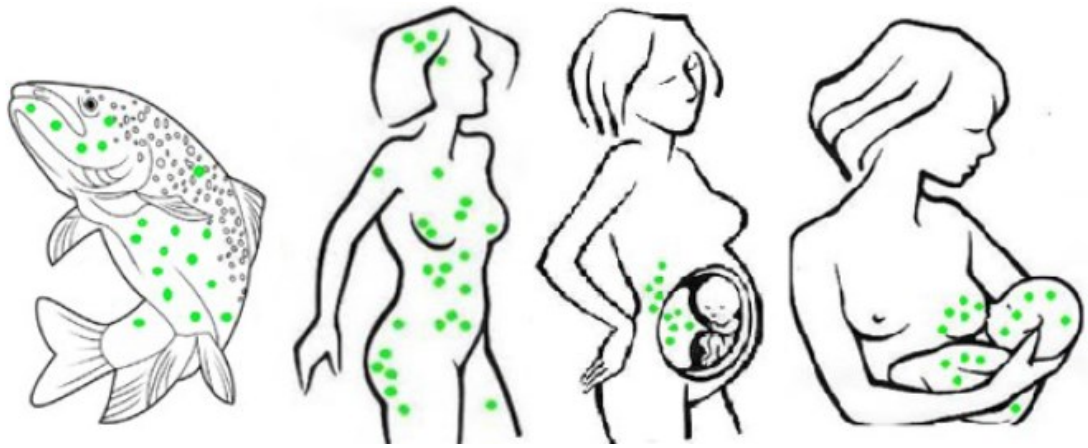
Maternal Seafood Consumption and Serum Levels of *p,p′*-DDE and PCB-153 in Mother and Child



Kaja Sørli

Master Thesis in Human Nutrition

May 2014

Institute of Medicine, University of Bergen (UiB)

National Institute of Nutrition and Seafood Research (NIFES)

Uni Helse

Front page, source (Chemical body burden, 2014)

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Bergen, May 2014

Kaja Sørli

Abstract

Background: While seafood is a natural part of a healthy and balanced diet, it may also contain some undesirable environmental contaminants such as persistent organic pollutants (POPs), which can have detrimental effects on human health. The risk-benefit discussion regarding seafood is under debate, especially for fatty fish. Recently the Norwegian Health Authority advised pregnant women to avoid eating more than two meals of fatty fish per week to reduce foetal exposure to contaminants that could interfere with development. The National Institute of Nutrition and Seafood Research (NIFES) on the other hand stated in a press release that young and pregnant women may eat more than two meals of oily fish per week.

Hypothesis: "A high maternal fish intake correlates with an elevated level of contaminants in serum of mother and child". This hypothesis were followed by specific aims; (1) to assess the mother's seafood intake according to the national recommendations; (2) to assess the study population's contaminant levels; (3) to see if breastfed children show higher serum contaminant levels compared to those who are not breastfed, and (4) to explore if there are other sources of seafood besides fish that contributes to an elevated serum contaminant level?

Method: This study is part of the prospective longitudinal population-based study "Nutrition, Mental Health and Infant Development" that took place in a municipality in Western-Norway. Eating habits were assessed by a short semi-quantitative food frequency questionnaire (FFQ), a 24-hour recall interview and an interviewer administrated FFQ. Blood were collected from mothers in their 28th gestation week and from both mother and child at 3, 6 and 12 months postpartum. By using a method recently established at NIFES 100 µl serum were analysed by High Resolution Gas Chromatography–High Resolution Mass Spectrometry for levels of 2,2',4,4',5,5'-Hexa-chlorobiphenyl (PCB-153) and *p.p'*-dichlorodiphenyldichloro-ethylen (*p.p'*-DDE).

Results and conclusions: Median seafood consumption was estimated to 224 g/week, where oily fish constituted of ~65 g/week. This is a low intake compared to the weekly recommendations. The study population's serum concentration of PCB-153 and *p.p'*-DDE was low compared to other countries. Mothers' PCB-153 and *p.p'*-DDE levels were affected by country of origin and fish liver consumption, while the child's serum concentrations were correlated with breastfeeding prevalence. A high fish intake in mother does not correlate with elevated serum contaminant levels in mother and child.

Sammendrag

Bakgrunn: Selv om sjømat blir sett på som en del av et sunt og balansert kosthold vil det også kunne inneholde noen uønskede forbindelser. Persistente organiske forbindelser (POPs) er en gruppe forbindelser som potensielt vil kunne gi skadelige effekter på menneskets helse ved kraftig eksponering Det er en pågående nytte-risiko debatt angående sjømat, og da spesielt med tanke på fet fisk ettersom den kan inneholde fettløselige POPs. Norske helse myndigheter har nylig rådet gravide kvinner til å unngå og spise mer enn to måltider fet fisk per uke. Dette for å redusere føtal eksponering som kan forstyrre barnets utvikling. Nasjonalt Institutt for Ernæring og Sjømatforskning (NIFES) på den andre siden var ikke enige i dette og uttalte i en pressemelding at unge og gravide kvinner gjerne kan spise mer enn to måltider fisk i uken.

Hypotese: «Et høyt fiskeinntak hos mor kan korreleres med et forhøyet nivå av fremmedstoffer i eget og barnets serum». De spesifikke målene var: (1) å vurdere mors sjømatinntak i forhold til de nasjonale anbefalingene; (2) å vurdere mor og barnas nivåer av fremmedstoffene i forhold til referanse verdier fra andre land; (3) se om ammede barn har høyere nivåer av fremmedstoffene enn de barna som ikke blir ammet; og tilslutt (4) undersøke om det er andre kilder ved siden av fisk som kan bidra til et høyt serum nivå av fremmedstoffene.

Metode: Denne studien er en del av en større prospektiv longitudinal populasjons basert studie kalt «Kosthold, mental helse og spedbarns utvikling» som finner sted i Fjell kommune. Spisevaner ble innhentet ved hjelp av et sjømat frekvensskjema (FFQ), et 24 timers intervju og et intervju basert FFQ. Blod ble samlet inn fra mor i løpet av uke 28og fra både mor og barn ved 3, 6 og 12 måneder etter fødselen. Ved bruk av en metode nylig utviklet på NIFES ble 100 µl 2,2',4,4',5,5'-Hexachlorobiphenyl og *p.p'*-dichlorodiphenyldichloroethylene ekstrahert og analysert av en High Resolution Gas Chromatography – High Resolution Mass Spectrometry (HRGC-HRMS).

Resultat og konklusjon: Sjømatinntaket ble estimert til 224 g/uke, der fet fisk bidro med ~65 g/uke. Dette er godt under de ukentlige anbefalingene. Studiepopulasjonen serums konsentrasjon av PCB-153 og *p.p'*-DDE var generelt lave i forhold til andre lands referanseverdier. Mødrenes PCB-153 og *p.p'*-DDE nivåer ble påvirket av opprinnelsesland og inntak av fiskelever, mens barnas serum konsentrasjon ble påvirket av om de ble ammet eller ikke. Et høyt fiskeinntak hos mor er ikke korrelert med et forhøyet serumnivå av fremmedstoffer hos mor eller barn.

