pate (Rogan et al., 1986; Schade et al., 1998; Caspersen et al., 2013; Morck et al., 2014). In the current project possible factors to consider were BMI, age, previous children, fish consumption, roe liver pate consumption, and fish liver consumption.

BMI had no impact on the concentrations of PCBs when the population was divided into above and below a BMI of 25 (normal weight or overweight). Overweight women will have more adipose tissue to distribute the PCBs over, thereby diluting the concentration of PCBs. Women with a high BMI have been found to have lower concentrations of PCBs in breast milk, and it is reasonable to expect this also in serum (Schade et al., 1998). The previous observations were not found in the present study, probably due to the low number of obese in the studied population.

Age had a significant impact on the serum PCB-concentrations. Among breastfeeding mothers a difference in PCB-concentrations between those over and under 30 years of age was detected only before birth. Mothers that did not breastfeed had a significant difference in concentrations throughout the first year after birth. Other studies have also found that PCB-concentrations are age dependent (Schade et al., 1998; Glynn et al., 2007; Schulz et al., 2011). The higher levels of PCBs in mothers over 30 years of age are probably due to a longer lifetime of bioaccumulation, and also from declining PCB-concentrations in nature and food during the last decades (Glynn et al., 2007; Axmon et al., 2008; EFSA, 2012b). This decline means that the eldest mothers have been exposed to higher concentrations of PCBs early in life than younger mothers. This higher PCB-concentration, among those over 30 years of age, might therefore be more of a birth cohort effect than an effect of aging (bioaccumulation and slower

metabolism) (Quinn et al., 2012). After birth the PCB-difference between mothers over and under 30 years of age disappeared in breastfeeding mothers. This is concurrent with other studies that states that previously breastfeeding women show lower levels of PCBs than women that have not breastfed (Wittsiepe et al., 2007; Bjermo et al., 2013).

There was also a difference in the PCB-distribution between those that had previous children and those that did not. This was only evident among those who breastfed their child. Breastfeeding mothers without previous children had a higher level of PCB-153 at 6 months than breastfeeding mothers with previous children. At 12 months the mothers without previous children, that still breastfed, had higher levels of both PCB-138 and -153 compared to breastfeeding mother with previous children. These are among the most abundant PCBs, and that might be the reason why only these PCBs show a difference. It has been established that women with previous children have lower PCB-concentrations, many new studies on PCBs therefore only use primiparous women (Schade et al., 1998; Glynn et al., 2007; WHO, 2007). There was no question in the seafood-FFQ about breastfeeding previous children. This may have masked a difference that might have occurred by looking at mothers that had previously breastfed against mothers that used formula.

4.3.2 Children

The majority of the studies in **Table A.10** (Appendix 5) are on children of school age, as there are few studies that assess PCB-concentrations in serum for infants. These serum PCB-levels are therefore more of a reference to the concentrations for the infants in this study. The median for all the infants are used here, since the other studies do no separate between breastfed and not breastfed children.

It is apparent that the German whole blood and cord blood PCB-levels, as well as the Spanish serum PCB-levels (**Table A.10** (Appendix 5)) are far higher than the median serum PCB-levels for the children in the present study (Lackmann, 2002; Grimalt et al., 2010; Schulz et al., 2011). The 6 to 11 year old children in the Danish study (Morck et al., 2014) are more comparable in median PCB-levels to the Norwegian infants studied in this project. In the Danish study the serum median level of PCB-138 was 0.086 ng/ml (Morck et al., 2014), while it in this project was 0.06 ng/ml 3 months after birth. Serum median level of PCB-153 was 0.118 ng/ml (Morck et al., 2014) in the Danish study and 0.1 ng/ml in the present project. For PCB-180 the serum median level was 0.072 ng/ml (Morck et al., 2014) in the Danish study and 0.06 ng/ml in the

present project 3 months after birth. As previously mentioned PCB-118 was not measured in the Danish study, but Spanish 4 year old children had an average serum concentration of 0.10 ng/ml (Grimalt et al., 2010), which was 3 times higher than the median serum level of 0.03 ng/ml measured in this project at 3 months after birth. There was only one child, in the present project, above the reference values for PCB-138 and PCB-180 suggested by Lackmann (2002) for serum concentrations in neonates. The child had been breastfed for more than 6 months, and a concentration over the neonate reference value is therefore not so unlikely (Verner et al., 2013). Based on current knowledge this is unlikely to cause an increased health risk since the PCB-levels of breastfed children tend to decrease as they grow and put on more fat (Verner et al., 2013). That both this and the Danish study population have lower serum PCB-levels than the German and Spanish concentrations, indicates geographical differences in PCB-levels, which are lower in Norway and Denmark than in other European countries. Both Grimalt et al. (2010) and Lackmann (2002) have values from children born in 1997-1998. These are born more than 10 years before the children in this project. This might contribute to a birth cohort effect, and might account for some of the difference between these values.

There was little difference in the median serum PCB-levels among the infants as they got older. This was also seen when the children were divided into breastfeeding and not breastfeeding. Other studies have not found the same results. In a toxicokinetic model by Verner et al. it was found that the child's PCB-concentrations would increase as long as the child was breastfed (Verner et al., 2009; Verner et al., 2013). In the Inuit Cohort Study children breastfed for more than 3 months had four times higher PCB-153 concentration at 6 months than those that were breastfed for less than 3 months (Ayotte et al., 2003). The difference between this cohort and the Inuit cohort is that 55% of the children continued with exclusive breastfeeding after 3 months in the Inuit cohort. Whereas, in this project only 7% of the children were exclusively breastfed at 6 months. The children in the Inuit cohort therefore probably received more breast milk than most of the children in this study, and consequently had a higher PCB-concentration.

Earlier studies have linked a high PCB-transfer to the infant during pregnancy to lower birth weight (Govarts et al., 2012; Papadopoulou et al., 2013). This was not found in this project. However, there was a tendency towards higher PCB-concentrations in mothers (28^{th} week in gestation) that had children below 2.8 kg at birth. There were only 7 children below 2.8 kg and 37 above 2.8 kg. Although the number of participants in this project was limited it is tempting

to suggest that high PCB-levels in mother leads to a reduction in birth weight as observed previously for PCB-153 (Govarts et al., 2012).

Breastfeeding children of mothers over 30 years of age had elevated levels of PCB-138, and -153 at 12 months. Older mothers often breastfeed longer than younger mothers (Lande et al., 2003; Betoko et al., 2013), which was also apparent in this study population. In this test 8 of the 11 children, that were still breastfeeding at 12 months, had mothers over 30 years of age. Longer duration of breastfeeding can lead to increased concentrations of PCBs in serum compared to children not breastfed for so long. This might be the reason why mothers over 30 years of age had children with higher serum levels for some PCBs at 12 months.

Although Quinn et al. (2012) found that the first-born child would have a higher prenatal PCB-transference than the second- to fifth-born child, parity had no impact on serum PCB-levels in the children in this project.

There was a significant difference in the median between breastfed and formula fed children, with higher serum PCB-concentrations among the breastfed infants. This is consistent with previous studies, where the increased PCB-concentrations in breastfed children could still be detected several years after the nursing period (Lanting et al., 1998a; Walkowiak et al., 2001; Ayotte et al., 2003; Carrizo et al., 2006). Breast milk is known to contain PCBs from the mother, and these will be transferred to the child (VKM, 2013). At the same time more children received omega-3 supplements in the breastfeeding group compared to the formula fed group. Ordinary Möllers tran, one of the most common cod liver oils in Norway, had 22 µg PCB_7/kg oil in 2010 (NIFES, 2011b). This supplement intake can possibly contribute to a higher PCB-intake among the breastfed children, but this is unlikely due to the small servings given to children. Some studies show a slight decline in PCB-concentrations in breast milk during the nursing period with about 1-3% per month, after a year the reduction can be up to 15 to 28% (Hooper et al., 2007; Thomsen et al., 2010). This can explain why the children levelled out in PCB-concentration; they are growing and accumulating more fat to store the PCBs in and at the same time their mothers are giving out declining amounts of PCBs through their milk. The breastfed children were still below other European countries in concentration. In addition the benefits from breastfeeding would likely outweigh any possible negative effect from this PCB-consumption, as was the conclusion of the Norwegian Scientific Committee for Food Safety in

2013 (VKM, 2013). This suggests that there is little reason for Norwegian mothers to not breastfeed to avoid PCB-transference from mother to child.

4.3.3 Comparison of PCB-concentration in mother and child

There were 20 children and 27 mothers that had longitudinal PCB-data from all the time-points. Since there was no difference in PCB-concentrations between these and the other participants these account for a representative selection of the study population and are therefore used to look at the difference in PCBs over the time-points.

In this study the not breastfed infants had lower serum PCB-concentrations at 3 months than mothers' serum PCB-concentrations at 28th week in pregnancy. The breastfed infants had concentrations equal to or higher than the mothers' serum PCB-concentrations at 28th week in pregnancy. This was also evident in the correlation between mother and child at 3 months where all the PCB-congeners showed a significant correlation with higher levels among most of the children. That the mothers have reduced some of their serum PCB-concentrations from 28th week in pregnancy to postpartum, might indicate a transfer of the PCBs from mother to child. It is normal that children have PCB-levels similar to the mothers at birth, and for the PCB-levels of breastfeeding infants to increase to above mothers' concentration during the breastfeeding period (Grandjean, 2003; Verner et al., 2013; VKM, 2013). The increase found in the breastfed children, but not in the formula fed, is consistent with what other studies have found (Ayotte et al., 2003; Lackmann et al., 2005).

At birth this PCB-concentration would be what was transferred over the placenta during the pregnancy. At 3 months after birth the amount of PCBs in the infants serum is both from transfer over the placenta and through breast milk.

The only significant correlation between mother and child at 6 months was for PCB-180, which also had the strongest correlation at 3 months. The median is still lower for PCB-118, - 138 and -153 for the mothers compared to the children at 6 months. This difference disappears before 12 months. At 12 months there were no longer any exclusively breastfed children, and close to 70% of the children were not breastfed at all. For those that stopped breastfeeding before, or right after 6 months, the growth of the child from this period to 12 months likely lead to a diluted body burden of PCBs (Verner et al., 2013). This dilution may cause the lack of difference between mother and child at this stage.

4.4 Seafood consumptions impact on serum PCB-concentrations

Mothers that consumed fish liver had increased PCB-concentrations compared to those that did not consume fish liver. Children of mothers that consumed fish liver only had an increase in serum PCB-138 concentration at 3 months. Fish liver has been found to have a high PCB-concentration, as is also documented in **Table 1.1** in part 1.3 in the Introduction. Fish liver and seagull eggs are especially high in PCBs, and MoBa found that pregnant women that consumed sea gull eggs and roe liver pate, containing fish liver, had higher serum level of PCB-153 compared to the rest of the study population (Caspersen et al., 2013). Fish liver is traditionally consumed in many coastal communities, and the study population here do live by the coast. Both MoBa and the Norwegian Fish and Game Study considered fish liver in their intake, but found that it had little impact on overall median and total intake (Kvalem et al., 2009; Caspersen et al., 2013).