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Abbreviations

ω-3 PUFA	Omega-3 Poly Unsaturated Fatty Acid
BMI	Body mass index
CRM	Certified Reference Material
CVD	Cardiovascular disease
p.p'-DDE	*p.p'*-dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
df	Degree of freedom
DFS	Double Focusing System
DHA	Docosahexaenoic acid
dl-/ndl PCBs	Dioxin-like/non-dioxin like polychlorinated biphenyls
EFSA	European Food Safety Authority
EPA	Eicosapentaenoic acid
EEA	European Environment Agency
FFQ	Food Frequency Questionnaire
HBS	Household Budget Surveys
HRGC-HRMS	High Resolution Gas Chromatography-High Resolution Mass Spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
MeHg	Methyl mercury
MoBa	Norwegian Mother and Child Cohort Stud
NIFES	National Institute of Nutrition and Seafood Research
PCB	Polychlorinated biphenyl
PCB-153	2,2',4,4',5,5'-Hexachlorobiphenyl
rpm	Revolutions per minute
RV_{95}	Reference value from the German Human Biomonitoring Committe
SPSS	Statistical Package for Social Sciences
TWI	Tolerable Weekly Intake
VKM	Norwegian Scientific Committee for Food Safety
UNEP	United Nations Environment Programme
WHO	World Health Organization

1 Introduction

1.1 Background

Based on current risk-benefit reports on fish consumption from Denmark (Fødevaredirektoratet, 2003), UK (SACN, 2004), Europe (EFSA, 2005a), Norway (VKM, 2006), US (CNRS, 2007) and Sweden (Livsmedelverket, 2007) it is evident that fish and seafood are part of a healthy and balanced diet. The distribution of nutrients varies in different types of seafood, depending on factors such as the nutrition for the particular organism, and its fat content. The Norwegian consumption of fish and other seafood is different from many other countries in that both the total seafood consumption, aswell as the consumption of fatty fish in the form of cold cuts and spread, is high.

The Norwegian Directorate of Health recommends eating 300-450 grams (g) of fish weekly, where of 200 g should come from oily fish (Directorate of Health, 2011). This corresponds to two to three dinner portions per week. Average daily fish consumption in Norway is approximately 65 g/day for women and 75 g/day for men (Meltzer et al., 2002; Totland, 2012). Fatty fish accounts for 1/3 of the seafood intake, while fish spread (mostly fatty fish species) accounts for 14 %. There are large differences in the distributions of fish consumption. Young women, ages 18 to 29, eat less fish than the rest of the adult population, approximately 36 g per day, which corresponds to 1.6 meals per week (Totland, 2012). These are women in their most fertile age, and a research study performed by Bloomingdale and co-workers (2010) have shown that young women generally have a lack of knowledge regarding the importance of seafood consumption during pregnancy. This can often be traced back to the media's vast coverage of toxicants in seafood (Dagbladet, 2013; Greiner et al., 2010; IFIC, 2007; NRK, 2013; VG, 2013). In mid June 2013, after a big media storm regarding contaminants in farmed salmon, the Norwegian Directorate of Health published a clarification containing dietary advice of fish intake (Directorate of Health, 2013). This publication stated that young and pregnant women should, over time, avoid eating more than two meals of oily fish per week, to reduce fetal exposure to contaminants. The National Institute of Nutrition and Seafood Research (NIFES) on the other hand stated

that young and pregnant women may eat more than two meals of oily fish per week (NIFES, 2013a). The Norwegian health authorities based their announcement on a report with contaminant content found in farmed salmon in 2004 (VKM, 2006), while NIFES stated that the present (2013) content of environmental pollutants in oily fish does not support a limit of two meals of oily fish a week as a decline is observed through yearly monitoring the last decades. The introduction of vegetable feed to farmed fish have been one of the contributors to the observed decline (NIFES, 2013b). A revision of the report from 2006 is now in press and will be published in the fall of 2014 (VKM, 2014).

1.2 Health effects of seafood

Seafood is a unique dietary source to several important macro- and micronutrients that are reported to have beneficial effects on human health. Seafood contains marine omega-3 polyunsaturated fatty acids (ω-3 PUFAs); eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA), high quality proteins, vitamin D, vitamin B_{12} and trace minerals such as selenium and iodine (NIFES, 2013c; VKM, 2006). However, seafood may also contain contaminants such as dioxins, polychlorinated biphenyls (PCB), brominated flame-retardants (BFR), polycyclic aromatic hydrocarbons (PAHs) and multiple heavy metals (NIFES, 2013c) (**Figure 1.1**).

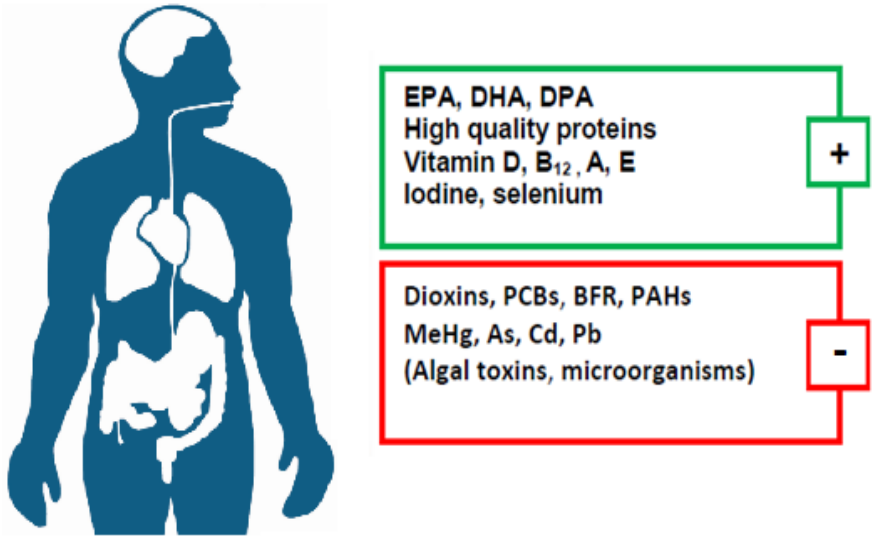

Figure 1-1: *Nutrients and contaminants found in seafood. Listings of the positive (green box) and negative (red box)(NIFES, 2013c; VKM, 2006).*

Epidemiological studies (Dyerberg et al., 1978; He et al., 2004; Whelton, 2004) show that seafood is an important factor in the prevention of cardiovascular diseases (CVDs). The intake of marine ω-3 PUFAs may reduce the risk of CVDs and complications by improving artery flexibility, preventing formation of blood clots, reducing blood pressure and stabilizing the heart rhythm. Research has shown that in addition to being an important source combating CVDs, marine ω-3 PUFAs possess anti-inflammatory effects (Galli & Calder, 2009) and may play a role in reducing the depression rate (Hibbeln et al., 2007; Markhus et al., 2013) diabetes mellitus (Djousse et al., 2011), and cancer (Norat et al., 2005; Rose & Connolly, 1999).