The only correlation that appeared between seafood and PCB-concentration in serum at 28th week of pregnancy was for shellfish (PCB-153, and -180) and this was an effect of medium size. Shellfish is normally low in PCBs (Fernandes et al., 2009; Gueguen et al., 2011), the correlation here is therefore difficult to explain. One possible explanation is the consumption of older shells, crabs and lobsters, which can have bioaccumulated more PCBs over time, as observed for shells where PCB-concentration increased with age (Grilo et al., 2013). However, the concentration of PCB_7 in Norwegian blue mussels, scallops and oysters were in 2010 found to be generally low, and comparable to that of lean fish (NIFES, 2011a). Shellfish consumers might have a more including eating pattern, with an increased consumption of different sorts of seafood. When removing the fish liver consumers from the data set the shellfish correlation disappeared. This might implicate fish liver consumption as the reason behind the shellfish correlation

Seafood intake, both total dinner intake and oily fish consumption, had little impact on serum PCB-concentrations in the mothers. The only influence from oily fish, found in this study, was for mothers that consumed more than 300 g per week at 6 months. This was only evident for PCB-180 and only among those that did not breastfeed. There were only three mothers that did not breastfeed and at the same time consumed more than 300 g of oily fish at this stage, the statistics here are therefore less reliable. Semi-oily and oily fish were found to be the largest contributor to PCB-intake, among those that did not consume seagull eggs and roe liver pate,

in the MoBa cohort (Caspersen et al., 2013). The majority of the women in this study population were breastfeeding, and the exogenous exposure from oily fish might be masked by this. This is concurrent with the observation that only non-breastfeeding mothers had a higher PCB-180 at 6 months due to oily fish consumption. At the same time, the consumption of oily fish was low in this study population, which might be why no relationship appears. When removing the 2 fish liver consumers that had an oily fish intake above 300 g at 6 months, the PCB-180 difference in serum concentration disappeared. Fish liver consumption is therefore also likely the cause of the higher PCB-180 serum concentration at 6 months for those that consumed over 300 g of oily fish per week.

4.5 Methodological considerations and potential improvements

4.5.1 Seafood-FFQ

This study is undertaken in pregnancy and nursing period, and during this time pregnancy complications may arise. This might be nausea, vomiting or bed rest. These are conditions that also affect the diet, but can be difficult to capture in an FFQ (Meltzer et al., 2008). In this study the seafood-FFQ only asked for habitual seafood intake for the last 3 months. Eventual pregnancy complications were therefore also reflected in these questions, perhaps better than in an FFQ considering the last 12 months. Still it would be useful with a question about particular food items, especially seafood, that the participant were not able to consume during the pregnancy, or in the months after, because of pregnancy nausea or the state of the pregnancy. This would make it possible to see if the women ate less fish during that period than they would normally.

The question on daily exercise (12D) in the seafood-FFQ should be changed from 20 minutes to 30 minutes per day. This is the recommended amount of moderate to high exercise needed per day. This is also applicable for pregnant women, but there only for 5 days a week (The Norwegian Directorate of Health, 2014). There should also be other options, like hard exercising for 60 minutes. Since some PCB-congeners are found in sweat, increased perspiration might lead to a higher elimination of these PCBs (Genuis et al., 2013). Very active women might therefore have lower PCB-levels than not so active women.

The Norwegian Health Authorities recommend eating fish for dinner 2-3 times per week (Nasjonalt Råd for Ernæring, 2011). In the seafood-FFQ the frequency is either 1-2 times per

week or 3 or more per week it is not possible to say if these women eat the recommended amount, or just 1 portion. This question should be changed according to the Norwegian recommendations.

4.5.2 Blood samples

Blood samples were in this study taken in a nonfasting state, which have been found to be as much as 29% higher in PCBs than fasting samples (Phillips et al., 1989). This difference evens out when the samples are corrected for total lipid concentration. Since PCBs are in a dynamic equilibrium among all tissues in the body, an alteration of lipid content in one of these tissues would change this equilibrium (Matthews et al., 1980). More serum lipids in the blood after a meal can thereby possibly extract some PCBs from the muscle and liver. Muscle and liver have a high perfusion rate and some affinity for PCBs but bind it weaker than lipoproteins in the blood (Matthews et al., 1980). The serum PCB-concentrations in this project were not adjusted for lipid concentration. There have been found good correlations between nonfasting PCB-concentrations in serum samples and their corresponding lipid adjusted values (Rylander et al., 2012). Good correlations have also been found between adipose tissue and serum (Stellman et al., 1998). The concentrations found in this project are therefore viable to be used as biomarkers of total body burden of PCBs.

Neither of the articles in **Table A.10** (Appendix 5) state if the blood sampling were done from fasting or non-fasting subjects. In this project blood was drawn in a nonfasting state. It is normal to collect blood samples in a fasting state, especially when studying lipoprotein and metabolic processes, since this is thought to give a more defined baseline and be more reproducible (Cohn et al., 1988).

Blood from children are rarely collected in a fasting state. This should therefore not affect the measured PCB-concentrations. Considerably fewer serum samples were analysed for the children than the mothers. Drawing blood from infants is more challenging than from adults. Therefore there are some children that have not had their blood taken at some of the time-points. The serum samples have also been used previously for other studies. Some vials were therefore empty when they were to be analysed and had to be excluded.

4.5.3 PCB-analysis

The analytical method used for analysing the four PCBs has not been fully validated or accredited. Hence, its associated uncertainty has not yet been established. To assure that the analytical quality of the method was sound some parameters needed to ensure reliable results were implemented, as explained in Materials and Method section 2.7. Two samples of Certified Reference Material (CRM) were run almost every day. Not all of them came out inside the desired concentrations. The majority of times this happened for only one of the two CRMs, or the CRM was incorrect for only one analyte. The decision was therefore made that it would not be correct to disregard the samples made that day. In future analysis with this method more CRM-samples (3+) should be analysed together with the samples. CRM and BL-values are illustrated with bar graphs (**Figure A.4** to **A.11**) in Appendix 4. A t-test was also completed on the recovered values of the CRM. Samples below 30% and above 130% recovery were removed from the dataset. Inside this range previous results from HRGC-HRMS analysis have been relatively robust. Some parallel samples showed too high variation. New samples were prepared for these and other samples that were excluded due to abnormal elution of compounds of interest in the chromatogram. The HRGC-HRMS developed a malfunction before these could be run and could not be fixed in time to get the results into this project. Mean values for the parallels were therefore used in this project. The amount of congeners found, with PCB-153 being the largest concentration, and PCB-118 being the smallest, are coherent with what other studies have reported. The PCB-concentrations were also different between older and younger mothers, as well as increasing with breastfeeding length for the children. This is also in agreement with other studies (EFSA, 2005; VKM, 2013). These observations strengthens the validity of the method.

The statistics used in this project does not fully account for possible confounding factors (BMI, parity, smoking, alcohol consumption, seafood intake) that can influence the PCB-levels studied in these mothers and children. In the future, a multilevel linear model should be used to get a complete view of the impact from these factors. Unfortunately, constructing a multilevel linear model is a time consuming task that could not be considered in the present master project. A recoding with the established and missing values were performed so that it was possible to observe the differences between those in the study population that had a complete sample set, and those that had not.

5. Summary and conclusion

Maternal fish consumption was lower than the recommendations given by the Norwegian Health Authority for both total fish and oily fish.

The newly established method for measuring PCBs in small serum volumes appears reliable for detection of PCB-118, -138, -153 and -180.

The current serum levels of PCB-118, -138, -153 and -180 in Norwegian mothers and children were low compared to other European countries.

There was a correlation between serum levels of PCB-118, -138, -153 and -180 in mother and child 3 months after birth. The only other serum PCB correlation between mother and child was for PCB-180 at 6 months after birth.

Whereas breastfeeding in itself had little impact on maternal PCB-concentrations in serum, breastfed children had an increase in serum PCB-levels compared to not breastfed children.

The serum PCB-levels increased with age and decreased with parity in not-breastfeeding and breastfeeding mothers respectively.

Fish liver consumers were found to have higher serum PCB-levels than the rest of the study population. Children of fish liver consumers only differed in serum concentration of PCB-138 at 3 months.

To achieve the overall beneficial health effects from seafood, young and pregnant women should increase their seafood consumption, but avoid fish liver products. This demonstrates the importance of specific dietary advice for young and pregnant women concerning fish liver products.

When fish liver consumers were excluded, there were no relation between maternal fish intake and serum levels of PCB-118, -138, -153 and -180 in mother or child.

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Appendix

Appendix 1: 6 month FFQ and Seafood-FFQ

Children: 6 month short FFQ:

Spørreskjema - barn **Dato:_____**

Mors ID-nr: _____

Barnets ID-nr: _____

Barn

Født i svsk.uke:_____

Ved fødsel: Vekt:_____ Lengde_____ Hodeomkrets _____

Nå, dato: _____Vekt:_____ Lengde_____ Hodeomkrets _____

Får barnet :	**Daglig**	**2-3/uke**	**Sjelden**				
Vitamintilskudd	☐	☐	☐	Biovit	Sanasol	Folinsyre	_____
Vitamin-D	☐	☐	☐				
Tran/omega-3	☐	☐	☐	Hvilke type tran/omega-3: _____			
Jern	☐	☐	☐				
Morsmelk:	☐	☐	☐				
Kumelk	☐	☐	☐				
Tillegg	☐	☐	☐				

Fullammet i antall mndr.:_____mndr

Kun melk i antall mndr.:_____mndr

Alder ved introduksjon av grøt.:_____mndr

Alder ved introduksjon av middag.:_____mndr

Type tillegg:_____

Ant. måltider/dag pr. i dag: Grøt_____ Middag_____ Skive_____ Frukt _____

Medikamenter: Ja Nei Hvilke _____ Ant. mndr.___

Spiser barnet fisk/sjømat til middag? Ja Nei

Hvis ja, hvilke type fisk/sjømat og hvor ofte? _____

Spiser barnet fisk/sjømat som pålegg? Ja Nei

Hvis ja, hvilke type(r) pålegg og hvor ofte? _____

Sjømatinntak

Her vil vi gjerne få informasjon om sjømatinntaket ditt. Ha de ***3 siste månedene*** i bakhodet når du fyller ut skjemaet. Med sjømat mener vi fisk, fiskeprodukter og andre sjømatprodukter som for eksempel skjell og skalldyr. Vi er klar over at kostholdet varierer fra dag til dag. Prøv likevel så godt du kan å gi et "gjennomsnitt" av ditt sjømatinntak spist til middag, som pålegg, i salat og eller spist som mellommåltid. Du skal bare sette ETT kryss på hvert spørsmål med mindre noe annet er spesifisert, og krysset skal være inne i en boks, ikke mellom boksene.

1. Hvor ofte bruker du fisk, fiskeprodukter eller annen sjømat som middagsmat?

Mer enn 5 ganger / uke	3 ganger eller mer/ uke	1-2 ganger/uke	1-3 ganger/ måned	Sjeldnere enn 1 gang/måned	Aldri
☐	☐	☐	☐	☐	☐

2. Hvis du spiser fisk, fiskeprodukter eller annen sjømat til middag, hvor mye spiser du vanligvis?

(1 porsjon = 150 gram, tilsvarer for eksempel 1 laksekotelett eller 3 fiskekaker eller 2 dl reker u/skall)

1/2 porsjon eller mindre	1 porsjon	1 ½ porsjon	2 porsjoner	3 porsjoner
☐	☐	☐	☐	☐

3. Hvor ofte bruker du sjømat som pålegg, i salat, mellommåltid, snacks eller lignende?

Mer enn 5 ganger / uke	3-5 ganger / uke	1-2 ganger / uke	1-3 ganger/ måned	Sjelden	Aldri
☐	☐	☐	☐	☐	☐

4. Hvis du bruker sjømat som pålegg, i salat, mellommåltid, snacks eller lignende, beskriv hvor mye du vanligvis spiser?

(for eksempel boks makrell i tomat, antall fiskekaker, dl reker til antall brødskiver/knekkebrød)

_____

_____

_____

5. Hvor ofte spiser du vanligvis følgende sjømat som middag?

	3 ganger eller mer/uke	1-2 ganger/uke	1-3 ganger /mnd	Sjeldnere enn 1 gang/mnd	Aldri
Laks, ørret	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐
Kveite	☐	☐	☐	☐	☐
Uer	☐	☐	☐	☐	☐
Steinbit	☐	☐	☐	☐	☐
Flyndre, rødspette	☐	☐	☐	☐	☐
Torsk	☐	☐	☐	☐	☐
Sei	☐	☐	☐	☐	☐
Hyse	☐	☐	☐	☐	☐
Abbor, gjedde (ferskvann)	☐	☐	☐	☐	☐
Røye, sik (ferskvann)	☐	☐	☐	☐	☐
Reker	☐	☐	☐	☐	☐
Krabbe	☐	☐	☐	☐	☐
Hummer	☐	☐	☐	☐	☐
Blåskjell	☐	☐	☐	☐	☐
Kamskjell	☐	☐	☐	☐	☐
Fiskekaker	☐	☐	☐	☐	☐
Fiskeboller	☐	☐	☐	☐	☐
Fiskepudding	☐	☐	☐	☐	☐
Fiskegrateng	☐	☐	☐	☐	☐
Fiskepinner	☐	☐	☐	☐	☐
Fiskesuppe	☐	☐	☐	☐	☐
Klippfisk	☐	☐	☐	☐	☐

6. Hvor ofte spiser du vanligvis følgende sjømat som pålegg?

	3 ganger eller mer/uke	1-2 ganger/uke	1-3 ganger /mnd	Sjeldnere enn 1 gang/mnd	Aldri
Makrell i tomat	☐	☐	☐	☐	☐
Sardin på boks	☐	☐	☐	☐	☐
Brisling	☐	☐	☐	☐	☐
Ansjos	☐	☐	☐	☐	☐
Røkt laks, ørret	☐	☐	☐	☐	☐
Gravet laks, ørret	☐	☐	☐	☐	☐
Tunfisk på boks	☐	☐	☐	☐	☐
Sild (sursild, rømmesild, kryddersild el.lign.)	☐	☐	☐	☐	☐
Kaviar	☐	☐	☐	☐	☐
Crabsticks	☐	☐	☐	☐	☐
Svolværpostei	☐	☐	☐	☐	☐
Lofotpostei	☐	☐	☐	☐	☐
Annet sjømat (spesifiser):	☐	☐	☐	☐	☐

_____

_____

_____

7. Spiser du innmat av fisk?

☐ Ja ☐ Nei

Dersom ja, hvor mange ganger per år spiser du fiskeinnmat?

	1-3 ganger/år	4-6 ganger/år	7-9 ganger/år	≥ 10 ganger/år
Rogn	☐	☐	☐	☐
Fiskelever	☐	☐	☐	☐

8. Andre generelle spørsmål om kostholdet ditt

A. Hvor ofte spiser du frukt og grønnsaker?

	Flere ganger/dag	Hver dag	4-6 ganger/uke	1-3 ganger/uke	Sjelden	Aldri
Frukt	☐	☐	☐	☐	☐	☐
Grønnsaker	☐	☐	☐	☐	☐	☐

B. Spiser du meieriprodukter (melk, yoghurt, ost) daglig?

☐ Ja ☐ Nei (gå til spørsmål C)

Dersom ja, hvor mange ganger per dag spiser du meieriprodukter *(en gang er for eksempel ett glass melk eller en yoghurt eller ost til en skive brød)*?

1-3 ganger/dag	4-6 ganger/dag	7-9 ganger/dag	≥ 10 ganger/dag
☐	☐	☐	☐

Dersom ja: Når det finnes vitamin D berikete varianter av de ulike melkeproduktene, hvor ofte velger du da disse?

Alltid	Som oftest	Noen ganger	Sjelden	Aldri	Vet ikke
☐	☐	☐	☐	☐	☐

C. Bruker du smør eller margarin?

☐ Ja ☐ Nei (gå til spørsmål D)

Fyll inn til hvor mange brødskiver/knekkebrød/rundstykker du vanligvis bruker smør/margarin per uke

____ Margarin ____ Lettmargarin ____ Smør

Hvor mye smører du pr. brødskive/knekkebrød/rundstykke?

En porsjonspakning på 10-12 g rekker til antall skiver/knekkebrød/rundstykke:

☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5

Når det finnes vitamin D berikete varianter av smør eller margarin, hvor ofte velger du da disse?

Alltid	Som oftest	Noen ganger	Sjelden	Aldri	Vet ikke
☐	☐	☐	☐	☐	☐

D. Angi hvilken type fett du vanligvis bruker til matlaging?

	Daglig	Ukentlig	Månedlig	Sjelden	Aldri
Margarin	☐	☐	☐	☐	☐
Lettmargarin	☐	☐	☐	☐	☐
Smør	☐	☐	☐	☐	☐
Oljer	☐	☐	☐	☐	☐

Hvis du bruker oljer, hvilken type olje bruker du vanligvis?

☐ Olivenolje ☐ Soyaolje ☐ Rapsolje ☐ Solsikkeolje

☐ Maisolje ☐ Annen olje (spesifiser): _____

Når det finnes vitamin D berikete varianter av oljer, hvor ofte velger du da disse?

Alltid	Som oftest	Noen ganger	Sjelden	Aldri	Vet ikke
☐	☐	☐	☐	☐	☐

9. Kosttilskudd

A. Bruker du tran, fiskeolje- eller omega-3 tilskudd (flytende eller som kapsler)?

	Ja, hele året	Ja, men bare om vinteren	Nei
Flytende	☐	☐	☐
Kapsler	☐	☐	☐

Dersom du tar flytende tilskudd: Hvor mye tran, fiskeolje eller omega-3 tar du per gang?

1 teskje (3 ml) ☐

1 barneskje (6 ml) ☐

1 spiseskje (11 ml) ☐

Dersom du tar kapsler: Hvor mye tran, fiskeolje eller omega-3 tar du per gang?

1-2 kapsler ☐

3-4 kapsler ☐

5 eller flere kapsler ☐

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Appendix 1

Hvilken type tran- eller fiskeolje/omega-3 tilskudd pleier du å bruke og hvor ofte tar du tilskuddet?

	HYPPIGHET				
	Daglig	4-6 ganger/uke	1-3 ganger/uke	1-3 ganger/måned	Sjelden/aldri
Møllers tran	☐	☐	☐	☐	☐
Møllers dobbel	☐	☐	☐	☐	☐
Triomar	☐	☐	☐	☐	☐
Eskimo omega-3	☐	☐	☐	☐	☐
Selolje	☐	☐	☐	☐	☐
Triomega	☐	☐	☐	☐	☐
Vitomega	☐	☐	☐	☐	☐
Sunkost omega3	☐	☐	☐	☐	☐
Eldorado	☐	☐	☐	☐	☐
Pikasol	☐	☐	☐	☐	☐
Friflyt	☐	☐	☐	☐	☐
Annen (spesifiser)	☐	☐	☐	☐	☐

Spesifiser annet omega-3 supplement:……

B. Bruker du annet kosttilskudd (vitaminer og mineraler)?

☐ Ja ☐ Nei (Gå til spm 10)

Hvis ja, hvilke type kosttilskudd bruker du og hvor ofte?

	HYPPIGHET			
	Daglig	4-6 ganger/uke	1-3 ganger/uke	1-3 ganger/måned
Multivitamin og mineral	☐	☐	☐	☐
Jern	☐	☐	☐	☐
B-vitaminer	☐	☐	☐	☐
Kalsium og vitamin D	☐	☐	☐	☐
Annet	☐	☐	☐	☐

Dersom du tar kosttilskudd spesifiser hvilket og hvor mye du tar hver gang:

_____

_____

_____

10. Bosted og etnisitet

A. Bor du mesteparten av året i Norge?

☐ Ja, Sør-Norge ☐ Ja, Midt-Norge ☐ Ja, Nord-Norge ☐ Nei, bor i annet land, spesifiser……

B. Er du av kaukasisk avstamning (har hvit hudfarge)?

☐ Ja ☐ Nei ☐ Vet ikke

C. Hvilket språk snakket dere i ditt barndomshjem?

☐ Norsk ☐ Annet, spesifiser…….

11. Solvaner

A. Hvor ofte bruker du solarium?

☐ 1-2 ganger i uken ☐ 2-3 ganger i mnd ☐ 1 gang i mnd ☐ Sjeldnere enn 1gang i mnd ☐ Aldri

B. Hvor mange uker de tre siste månedene har du vært på badeferie (Norge eller Syden)?

☐ 7 uker eller mer ☐ 4-6 uker ☐ 2-3 uker ☐1 uke ☐ Har ikke vært på badeferie

C. Hvor mange uker de tre siste månedene har du vært på fjellet i snø?

☐ 4 uker eller mer ☐ 2-3 uker ☐7-13 dager ☐1-6 dager ☐ Har ikke vært på fjellet i snø

D. Hvor mye utendørsaktivitet har du om sommeren (turer, hagearbeid, jobb)?

☐ Ute nesten hele tiden ☐ Ganske mye ☐ Middels ☐ Lite

12. Andre spørsmål

A. Alder _____år **Høyde** _____cm **Vekt**_____ kg

B. Spørsmål kun til mor (Spørsmålene under 12B gjelder bare for dem som svarer på dette skjemaet for første gang)**:**

Hvor mye veide du før du ble gravid (dette svangerskapet)? _____ kg

Har du vært gravid tidligere ☐ Ja ☐ Nei (Gå til spørsmål C)

Antall svangerskap: _____ svangerskap **Antall levendefødte barn:**_____barn

Fødselsdato for barnet/barna:_____

C. Røyker du? ☐ Ja ☐ Nei

Hvis ja, hvor mange sigaretter/piper røyker du pr. dag? _____

Bruker du snus? ☐ Ja ☐ Nei

Hvis ja, hvor mange ganger pr. dag? _____

D. Hvor ofte mosjonerer du i minst 20 minutter (går, jogger, sykler, svømmer, fotball, aerobic, styrketrening eller lignende)?