Seafood is regarded a good source of protein, as all the essential amino acids are present. The protein content in fish is at a fairly stable level, around 15-20 g proteins/100 g fish (VKM, 2006). In addition to the macro nutrients already mentioned, seafood is a good source of several vitamins, minerals and trace elements (**Table 1-1**).

***Table 1-1**: Micro nutrients found in seafood.*

Micro nutrient	Function	Reference
Vitamin D	Essential for bone metabolism. Potential role in immune system, brain development. Prevention of cancer and mental diseases.	(Holick, 1996, 2007)
Vitamin E	Antioxidant in especially fatty tissue.	(Traber & Atkinson, 2007)
Vitamin A	Important for a range of functions like eyesight, immune responses, growth and development.	(Thorne-Lyman & Fawzi, 2012)
Vitamin B_{12}	Important role in energy metabolism.	(Huskisson et al., 2007)
Iodine	Component of the thyroid hormones. Necessary for the neural maturity and development of the fetal brain.	(Dahl et al., 2004; Zimmermann, 2009)
Selenium	Cofactor in the enzyme glutathione peroxidase. Part of selenoproteins. Detoxification of certain heavy metals.	(Folven et al., 2009)

Several environmental contaminants are present in low concentrations in oceans, seas, lakes, rivers and sediments. Seafood may therefore represent a major source of human exposure to contaminants. Besides organic persistent pollutants (POPs), which is elaborated in section 1.3, mercury, in the form of methyl mercury (MeHg), is the contaminant that has elicited most concern among consumers of seafood (CNRS, 2007). MeHg is not lipophilic and is thus present in the largest concentrations in the muscle tissue rather than in fat deposits (Olsvik et al., 2011). Metal contaminants such as lead, manganese, chromium, cadmium, and arsenic may be present in seafood,

although on population basis, seafood consumption does not appear to be a major route of exposure to these metals (CNRS, 2007). Seafood can also contain certain microbiological organism, viruses, toxins or parasites, which can be harmful for the human body. However, this is mostly due to improper treatment of the foodstuff (CNRS, 2007).

1.3 Persistent organic pollutants (POPs)

The United Nations Environment Programme (UNEP, 1999) defines POPs as "chemical substances that persist in the environment, bio accumulate through the food web, and pose a risk of causing adverse effects to human health and the environment". POPs are, by definition, persistent, a property that generally is correlated to their chemical stability. The chemical stability originates from the presence of aromatic systems in which one or more hydrogen atoms are substituted by halogens (Miniero et al., 2005). The carbon-halogen bond is very resistant to hydrolysis, and the larger the number of halogen atoms, the greater the resistance to biological, chemical and photolytic degradation, and therefore more toxic. POPs are typically 'water-hating' and 'fat-loving' chemicals, i.e. hydrophobic and lipophilic (Jones & de Voogt, 1999). In organisms, they will partition into lipids and be stored in fatty tissue, making bioaccumulation possible (Miniero et al., 2005) (**Figure 1-2 A**).

Organic persistent substances are subject to accumulation in the food web described as biomagnification (Miniero et al., 2005) (**Figure 1-2 B**). This means that these molecules are found at higher concentrations in animals at the highest levels of the food chain. Predatory animals eat hundreds of times their own weight in their prey and thus the persistent chemicals accumulate to concentrations far higher in the predators than in their prey. This includes fatty fish such as herring, mackerel, salmon and trout, but also piscatorial birds and humans (Kvalem et al., 2009). Muscle tissue is less contaminated, depending on the fat content of the muscle, which is likely to be greater in the older, larger, and oily fish (CNRS, 2007).

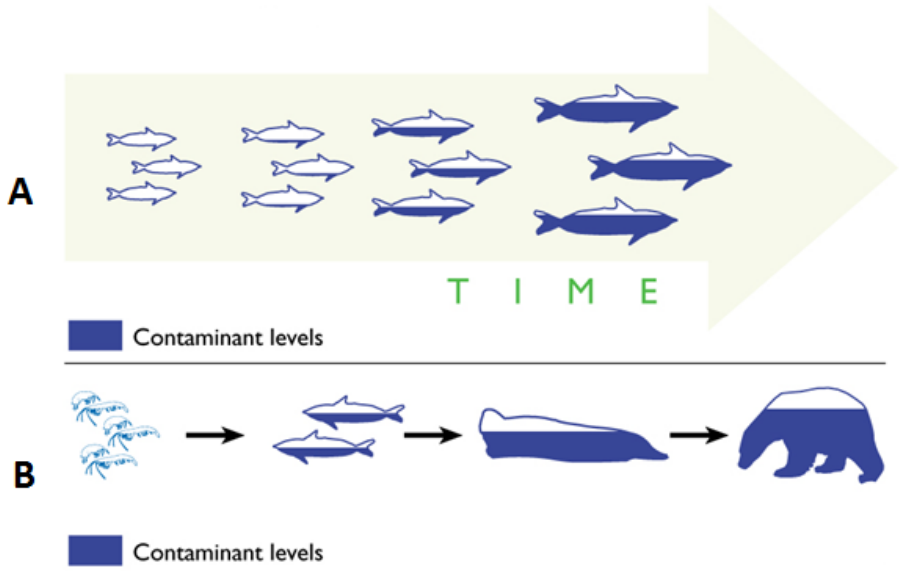

Figure 1-2: *A: Bioaccumulation: If the absorbation rate is greater than the excretion rate POPs will partition into lipids and be stored in fatty tissue.* ***B****: Biomagnification: POPs are found at higher concentrations in animals at the highest levels of the food chain. Modified figure (MIT, 2013).*

POPs have the tendency to enter the gaseous state under atmospheric pressure and varm temperatures (Jones & de Voogt, 1999). Consequently, they may vaporize from soils, vegetation and water bodies into the atmosphere and – because of their resistance to breakdown reactions in the air – travel long distances before they are deposited. Pollutants vaporize at warm temperatures and are carried by winds to polar regions where they condense and accumulate in the local food web (Gouin et al., 2004; Miniero et al., 2005). Hence, POPs can be found in every part of the world, even in areas where human activities are almost completely absent. This phenomenon is better known as global distillation or as the grasshopper effect (Gouin et al., 2004).

POPs can be divided into two categories. Either they are intentionally produced for one or multiple purposes, or they are unintentionally formed as by-products in other (industrial) processes (Breivik et al., 2004). Further exposure occurs from leaks from transformers and capacitors, volatilization of POPs in cities and buildings, from sewage, landfills and waste sites, and from combustion of materials containing POPs (Dyke et al., 2003; IARC, 2012). It is difficult to assess and evaluate the relative importance of primary emissions of POPs, versus the environmental recycling of previously emitted chemicals (Breivik & Alcock, 2002).