Hver dag	4-6 ganger/uke	2-3 ganger/uke	1 gang/uke	Sjeldnere enn 1 gang /uke	Aldri
☐	☐	☐	☐	☐	☐

E. Hvor stor vekt legger du på å ha et sunt kosthold?

Svært stor	Stor	Middels	Liten	Svært liten
☐	☐	☐	☐	☐

F. Er ditt kosthold representativt for resten av familien?

☐ Ja ☐ Nei

G. Spiser du vanligvis ett eller flere måltider om dagen sammen med resten av familien?

☐ Ja ☐ Nei

H. Hva er din høyeste fullførte utdanning?

9-årig grunnskole	Videregående	Høyskole/ universitet inntil 4 år (bachelor, lærer, ingeniør, sykepl.)	Høyskole/ Universitet mer enn 4 år (master, embetseksamen)
☐	☐	☐	☐

Kryss av for feltene under som ev. gjelder for deg

☐ Er vegetarianer

☐ Har diabetes (sukkersyke)

☐ Har matvareallergi/intoleranse

☐ Spiser ikke melprodukter

☐ Spiser ikke melkeprodukter

☐ Spiser ikke kjøttprodukter

☐ Spiser ikke grønnsaker

☐ Bruker medisiner Spesifiser gjerne produktnavn:

_____

_____

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Appendix 2: Chemicals and solution-preparations

Table A.1 Chemicals and preparation of internal standard and recovery standard

13C12 intern standard	Art.nr	Content				ng/ml
	CLM-1627	13C12 pp-DDE		Deluted with		50
	EC-1435	13C12 PCB-118				20
	EC-1436	13C12 PCB-138		isopropanol		20
	EC-1406	13C12 PCB-153		to		20
	EC-1407	13C12 PCB-180				20
	EO-4982	13C12 PBDE-47				25
	Art.nr	Content				ng/ml
Recovery standard	EC-1415	13C12 PCB-111 Rec		Deluted with nonane to		40
All standards are bought from Cambrigde Isotope Laboratories via LGC Sweden						

Table A.2 Chemicals used in the sample preparation for PCB-analysis

Name	Supplier
Formic acid (HCOOH)	Merck Index-No: 607-001-000
Acetone (C_3H_6O)	Sigma-Aldrich Lot # SZBD119AV
Dichloromethane (CH_2Cl_2)	Merck Index-No:602-004-00-3
Isohexane (C_6H_{14})	Merck Index-No: 401-007-00-7
Sulphuric acid (H_2SO_4)	Sigma-Aldrich Lot # SZBD3190V
Nonane (C_9H_2O)	Sigma-Aldrich Lot # STBC5938V

Table A.3 Chemicals and preparation of HRGS-HRMS standard solutions

	Standard 5	Standard 4	Standard 3	Standard 2	Standard 1
Native stock B mix	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml
p.p'-DDE	80	40	20	8	1.6
PCB-118	40	20	10	4	0.8
PCB-138	40	20	10	4	0.8
PCB-153	40	20	10	4	0.8
PCB-180	40	20	10	4	0.8
13C12 stock B mix					
p.p'-DDE	100	100	100	100	100
PCB-118	40	40	40	40	40
PCB-138	40	40	40	40	40
PCB-153	40	40	40	40	40
PCB-180	40	40	40	40	40
All standards are bought from Cambrigde Isotope Laboratories via LGC Sweden					

Appendix 3: Results

Table A.4 Healthy diet and seafood intake: Spearman's correlation on Focus on a Healthy Diet (Healthy) against different categories of seafood from the S-FFQ at 28th week (w) in pregnancy and 3-, 6- and 12 months (m) postpartum

		Sea-food dinner	Sea-food spread	Supple-ment	Total sea-food[b]	Salmon /Trout	Cod	Oily fish	Lean fish	Shell-fish	Proc esse d seafo od
Healthy 28w - S-FFQ 28w	ρ	0.303*	0.197	0.222	0.427**	0.286*	0.182	0.168	0.235	0.111	0.265
	p	0.025	0.158	0.103	0.001	0.034	0.184	0.219	0.091	0.432	0.066
	n	55	53	55	53	55	55	55	53	52	49
Healthy 3m - S-FFQ 3m	ρ	0.246*	0.231*	0.279**	0.367**	0.141	0.221*	0.073	0.136	0.109	0.197
	p	0.020	0.028	0.008	0.000	0.188	0.036	0.499	0.213	0.316	0.072
	n	90	90	90	90	89	90	88	86	87	84
Healthy 6m – S-FFQ 6m	ρ	0.207	0.096	-0.099	0.115	0.222*	0.102	0.123	0.077	-0.042	-0.004
	p	0.062	0.389	0.601	0.547	0.045	0.360	0.269	0.491	0.708	0.972
	n	82	82	30	30	82	82	82	82	82	81
Healthy 12m - S-FFQ 12m	ρ	0.222	0.078	0.097	0.172	0.174	0.205	0.100	0.138	0.001	0.083
	p	0.054	0.504	0.426	0.155	0.135	0.078	0.391	0.239	0.996	0.479
	n	76	76	70	70	75	75	75	75	75	75

[a]: *Dinner + spread + omega-3 supplement*
** p<0.05*
*** p<0.001*

7.3.1 ROUT-test

Since there are some values that are well above the mean and median an outlier-test (ROUT) was perform to take out outliers and see if there were any difference in mean and median values with and without these (**Table A.5, Figure A.1, Figure A.1** is made for easier visual comparison of median differences).

The only significant differences in distribution between with and without ROUT-test are for PCB-118 for mothers at 28 weeks (p= 0.033, $\chi^2(1)$= 4,571) and PCB-138 (p=0.009, $\chi^2(1)$= 6.914) for mothers at 3 months. At the most 7 values were taken out from a sample set, this was for PCB-118 for mothers at 6 months.

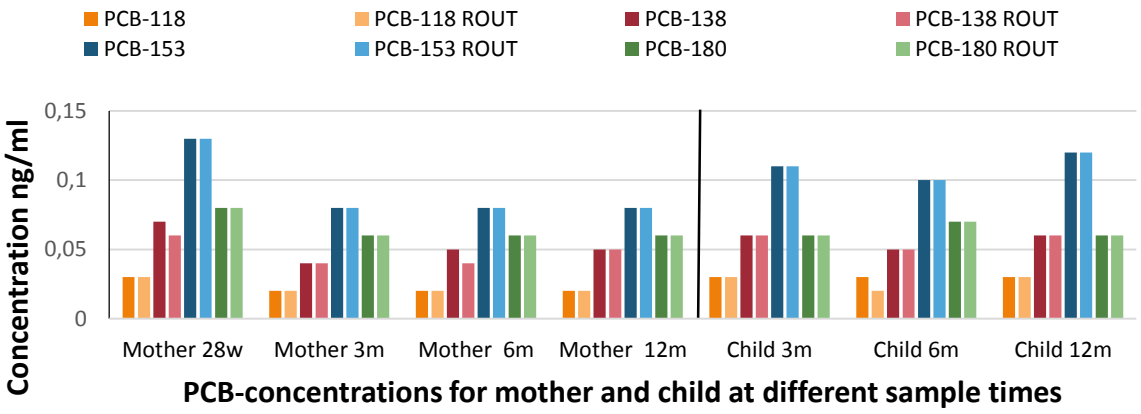

Figure A.1 Rout-test median: Median of PCB-concentrations (PCB-118,-138,-153,-180) in ng/ml for 28th week in pregnancy and 3-, 6- and 12 months (m) after birth with and without ROUT-test. Mothers to the left and children to the right

Table A.5 Descriptive with and without outliers: Comparison of number of participants (n), mean, standard deviation (SD) and median PCB-118, -138, -153 and -180 before and after ROUT-test for mothers in 28th week in pregnancy, and for mothers and children at 3-, 6-, and 12 months after birth.

	With outliers				Without outliers			
	n	Mean	SD	Median	n	Mean	SD	Median
PCB 118								
Mother 28 weeks	61	0.03	0.02	0.03	60	0.03	0.01	0.03
Mother 3 months	82	0.02	0.02	0.02	80	0.020	0.009	0.02
Mother 6 months	85	0.02	0.02	0.02	78	0.020	0.009	0.02
Mother 12 months	73	0.02	0.01	0.02	70	0.019	0.009	0.02
Child 3 months	47	0.03	0.02	0.03	47	0.03	0.02	0.03
Child 6 months	61	0.03	0.02	0.03	59	0.03	0.02	0.02
Child 12 months	52	0.03	0.02	0.03	52	0.03	0.02	0.03
PCB 138								
Mother 28 weeks	61	0.08	0.06	0.07	59	0.07	0.04	0.06
Mother 3 months	82	0.06	0.06	0.04	76	0.04	0.03	0.04
Mother 6 months	85	0.06	0.07	0.05	79	0.05	0.03	0.04
Mother 12 months	73	0.06	0.04	0.05	71	0.05	0.03	0.05
Child 3 months	47	0.08	0.06	0.06	45	0.07	0.05	0.06
Child 6 months	61	0.07	0.06	0.05	59	0.06	0.05	0.05
Child 12 months	52	0.08	0.09	0.06	51	0.07	0.06	0.06
PCB 153								
Mother 28 weeks	61	0.14	0.08	0.13	60	0.13	0.06	0.13
Mother 3 months	82	0.09	0.06	0.08	80	0.09	0.04	0.08
Mother 6 months	85	0.10	0.09	0.08	83	0.09	0.06	0.08
Mother 12 months	73	0.10	0.07	0.08	69	0.08	0.04	0.08
Child 3 months	47	0.12	0.09	0.11	47	0.12	0.09	0.11
Child 6 months	61	0.12	0.09	0.10	60	0.12	0.08	0.10
Child 12 months	52	0.1	0.1	0.12	52	0.1	0.1	0.12
PCB 180								
Mother 28 weeks	61	0.09	0.05	0.08	61	0.09	0.05	0.08
Mother 3 months	82	0.07	0.05	0.06	79	0.06	0.03	0.06
Mother 6 months	85	0.07	0.05	0.06	81	0.06	0.03	0.06
Mother 12 months	73	0.07	0.06	0.06	69	0.06	0.03	0.06
Child 3 months	47	0.07	0.05	0.06	46	0.07	0.05	0.06
Child 6 months	61	0.08	0.06	0.07	61	0.08	0.06	0.07
Child 12 months	52	0.1	0.1	0.06	51	0.08	0.06	0.06

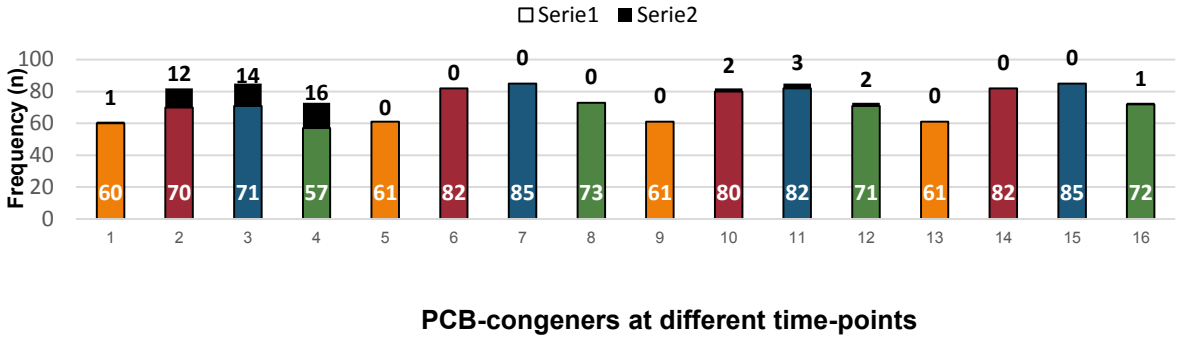

Figure A.2 Mothers over/under PCB-LOQs: Serum samples above and below LOQ-values for mothers (M) for PCB-118,-138,-153, and -180 at 28th week (w) in gestation, and 3, 6, and 12 months (m) after birth.

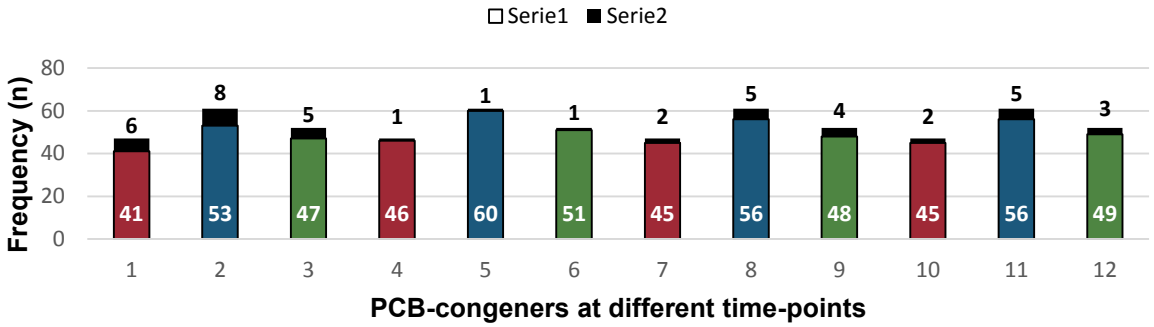

Figure A.3 Children over/under PCB-LOQs: Serum samples above and below LOQ-values for children's (C) for PCB-118,-138,-153 and -180 at 3, 6, and 12 months (m) after birth.

Table A.6 Longitudinal differences in PCB-concentrations: Statistics with probability (p), number of mothers or children (n), test statistics (χ^2) with degree of freedom in brackets, and median for the PCB-difference between 28th week (w) in pregnancy, and 3, 6 or 12 months (m) after birth for PCB-118, -138,-153 and-180 for the 27 mothers and for the 20 children

	Mothers				Children			
	PCB-118	**PCB-138**	**PCB-153**	**PCB-180**	**PCB-118**	**PCB-138**	**PCB-153**	**PCB-180**
Median[a]								
28w	.03	.08	.14	.10				
3m	.02	.05	.08*	.06	.02	.08	.1	.06
6m	.017*	.05	.08**	.06**	.03	.06	.1	.08
12m	.02*	.05	.08**	.06**	.03	.06	.1	.07
Test Statistics[b]								
n	27	27	27	27	20	20	20	20
p	0.003	0.079	<0.001	<0.001	0.987	0.350	0.271	0.861
χ^2(3)	14.2	6.8	24.6	19.8				
χ^2(2)					0.03	2.1	2.6	0.3
Follow-up[c]								
28w-3m								
p	>0.05	>0.05	0.013	>0.05				
χ^2(1)			1.074					
28w-6m								
p	0.002	>0.05	<0.001	0.001				
χ^2(1)	1.241		1.593	1.333				
28w-12m								
p	0.022	>0.05	<0.001	0.001				
χ^2(1)	1.019		1.407	1.37				

[a] Median of the 27 valid mothers and 20 valid children
[b] Friedman two-way analysis, test statistics is omitted for the children since there is no significance
[c] Pairwise Follow-up test
*Significant difference between this concentration and 28th week concentration, p≤0.05
**Significant difference between this concentration and 28th week concentration, p≤0.001

Table A.7 Differences in PCB-concentrations between mother and child: Statistics for the significant differences in PCB-concentrations between mother and child for PCB-118, -138 and -153 at 3 and 6 months, with probability (p) and test statistics (z or χ^2). For Friedman test this includes degree of freedom in brackets behind the test statistics

	Wilcoxon Signed Rank test		**Friedman Two-way test**	
	p	**z**	**p**	**χ^2(1)**
PCB-118: 3 months	0.005	-2.831		
PCB-138: 3 months	0.001	-3.445	0.014	6.095
PCB-153 3 months	0.003	-2.945		
PCB-118: 6 months	0.007	-2.697		
PCB-138: 6 months	0.003	-2.952	0.014	6.333
PCB-153 6 months	0.012	-2.504	0.033	4.751

Appendix 4: Analytical evaluation

The analytical evaluation of the method is discussed in part 2.7 in the Material and Method section.

Certified Reference Material (CRM)

CRM bar graphs are found in **Figure A.4-7**, with concentrations for PCB-118, -138, -153 and -180 listed in **Table A.8**. Red bars in the figures are values above 130% of the true value plus the uncertainty value (U) and these also fail the t-test. Green bars are above 130% of the true value plus the uncertainty value, but passed the t-test.

Table A.8 True value concentrations for certified reference material (CRM): with concentration in ng/g, uncertainty value (U), 30% of CRM minus U and 130% of CRM plus U for PCB-118, -138,-153 and -180

Concentration (ng/g)	PCB118	PCB153	PCB138	PCB180
True value	0,0185	0,0572	0,0369	0,0544
U	0,0027	0,0033	0,0054	0,0013
30%-U	0,00474	0,01617	0,00945	0,01593
130% + U	0,02756	0,07865	0,05499	0,07241

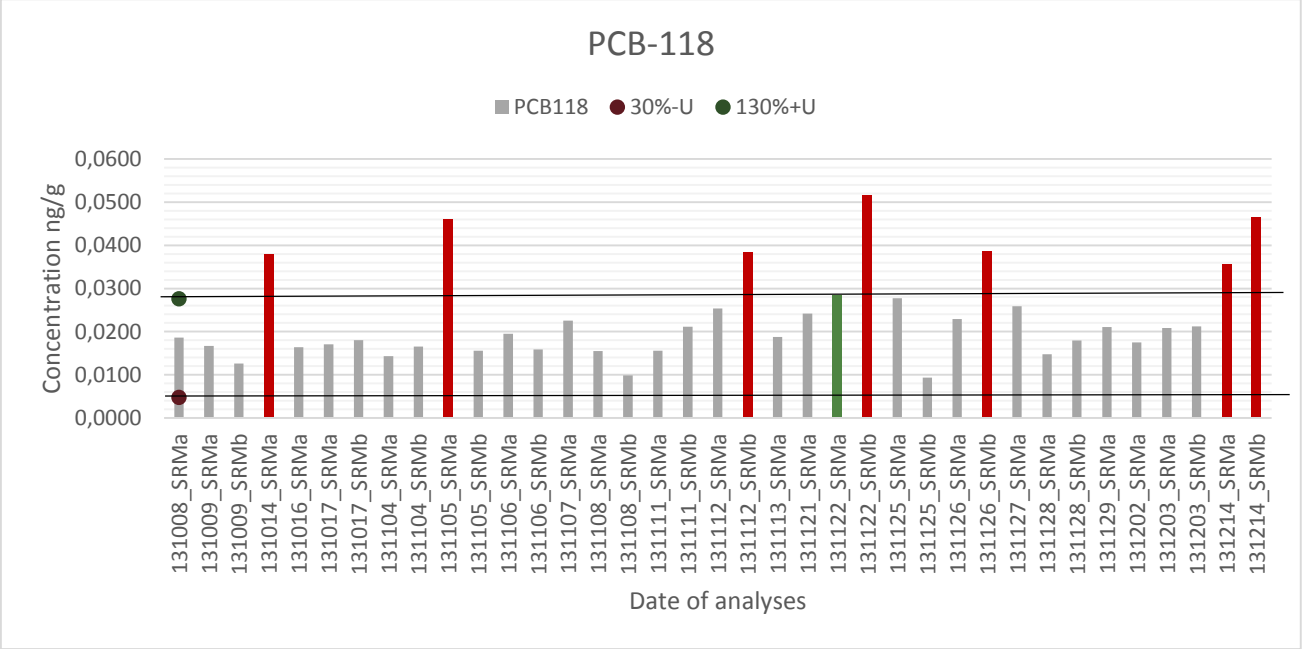

Figure A.4 CRM (SRM) for PCB-118 in ng/g for the different sample preparation dates. Parallels are denoted a and b. Lines are drawn in for concentrations representing 30% of the true value concentration minus the uncertainty value, and 130% of the true value concentration plus the uncertainty value.

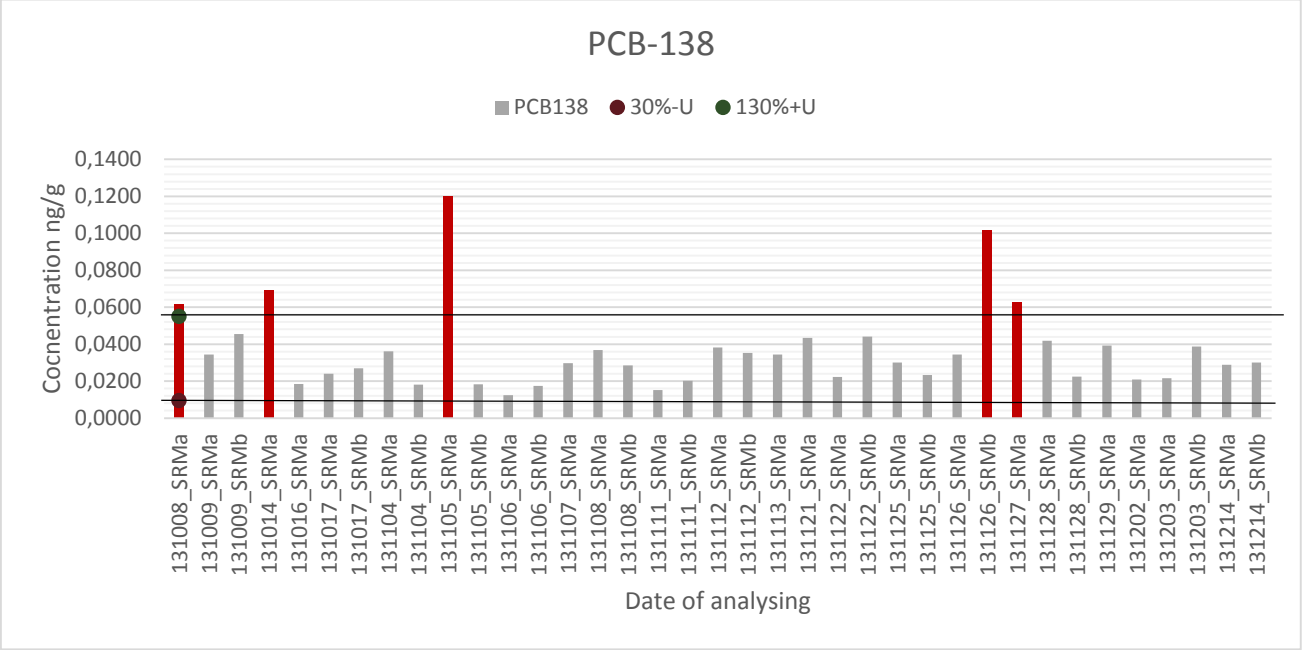

Figure A.5 CRM (SRM) for PCB-138 in ng/g for the different sample preparation dates. Parallels are denoted a and b. Lines are drawn in for concentrations representing 30% recovery concentration minus the uncertainty value, and 130% recovery concentration plus the uncertainty value.