Because POPs persist in the environment and have the capacity to travel long distances from the point of release, concerted measures are necessary in order to minimise further environmental exposures (Breivik & Alcock, 2002; Miniero et al., 2005). Two international legally binding treaties have, to this day, been negotiated and finalized: the global Stockholm Convention on POPs (2009) that entered into force on May 17th 2004, and the Protocol to the Regional United Nations Economic Commission for Europe (UNECE) Convention on Long-Range Transboundary Air Pollution on POPs that entered into force October 23rd 2003. In the period 1990 to 2011 the European Environment Agency (EEA) reported that PCBs have decreased by roughly 73 % while dioxins and furans have decreased by 84 % (**Figure 1-3**) (EEA, 2013).

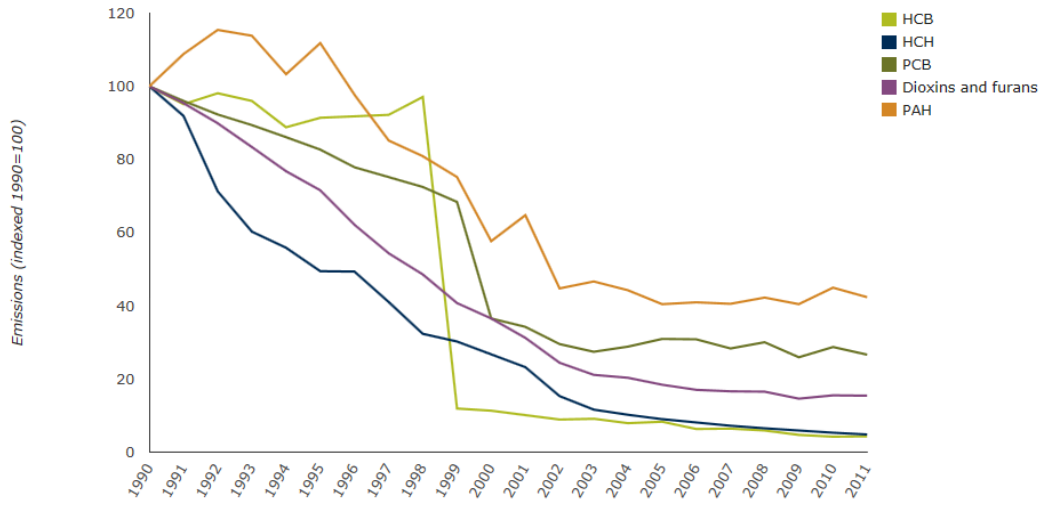

Figure 1-3: *Emission trends of POPs the past years (1990-2011) (EEA, 2013).*

The toxicity of POPs is more related to the body burden than to recent dietary exposure (Papadopoulou et al., 2013).The body burden is, as stated by VKM (2013) "the total amount of a substance in the body, expressed per kilo body weight". In an individual with constant exposure, equilibrium between intake and excretion will be reached after three to five half-lives, and the body burden is thereafter constant (Hansen, 1998). Children are born with approximately the same blood concentration as their mothers (VKM, 2013). After birth the blood concentration increases with their dietary exposure (Lackmann et al., 2005; Patandin et al., 1999a). This increase is, however, not as steep as adults would have had with the same contaminant intake (VKM, 2013). This is because of the child's body weight gain and change of body composition. Exposure duration in childhood is too short to reach a steady state (VKM, 2013).

1.4 Chlorine substituted organic compounds

The greatest concern with organic contamination in relation to exposure from fish and other seafood is POPs; especially the chlorinated substituted organic compounds. These include polychlorinated dibenzodioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCBs), dichlordiphenyltrichlorethane (DDT) and its metabolites (DDE and DDD), chlordane and dieldrin to mention a few. All these are under supervision of the EEA and UNEP. The most severe effects of exposure (both acute and long-term) to chlorinated organic compounds is linked to dioxins and dioxin-like PCBs (dl-PCBs), but also pesticides and non-dioxin like PCBs (ndl-PCBs) have shown detrimental effects (VKM, 2006). The tolerable weekly intake (TWI) levels are the amounts that can be consumed safely throughout a person's lifetime without any significant risk of adverse health effects (WHO, 2006). The TWI for dioxins and dl-PCBs is set to 14 pg TEQ[1]/body weight/week (WHO, 2006). It is not possible to distinguish between the effects resulting from ndl-PCBs alone because of the simultaneously exposure to dioxin-like compounds that hampers the interpretation of the results. The European Food Safety Authority's (EFSA) Scientific Panel on Contaminants in the Food Chain have because of this concluded that it is not possible to establish a TWI level for any ndl-PCBs (EFSA, 2005b). Since no human data is available, quantitative risk assessment is based on animal studies. Cases in which the TWI has been derived from animals a default uncertainty factor of 10 is used for extrapolation from animals to humans and a factor of 10 is used for variations among humans (100 altogether). Many children, especially the youngest, exceed the TWI for dioxins and dl-PCBs when considering the total diet. This is because childrens intake of contaminants per kg body weight is greater for children than adults.

More than 90 % of the global human exposure to POPs is via food (EFSA, 2005b; VKM, 2006).The largest contributors in the Norwegian diet are semi-oily and oily fish, where of raw cod liver oil, cod liver roe pâté (known as lofot- and svolværpostei) and herring gull eggs have the highest levels of these particular compounds (Kvalem et al., 2009). The latter are however only consumed by a very small percentage of the population and on a seasonal basis.

[1] TEQ = Toxic Equivalent. Report the toxicity-weighted masses of mixtures of PCDD/Fs, and PCBs

1.4.1 Selected POPs

Chlorine substituted organic compounds are the contaminants studied in this thesis, with special attention given to the pesticide metabolites *p.p'*-dichlorodiphenyldichloroethylene (*p.p'*-DDE) and the ndl-PCB, 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153). Both compounds occur in relatively high concentrations in serum and a large number of analysis have shown to find the congeners with good precision and high accuracy. Some important information regarding these particular compounds is given in the upcoming sections, but first some general information on pesticides and PCBs is given.

Pesticides are a large group of chemical or biological agents used to prevent weeds, mould, insects and other pests from growth and propagation (VKM, 2006). Many of these substances went out of use several decades ago, but are still found in the environment as a result of their long decomposition time and the fact that they are relatively fat-soluble (Breivik et al., 2002). The Stockholm convention on Persistent Organic Pollutants have categorised 9 of the 12 most dangerous and persistent organic chemicals as pesticides (Stockholm Convention, 2009). Pesticides can be grouped into different chemical families based on target pest group, chemical structure, physical state or their mechanism of action.

PCBs consist of 209 congeners, were 12 of these are considered dioxin-like (VKM, 2006). PCBs were widely used from the 1930's to the 1980's and later, with an estimated total production of about 1.3 million metric tons (Breivik & Alcock, 2002; Breivik et al., 2002). In Norway PCBs have been restricted to closed systems since 1971, and a ban was imposed in 1979 (Skaare et al., 1988). The presence of PCBs is often calculated on the basis of the sum of three ndl-PCBs (PCB-138, 153 and 180) or PCB6, which is the sum of six ndl-PCB (PCB-28, 52, 101, 138, 153 and 180).