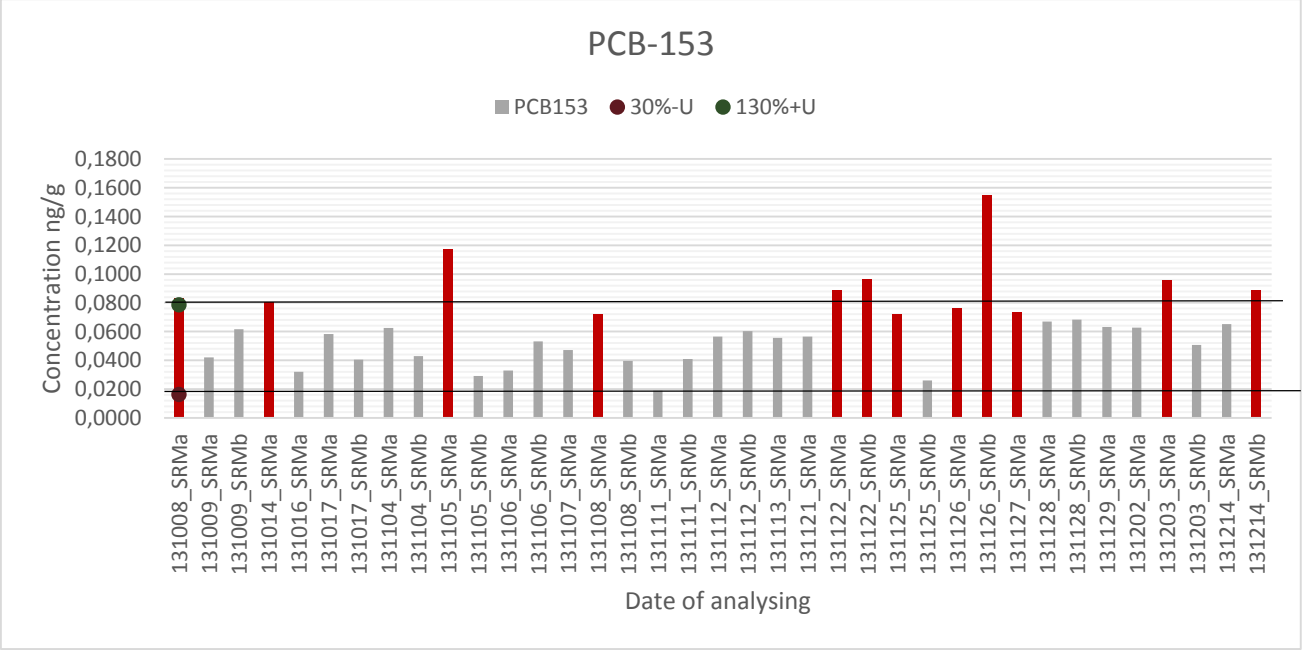

Figure A.6 CRM (SRM) for PCB-153 in ng/g for the different sample preparation dates. Parallels are denoted a and b. Lines are drawn in for concentrations representing 30% recovery concentration minus the uncertainty value, and 130% recovery concentration plus the uncertainty value.

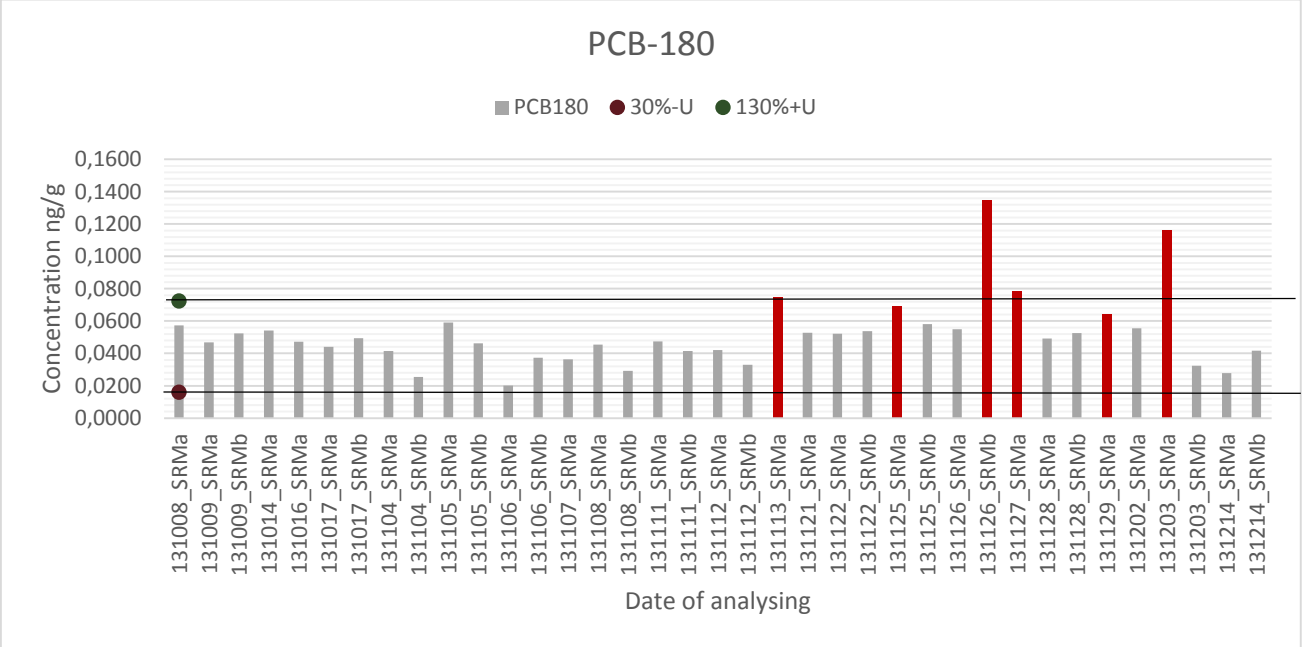

Figure A.7 CRM (SRM) for PCB-180 in ng/g for the different sample preparation dates. Parallels are denoted a and b. Lines are drawn in for concentrations representing 30% recovery concentration minus the uncertainty value, and 130% recovery concentration plus the uncertainty value.

Blank

Bar graphs of blank-values measured in distilled water are found in Figure A8-11, with a suggested limit of 30% of the true value of the CRM drawn in. Orange bars are values above 30% of the true CRM value.

Table A.9 Blank values: Limit 30% of certified referende material (CRM): concentrations in ng/ml for PCB-118, -138, -153 and -180

Concentration (ng/ml)	PCB118	PCB138	PCB153	PCB180
30% of CRM	0,006	0,012	0,018	0,015

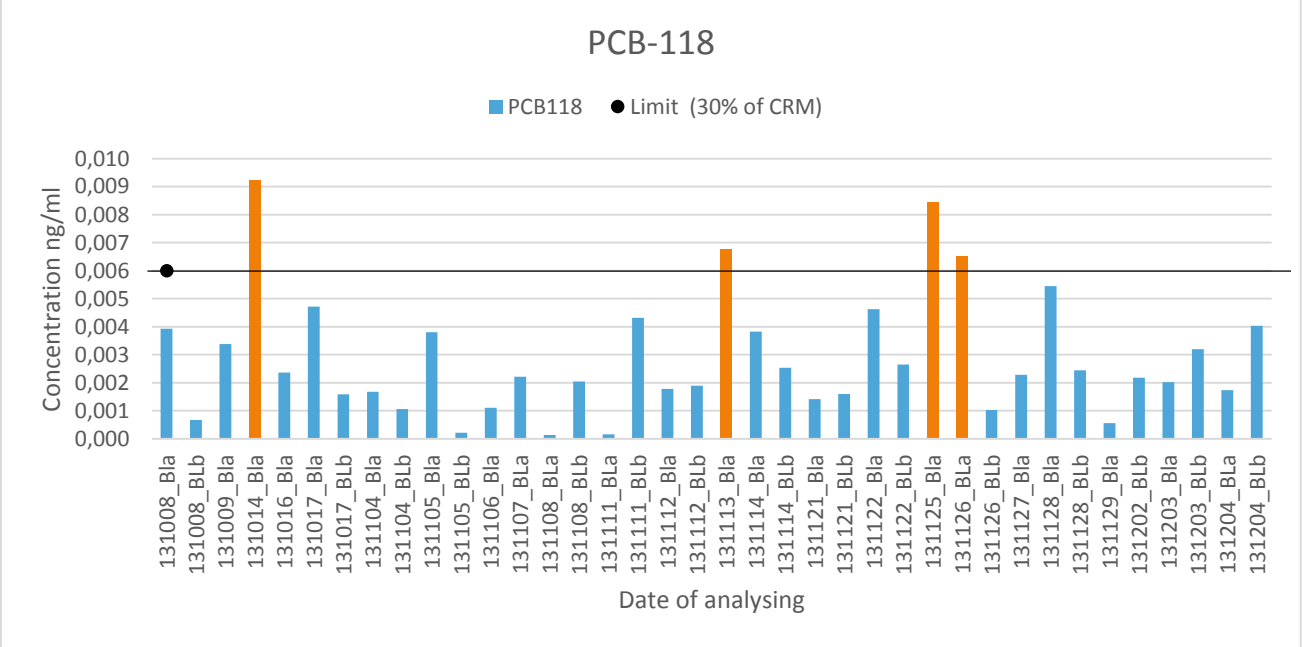

Figure A.8 Blank-values (BL) for PCB-118 in ng/ml for the different sample preparation dates. Parallels are denoted a and b. Line is drawn in representing a concentration of 30% of the certified reference material.