Both dl-PCBs and non-dl-PCBs bioaccumulate to varying extent and can cause a number of acute and chronic toxicological effects in the human body. This was evident after two mass poisoning incidents of rice bran cooking oil in Japan and Taiwan (Furue, 2005), and from consumption of polluted fish from Lake Michigan (DeVault et al., 1988; Hanrahan et al., 1999), respectively. The effects from PCBs poisoning ranges

from chloracne, weight loss and skin mucosa to neurological symptoms such as memory loss, numbness and neuralogia of the limbs in adolescents and adults (Furue, 2005). Other effects have been observed in the liver and thyroid gland (Patandin et al., 1999b; Walkowiak et al., 2001) . The International Agency for Research on Cancer (IARC, 2012) has classified PCBs as probably carcinogenic to humans (Group 2A). The most important health effect associated with PCB exposure is the one associated with the perinatal period (Hibbeln et al., 2007; Longnecker et al., 2005; Torres-Sanchez et al., 2008). PCB exposure in this period may cause impairment of reproduction, delayed development of the central nervous system and a reduced immune system in the infant (Patandin et al., 1999b). Perinatal exposure have also been associated with lower psycho motor scores (Rogan et al., 1986) and detrimental effects on cognitive functions (Darvill et al., 2000; Walkowiak et al., 2001).

p.p'-Dichlorodiphenyldichloroethylene (p.p'-DDE)

p.p'-DDE is a chemical compound that is formed by the release of a hydrogen chloride (HCl), in a prosess called dehydrohalogenation, from the more known compound dichlorodiphenyltrichloroethane (DDT, $C_{14}H_9Cl_5$) (**Figure 1-5 A**). DDT is an organochlorine pesticide, first synthesized in 1874, that was widely used during the Second World War (WWII) to control malaria and typhus among the troops and civilians (ATSDR, 2009). It operates by disrupting the sodium/potassium (Na^+/K^+) balance of the nerve fibre, forcing the nerve to transmit continuously (ATSDR, 2009). Since WWII it has been estimated that 1.8 million tonnes have been released into the environment (U.S. Department of Health and Human Services, 2002). The use of DDT as a disease vector control is still allowed in the most endemic areas until safe, effective, and affordable alternatives become available (ATSDR, 2009; Stockholm Convention on Persistant Organic Pollutants, 2008).

DDT and its main metabolite, *p.p'*-DDE, have much of the same chemical and physical properties (U.S. Department of Health and Human Services, 2002). *p.p'*-DDE is more persistent than DDT, which means serum *p.p'*-DDE levels may be an indicator of historic exposure and may be higher than the DDT levels in the same person (ATSDR, 2009). The reported half-life of *p.p'*-DDE is 5.7 years, and a steady state in the body will not be reached until approximately 20 years, of constant exposure, have passed. DDT is besides seafood, also found in eggs and milk (EFSA, 2010) (**Figure 1-4**).

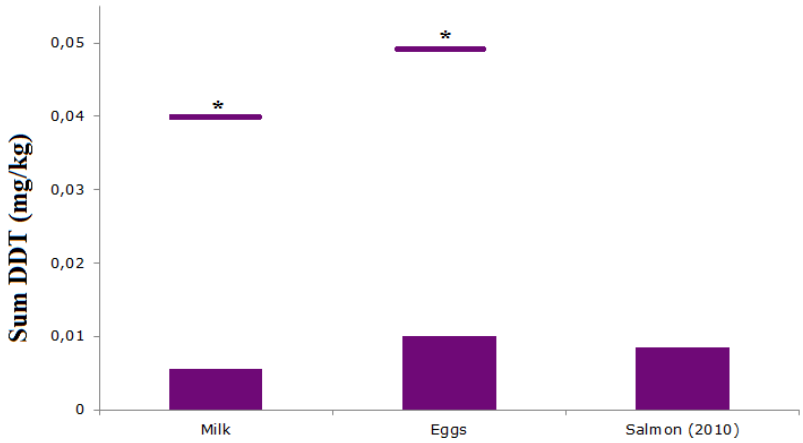

***Figure 1-4**: Sum DDT (mg/kg) found in milk, eggs and salmon. Asterisk (*) denotes EU's upper limit at 0.04 and 0.05 for milk and eggs, respectively (EFSA, 2010; NIFES, 2013c). No limit is set for salmon.*

In humans, studies have indicated that *p.p'*-DDE is a weak anti androgen (Gray et al., 2001; Torres-Sanchez et al., 2008), an endocrine disruptor (Kezios et al., 2013), have genotoxic effects (De Coster et al., 2008; Ennaceur et al., 2008) and is also linked to diabetes (Ruzzin et al., 2010). As for carcinogenicity, it is classified by the IARC as a "possible" human carcinogen (IARC, 2012). Epidemiologic studies of children with environmental exposure to *p.p'*-DDE have not demonstrated neurologic or developmental abnormalities (Jusko et al., 2006; Longnecker et al., 2002; Mariussen & Fonnum, 2006). A risk of preterm delivery, and therefore a major contributor to infant mortality, may however be related to maternal *p.p'*-DDE levels (Longnecker et al., 2001).

2,2',4,4',5,5'-Hexachlorobiphenyl - PCB-153

PCB-153 is a ndl-PCB which has its 6 chlorine atoms distributed in di-ortho positions (**Figure 1-5 B**). This means it will get a non-coplanar steric conformation and will not be able to interact with the dioxin-receptor. PCB-153 is one of the more persistent PCB congeners (half-life 14.4 years), and the most abundant in both human serum, fat tissue and breast milk (Herrick et al., 2011; Ritter et al., 2011). Because of the long half-life it will not reach steady state before more than 40 years of constant exposure have passed. Its biggest source of entry is through the diet, particularly fatty fish. PCB-153 accounts for nearly 15 % of the total PCB burden determined in human serum (Lin et al., 2013), and have shown the highest carry-over into breast milk of all the PCB congeners with 27 % (EFSA, 2005b). In this study PCB-153 is used as a biomarker of

PCB exposure because the concentration of this PCB congener are relatively high and highly correlated with the total molar concentration of PCBs (Govarts et al., 2012; Needham et al., 2011; VKM, 2013). A study performed by Lee and co-workers (2006) have shown a strong association between serum levels of PCB-153 and diabetes. Prenatal exposure to di-ortho PCBs has been associated with lower birth weight (Govarts et al., 2012; Papadopoulou et al., 2013). A decreased sperm motility have also been correlated with high PCB-153 serum concentration in Swedish men (Richthoff et al., 2003).

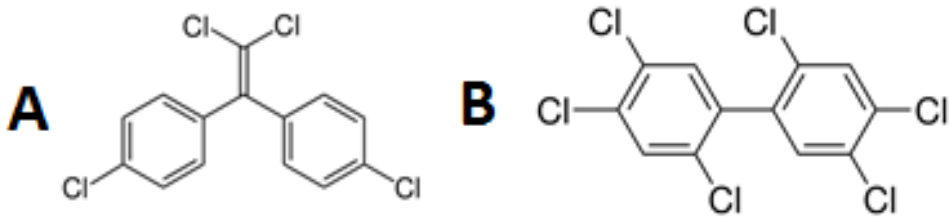

Figure 1-5: ***A****: p.p'-DDE: formed by the release of HCl from the more known compound DDT.* ***B****: PCB-153: chlorine atoms distributed in di-ortho positions, and have a non-coplanar steric conformation. Modified figure (Sigma-Aldrich, 2014).*

1.4.2 Bio monitoring

Bio monitoring studies (Anderson et al., 1998; Saoudi et al., 2014; Schulz et al., 2009) on contaminant levels provide public health organizations in the applicable countries/areas with reference values that indicate the upper margin of background exposure to a given pollutant in a given population at a given time (ATSDR, 2009; Schulz et al., 2009). Although biomonitoring studies do not represent toxicologically derived biological exposure limits, they can be used as criteria to classify the measured values as being "elevated" or "not elevated". Germany have come a long way in monitoring the contamination concentration in human biological media in the population. **Table 1-2** shows the reference values (RV_{95}) for PCB-153 and *p.p'*-DDE in whole blood (ng/ml). The lipid fraction (where the POPs are located) should not be affected by the reduction of whole blood, making a comparison to serum possible. The RV_{95}, set by the Commission, is based on the 95 % confidence interval for the 95th population percentile. The German Human Biomonitoring Commission states that if a sample exceeds the PV_{95}, it stands out from the general population and possible explanations should be checked. The RV_{95} set for *p.p'*-DDE is based on values gathered in what was formerly known as West Germany as Norway banned the use of DDT at approximately the same time, in 1972 and 1970 respectively (Schulz et al.,

2009; SNL, 2009a). In the former East Germany DDT was used for a much longer time, and shows values that are nearly threefold higher (Schulz et al., 2009).

***Table 1-2**: Reference values (RV_{95}) for PCB-153 and p.p'-DDE in whole blood of children and adults (Schulz et al., 2011)*

	Country	Age	PCB-153 (ng/ml) RV_{95}	*p.p'*-DDE (ng/ml) RV_{95}
Children	Germany	7-14	0.4	0.7
Adults	Germany	20-29	0.9	2.0
	Germany	30-39	1.6	4.0

Nøst and co-workers (2013) published a longitudinal study addressing the concentrations of contaminants in Norwegian men's serum the past 30 years. These scientists found that the decline of POPs in the men's serum between 1979 and 2009 are consistent with reduced environmental exposures in this period (**Figure 1-3**). A 68 % decrease in summed POP concentration was seen in Norwegian men from 1979 to 2007. The relative contributions of individual POPs to summed POP concentration (as percentage) are shown in **Figure 1-6**. The most prominent congeners were *p.p'*-DDE, PCB-153, PCB-138/163, PCB-180 and HCB, respectively (Nost, et al., 2013). The *p.p'*-DDE/*p.p'*-DDT ratio started at 12 in 1979 and ended at 55 in 2009 supporting the fact that *p.p'*-DDE is more persistent than *p.p'*-DDT and that it is therefore the result of old DDT exposure (ATSDR, 2009).

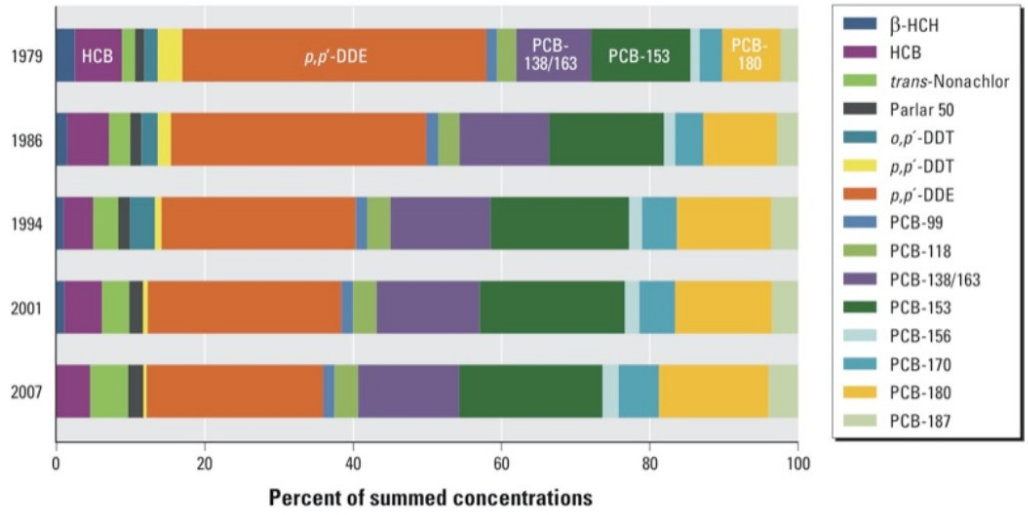

***Figure 1-6**: The relative contributions of individual POPs to summed POP concentration (Nost, et al., 2013). A 68 % decrease in summed POP concentration was seen in Norwegian men from 1979 to 2007. p.p'-DDE shows the biggest percentage decline.*

1.5 Pregnant and lactating women

Lately, there has been an increased interest in the relationship between nutrition during pregnancy and its correlation with growth, foetal development and the onset of diseases later in life (Moore & Davies, 2005). The wellbeing of mother, and complications associated with pregnancy are also affected by dietary factors (Meltzer et al., 2011; Oken et al., 2007). Breast milk is considered to be the optimal infant diet as it contains all nutrients to varying degrees. However, the composition of the breast milk is affected by the mothers' nutritional status, and the composition is therefore individual (Directorate of Health, 2012). The postpartum period represents a time when the maternal diet needs to meet physiological demands associated with lactation. Estimated nutrient requirements are considerably higher when breastfeeding than during time of pregnancy (Picciano, 2003). Research on nutritional status of postpartum women is however limited (Picciano, 2003).

Because POPs have lipophilic properties they are rarely excreted from the human body, and therefore the body levels of POPs tends to rise throughout life (Jones & de Voogt, 1999; Miniero et al., 2005). One exception is the excretion of POPs into the lipid rich breast milk (Jonsson et al., 2005; Lackmann et al., 2004; Verner et al., 2013). The importance of breast milk as an excretory route for women was pointed out in studies showing the fact that the body burden in multiparous women was lower than in the primi- or nulliparous women, and also when comparing men and womens serum contaminant levels (EFSA, 2005b; WHO, 2003). From 1986 to 2005 the breast milk concentrations of ndl-PCB 6 and ΣDDT has decreased by approximately 70-90 % (**Figure 1-7**). In Norway, exclusively breastfeeding is recommended the first 6 months. However, the occurrence of exclusively breastfeeding declines rapidly from 3 months onwards, reaching only 9% at 6 months (VKM, 2013).