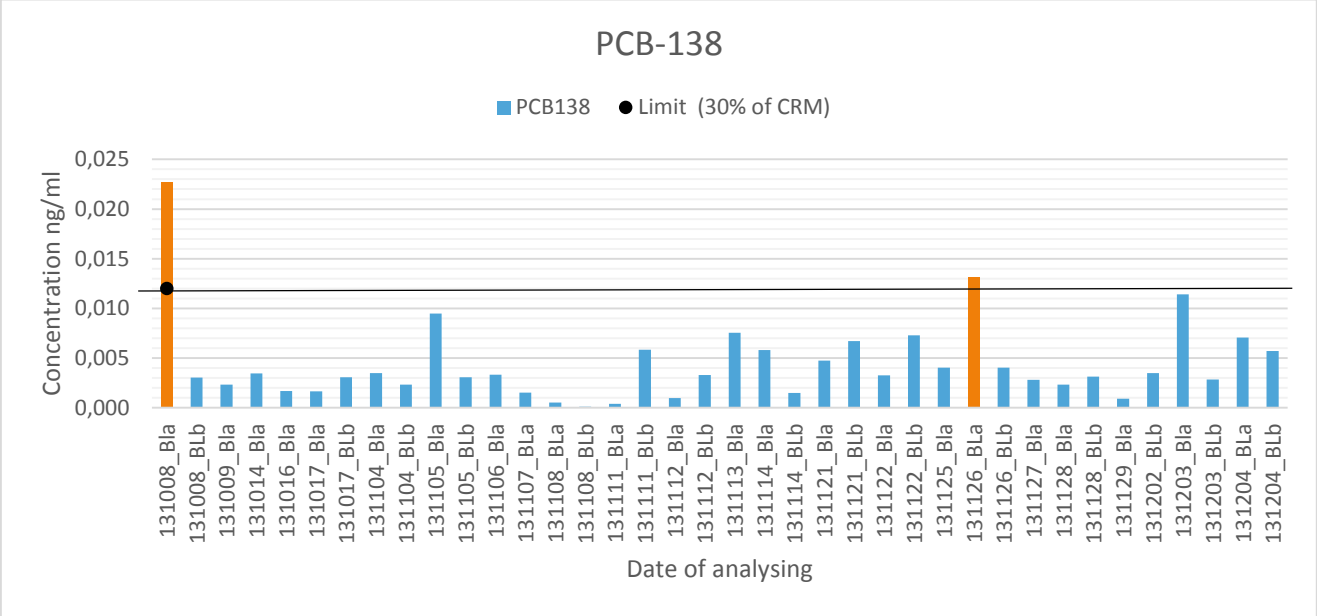

Figure A.9 Blank-values (BL) for PCB-138 in ng/ml for the different sample preparation dates. Parallels are denoted a and b. Line is drawn in representing a concentration of 30% of the certified reference material.

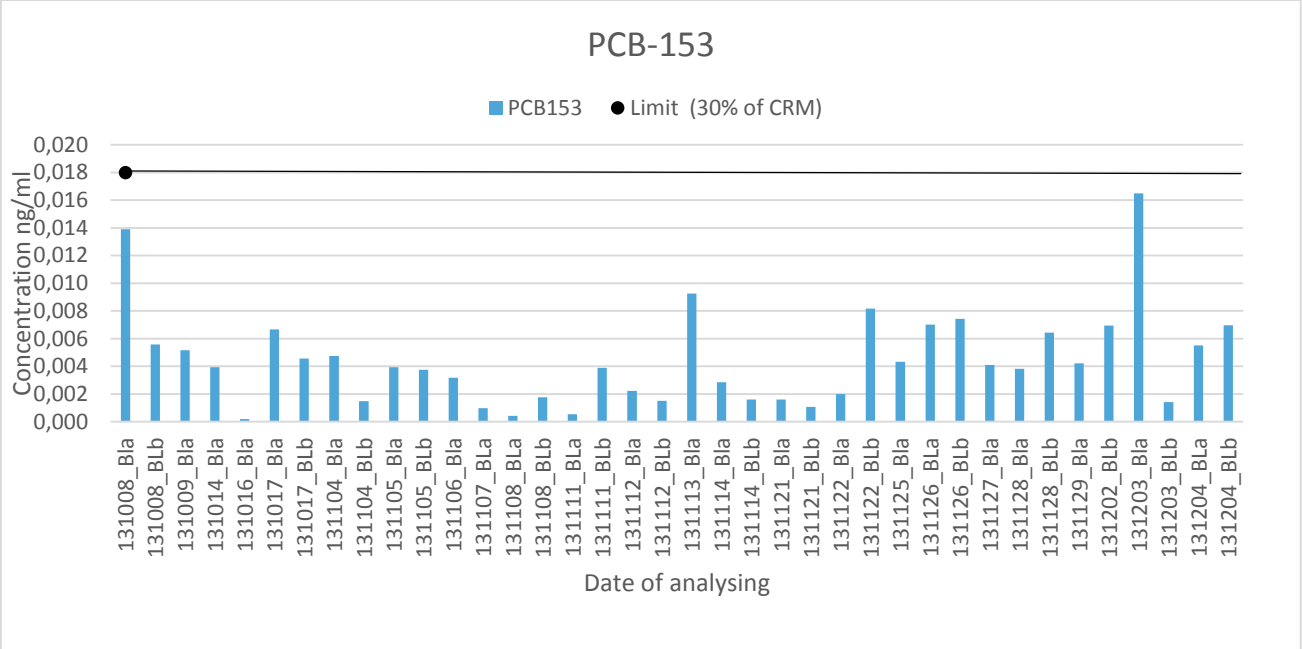

Figure A.10 Blank-values (BL) for PCB-153 in ng/ml for the different sample preparation dates. Parallels are denoted a and b. Line is drawn in representing a concentration of 30% of the certified reference material.

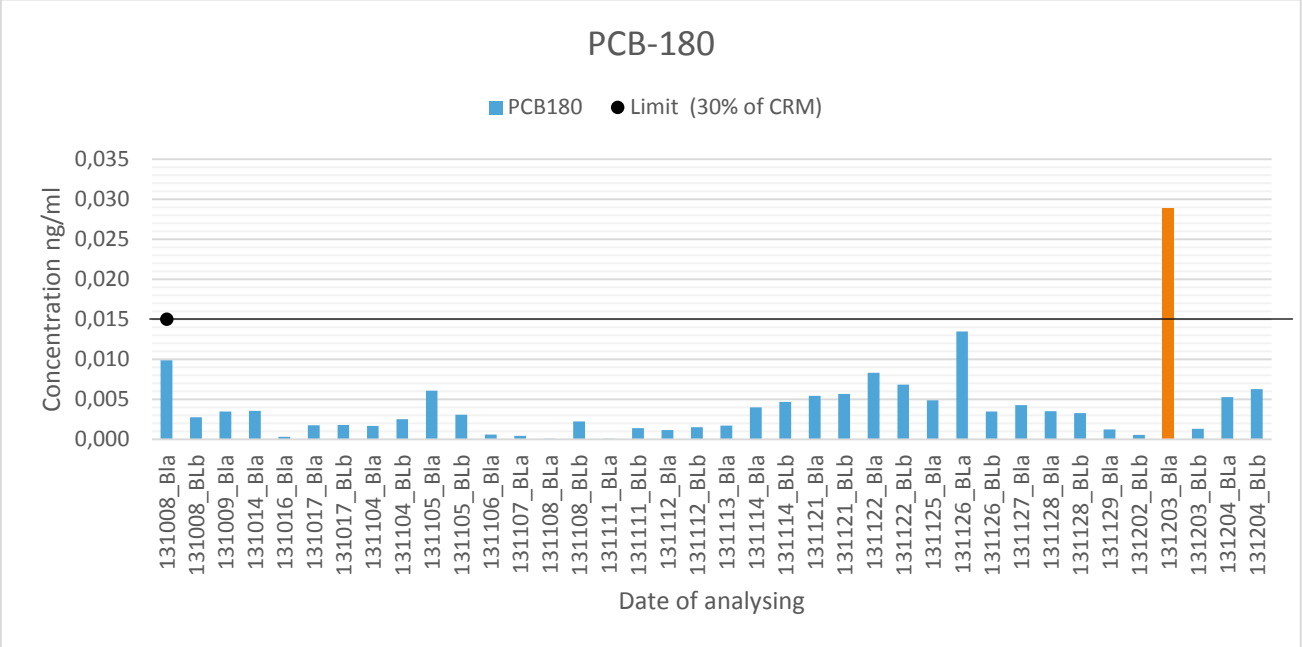

Figure A.11 Blank-values (BL) for PCB-180 in ng/ml for the different sample preparation dates. Parallels are denoted a and b. Line is drawn in representing a concentration of 30% of the certified reference material.

Standard 3 values

Standard 3 results for PCB-118, -138, -153 and -180 from 18.11.2013 to 11.02.2014 (Figure A.15 to A.18.).

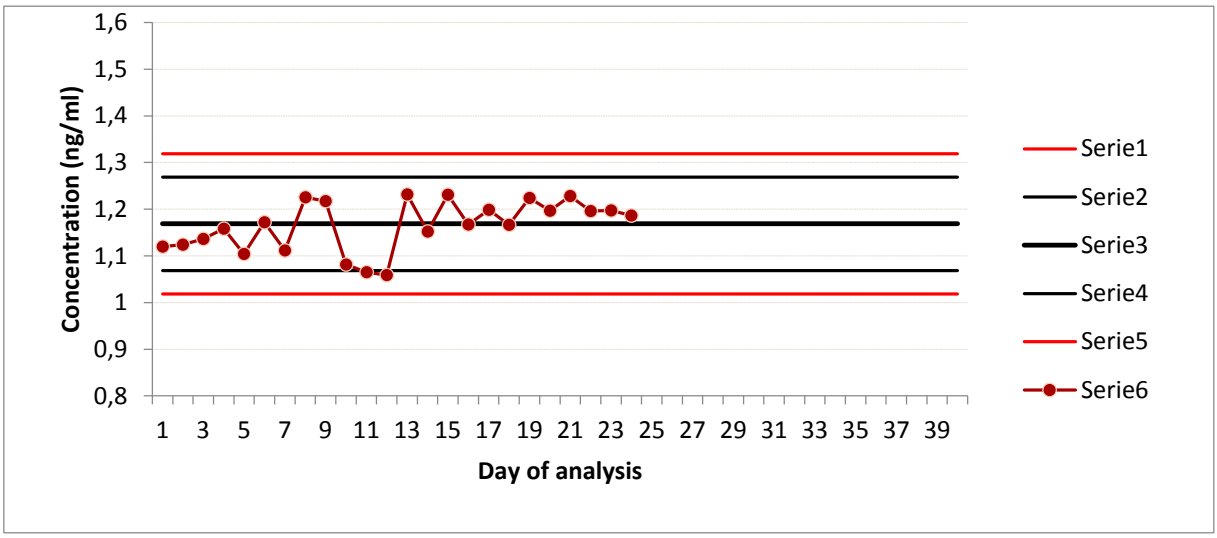

Figure A.12 PCB-118: Standard 3 used to ensure HRGC-HRMS calibration curve. Different analysis days are on the x-axis, concentration in ng/ml on the y-axis. Lines for +/- 2 and 3 standard deviations (sdev) from the true values are plotted in.