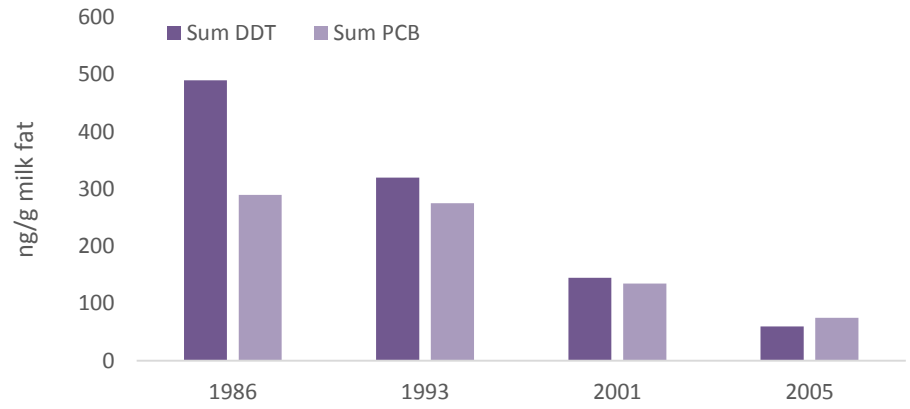

***Figure 1-7**: PCB 6 (ndl-PCBs) and ΣDDT in breastmilk samples from Norwegian primiparous women collected in 1986, 1993, 2001 and 2005 as part of the WHO surveillance programme on breast milk (Norwegian Institute of Public Health, 2009).*

Besides breast milk, nutrients and toxic chemicals can be transferred over the placental barrier, which causes the human exposure to POPs to start already in the uterus (Rogan et al., 1986). This transfer is similar to transfer across other biological membranes, and is known to increase as the fetal growth rate increases (Murphy et al., 2006). The placenta may prevent transfer of some pollutants but there is evidence that POPs, even the ones with high molecular weight can reach the foetuses (Needham et al., 2011; Vizcaino et al., 2014). The different PCB congeners cross the barrier to various extent, with the ones with the lowest amount of chlorine substituent showing the highest ratio between maternal serum and cord blood (maternal source that transfer the pollutant to the placenta and fetus) (Needham et al., 2011; Vizcaino et al., 2014). Data available indicates that *p.p'*-DDE (4 Cl) have a high C_{cb}/C_m[2] ratio (0.91) compared to PCB-153 (6 Cl) ratio (0.37) (Vizcaino et al., 2014).

The beneficial effects of seafood consumption prevail over the potential toxicological effects; hence continuous seafood consumption during pregnancy and lactation is recommended (Hibbeln et al., 2007; Koletzko et al., 2007; Oken et al., 2007). By consuming the amount of fish and seafood as recommended by The Norwegian Directorate of Health the desired intake of essential nutrients will be obtained, while remaining well below the TWI of contaminants (Directorate of Health, 2013; Sirot et al., 2012).

[2] *cb = cord blood, m = maternal serum*

1.6 Assessment of food intake

Dietary surveys are defined as registration of food consumption on either an individual level or for certain groups (SNL, 2009b). Insight into dietary patters can be obtained from food supply data, household budget surveys (HBS) and individual surveys. Food supply data and HBS provide gross estimates of type and amount of food available within a country (Claro et al., 2010). The total revenue of the different food groups will then be calculated as kilograms consumed per capita per year, and will therefore not provide adequate knowledge of the distribution of food and its nutrients within a household (Nelson & Bingham, 1997). Data on the individual level will, in contrast, provide detailed information on average food and nutrient intake as well as distribution. This facilitates the investigation of possible relationships between diet, health and disease (Bingham, 1991). Individual dietary surveys intend to describe dietary habits, assess nutritional quality, evaluate if dietary habits are consistent with the recommendations, consider factors that may explain differences in the diet, and obtain an overview of potentially hazardous effects in the food. This information can contribute to establishing a system that can monitor dietary habits over time (Lande et al., 2000). Dietary surveys are often categorised into short-term (< 7 days) and long-term (≥ 7 days) surveys when looking at different consumption patterns (Ortiz-Andrellucchi et al., 2009). Another useful categorisation is based on prospective and retrospective methods (Nelson & Bingham, 1997). The food frequency questionnaire (FFQ) is an example of a retrospective dietary method used to estimate long-term habitual intake.

By a FFQ the participants are asked to report the frequency of the consumption of habitual food intake over a prolonged time e.g. weeks, months or years (Cade et al., 2002; Nelson & Bingham, 1997). Whether or not portion sizes should be included in the surveys is under debate. Portion sizes will be specified as certain units e.g. a spoonful, cups, slices of bread and so on. These are units that may differ from household to household. Despite this, FFQ has become the dominant method for assessing food consumption in epidemiological studies, mainly due to convenience, ease of administration and low cost. Short FFQs can be customized to measure specific foods, e.g. seafood, but also the level of contaminants such as PCBs and pesticides. Long FFQs intend to give a comprehensive assessment of total dietary intake (Nelson & Margetts,

1997). Essentially it is the long-term diet that constitutes the important exposure related to health and disease.

Besides being highly dependent on the memory of the participants the major weaknesses with FFQs are related to the design. It is important to be fully aware of the objective when designing the FFQ, whether the primary objective is to rank individuals relative to each other and to compare low and high consumers, or to be able to measure absolute food or nutrient intake (Willett, 1998a). FFQs may perform better at ranking individuals than to estimate absolute intake (Thompson & Byers, 1994). Fundamental to a good FFQ design is the focus on the respondent's perception of the meaning of the questions (Subar et al., 1995), and the cognitive and behavioural processes involved with food intake (Livingstone & Black, 2003). Ultimately the design of a FFQ is a compromise between the degrees of how detailed questions one can ask within an acceptable level of participant burden.

1.7 Biomarkers

Biomarkers, also known as biochemical measurements, refer to a measured characteristic that may be used as an indication of a biological state or condition (SNL, 2009c). They are, as well as being important instruments in clinical settings, useful in several research areas. Biomarkers can be categorized into three types; markers of exposure, markers of effect and markers of susceptibility (ATSDR, 2009). In this study biomarkers of exposure to POPs are used, namely serum *p.p'*-DDE and serum PCB-153. Serum is the component in blood that contains neither white or red blood cells, nor clotting factors (fibrinogens). The lipid fraction of plasma is not affected by the removal of fibrinogen, which suggests that plasma also can be used.

The biggest advantage regarding using biomarkers in population studies is that measurement errors are uncorrelated (Andersen, 2000; Willett, 1998b). Biomarkers do not rely on the subject's perception or ability to give accurate information as opposed to dietary methods which will always be vulnerable to subjective reporting (Bingham, 1991; Hunter, 1998). However, there are a few negative aspects with the use of biomarkers as well. They are, for example, influenced by between-subject variations in nutritional physiology and metabolism e.g. digestion, absorption, distribution, uptake, turnover and excretion (Bates et al., 1997). Both genetic and environmental factors

might complicate the use, and most often the biomarker will better predict the bioavailability of a substance rather than accurately estimate the intake (Pearce et al., 1995). Biomarkers are also limited by the potential errors introduced in the sample preparation step and the instrument used in the analytical process (Cade et al., 2002).

1.8 Aims of the study

The main aim of this thesis was to test the following hypothesis: A high maternal fish intake prior to- and during pregnancy correlates with an elevated level of the environmental contaminants *p.p'*-DDE and PCB-153 in serum of mother and child.