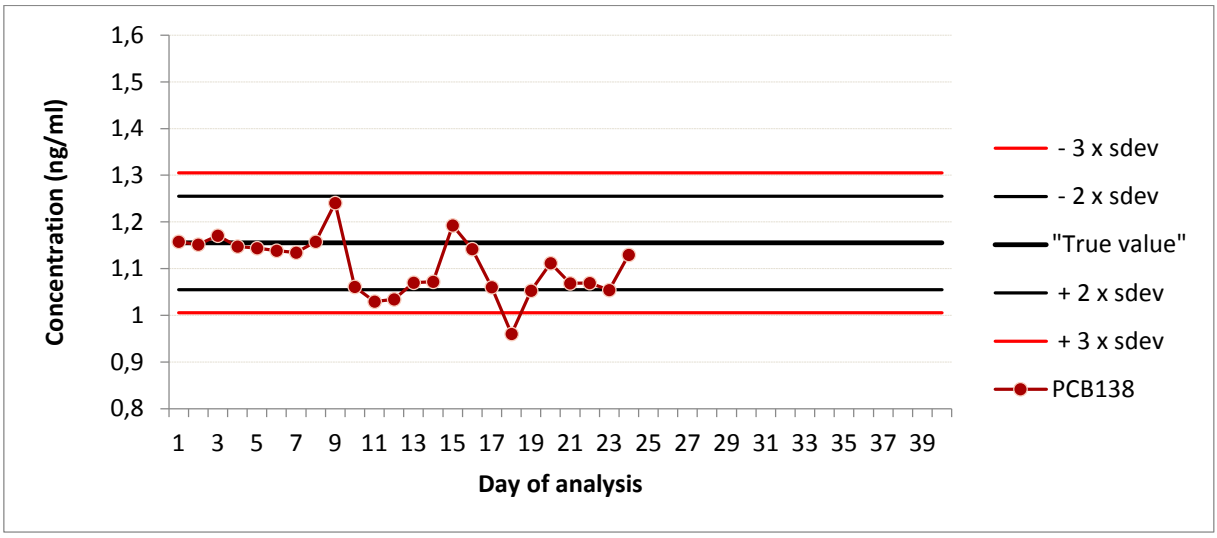

Figure A.13 PCB-138: Standard 3 used to ensure HRGC-HRMS calibration curve. Different analysis days are on the x-axis, concentration in ng/ml on the y-axis. Lines for +/- 2 and 3 standard deviations (sdev) from the true values are plotted in.

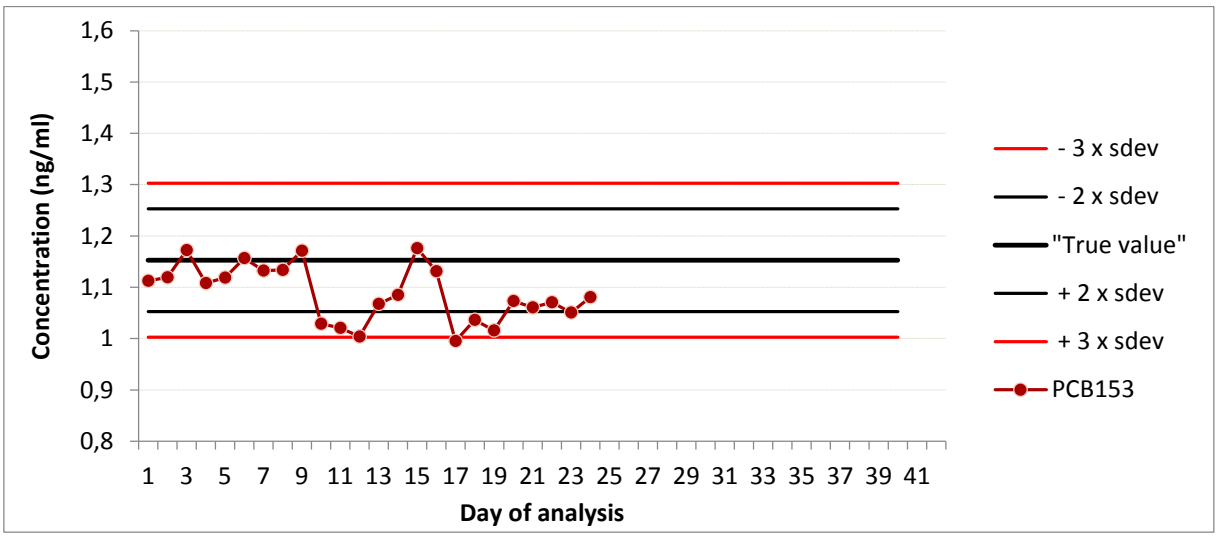

Figure A.14 PCB-153: Standard 3 used to ensure HRGC-HRMS calibration curve. Different analysis days are on the x-axis, concentration in ng/ml on the y-axis. Lines for +/- 2 and 3 standard deviations (sdev) from the true values are plotted in.

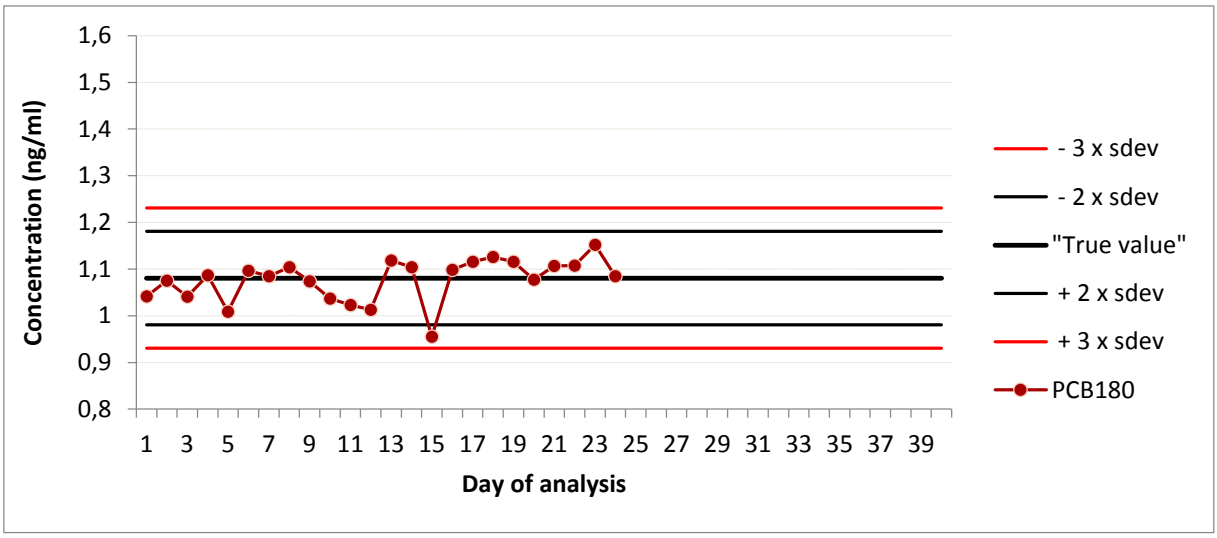

Figure A.15 PCB-180: Standard 3 used to ensure HRGC-HRMS calibration curve. Different analysis days are on the x-axis, concentration in ng/ml on the y-axis. Lines for +/- 2 and 3 standard deviations (sdev) from the true values are plotted in.

Appendix 5: Comparison between present study and other similar studies

Table A.10 Comparison of present project with other similar studies: Concentration of PCB-118, -138, -153, -180 and summed PCB (138+153+180) (Sum PCB) in the present project and other similar studies. Present project: for mothers at 28th week (w) in gestation and for mother and child at 3-, 6-, and 12 months (m) after birth, for mothers showing levels under 30 years (y) of age, over 30 years of age and total. Similar studies: at different times in life

	Reference	Country	Age	PCB-118 (ng/ml)	PCB-138 (ng/ml)	PCB-153 (ng/ml)	PCB-180 (ng/ml)	Sum PCB (ng/ml)
Women	**Present project ***	**Norway**	**28w 19-29y**	**0.02**	**0.07**	**0.13**	**0.08**	**0.3**
			30-41y	**0.03**	**0.07**	**0.16**	**0.11**	**0.3**
			19-41y	**0.03**	**0.07**	**0.13**	**0.08**	**0.3**
	Present project *	**Norway**	**3m 19-29y**	**0.02**	**0.03**	**0.07**	**0.05**	**0.2**
			30-42y	**0.02**	**0.05**	**0.09**	**0.07**	**0.2**
			19-42y	**0.02**	**0.04**	**0.08**	**0.06**	**0.2**
	Present project *	**Norway**	**6m 19-29y**	**0.02**	**0.04**	**0.08**	**0.05**	**0.2**
			30-42y	**0.02**	**0.05**	**0.10**	**0.07**	**0.2**
			19-42y	**0.02**	**0.05**	**0.08**	**0.06**	**0.2**
	Present project*	**Norway**	**12m 19-29y**	**0.02**	**0.05**	**0.08**	**0.05**	**0.2**
			30-42y	**0.02**	**0.05**	**0.08**	**0.06**	**0.2**
			19-42y	**0.02**	**0.05**	**0.08**	**0.06**	**0.2**
Adults	Morck et al., 2014*	Denmark	31-52y	-	0.121	0.181	0.024	0.326
	Schulz et. al., 2011[c]	Germany	20-29y	-	0.6	0.9	0.6	2.0
			30-39y	-	0.9	1.6	1.0	3.2
Infants	**Present project ***	**Norway**	**3m**	**0.03**	**0.06**	**0.1**	**0.06**	**0.3**
	Present project *	**Norway**	**6m**	**0.03**	**0.05**	**0.1**	**0.07**	**0.3**
	Present project *	**Norway**	**12m**	**0.03**	**0.06**	**0.1**	**0.1**	**0.3**
	Walkowiak et al., 2001*	Germany	42 m	-	-	-	-	1.22
	Lackmann, 2002[b]	Germany	Neonate	-	0.5	0.5	0.4	1.4
Children	Grimalt et al., 2010[a]	Spain	4 y	0.10	0.24	0.35	0.20	0.79
	Morck et al., 2014*	Denmark	6-11 y	-	0.086	0.118	0.072	0.276
	Schulz et al., 2011[c]	Germany	7-14 y	-	0.3	0.4	0.3	1.0

**Morck et al. (2014), Walkowiak et al. (Walkowiak et al., 2001), present project: median, ng/ml in serum samples*

[a]Grimalt (Grimalt et al., 2010) : average in serum ng/ml

[b]Lackman (Lackmann, 2002): cord blood: 95th percentile: reference value

[c]Schulz (Schulz et al., 2011): 95% confidence interval of 95th population percentile reference values from the German Human Biomonitoring Commission, whole blood samples