The specific aims of this study were as follows:

- Are mothers' seafood intake in accordance with the national recommendations?
- How are the levels of contaminants in mother and child compared to other reference/bio monitored values?
- Will children who are breastfed show higher serum contaminant levels compared to those who not receive breast milk?
- Are there other sources of seafood, besides fish, that contributes to an elevated serum contaminant level?

2 Materials and methods

The present study is part of the project "Nutrition, Mental health and Infant Development" (DMID), which is a prospective longitudinal population-based study in the municipality of Fjell, outside the city of Bergen, Norway. The project is led by the Regional Centre for Child and Youth Mental Health and Child Welfare (RKBU West).

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, 2011). The project was reported to and approved by the Regional Committees for Medical Health Research Ethics (REC West, Reference nr: 2009/570/REC, Project nr: 083.09) and the Norwegian Social Science Data Service (NSD, Ref nr: 21904). A specific bio-bank was established and approved for storage of biological samples. The master project was financed by NIFES and the University of Bergen (UiB).

2.1 Participants and recruitment

The target population included all pregnant women that gave birth within the period 2010-2011 in the municipality of Fjell (Markhus et al., 2013). Midwives and medical doctors at their local health centres recruited the participants between September 2009 and June 2011 at a routine visit during their 24th gestation week. There were no exclusion criteria for participation. Written informed consent was obtained from all participants before inclusion and the participants were free to withdraw from the study at any time without reason.

2.2 Data collection

Data collected in this thesis was obtained from a FFQ (Appendix I), a 24-hour recall interview and an interviewer administrated FFQ (Appendix II). Blood samples from the mothers were collected at their local health centre at 28 weeks pregnant and later from both mother and child at 3, 6 and 12 months postpartum. An overview of the present study is showed in **Figure 2-1**.

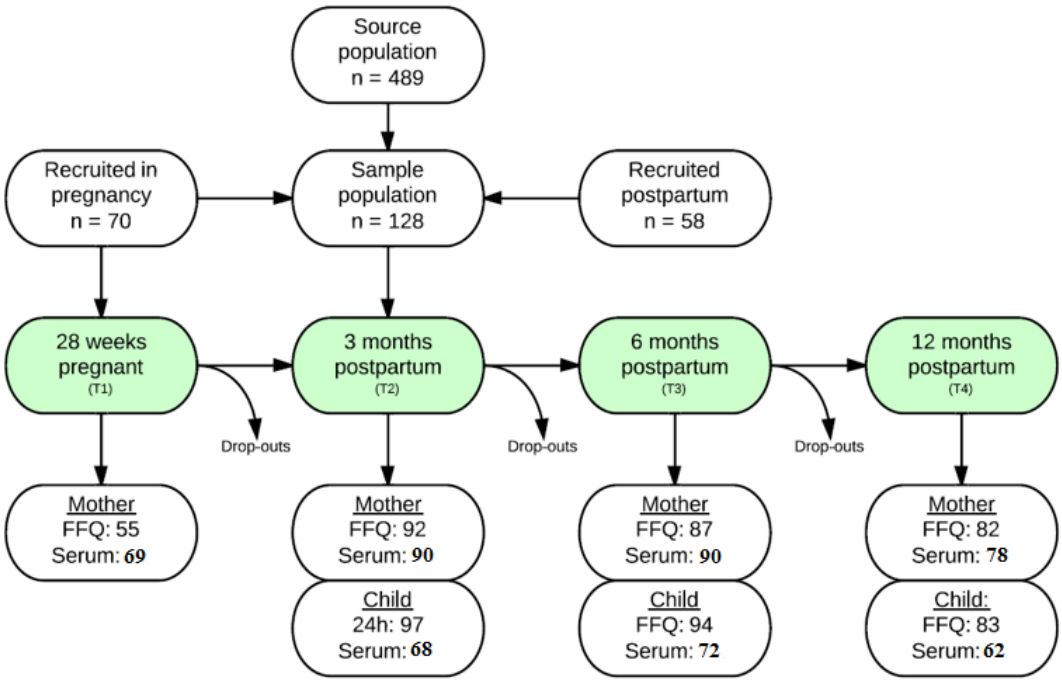

Figure 2-1: *Flow-chart describing the present study.*

2.2.1 Maternal diet

Maternal diet and habits concerning lifestyle were assessed by a validated short semi-quantitative FFQ, hereafter referred to as the Seafood-FFQ (Dahl, 2004; Markhus et al.,2013). A seafood index has been established and validated from the seafood-FFQ. Based on correlation analysis, the Seafood-FFQ has shown to capture information on the seafood intake of pregnant women considerably well. The Seafood-FFQ was provided electronically via a link generated through QuestBack © (2000-2008, Oslo, Norway) during pregnancy and 3 months postpartum, and as paper version at 6 and 12 months. The answers were automatically generated as a Statistical Package for the Social Sciences (SPSS) file for the first two time sets, and were manually computed for the last two time sets. The Seafood-FFQ was only provided in Norwegian.

The Seafood-FFQ addressed seafood consumption and other relevant dietary and lifestyle habits within the pregnancy for the FFQ at 28 weeks pregnant, and within the preceding 3 months for the FFQ at 3, 6 and 12 months. The questionnaire contained seven main questions about seafood intake, as dinner, spread, and salads or as snack meals[3], with regards to frequency, type and amount. The FFQ also contained questions

[3] *Seafood as spread, in salad or snack meals is hereby referred to as seafood as spread.*

about the intake of fruit, vegetables, milk and other dairy products. Residency, educational level, ethnicity, sun practice, social demographics and anthropometric measurements were also accounted for in the questionnaire. In the last section three questions on dietary habits were incorporated together with some conditions that the women were asked to state if had any relevance for them. The conditions were as follows: vegetarian, diabetic, food allergy/intolerance, medication and if they were still breastfeeding. The women also had to state if they avoided products containing milk and dairy, meat or vegetables. Open spaces for items not included in the list allowed for a better characterization of the participants diet. The frequency intervals ranged from never to more than three times a week for dinner related questions and from never to more than five times a week for questions regarding spread. The maximum frequency for spread was higher than for dinner due to a frequent intake of bread in Norway. The general questions were followed by frequency questions on 35 specific seafood types. Regarding portion sizes, the respondent could choose between one out of five sizes ranging from half a portion to three portions. One portion was specified as a standard Norwegian dinner size of 150 g. This corresponds to one slice of salmon fillet, three fishcakes or two dl of shrimps. The question about amount of seafood used as spread was open ended.

2.2.2 Infant diet

Infant diet was assessed through a 24-hour interview at 3 months and an interviewer administrated FFQs at 6 and 12 months. The infant FFQ included questions on the consumption and frequency of dietary supplements (e.g. cod liver oil, vitamin-D droplets and iron) and milk (breast milk, formula and/or cow's milk). These were followed by questions on breastfeeding, introduction of complementary feeding (solids), and frequency of cereal, dinner, bread, and fruit meals. Finally infant seafood consumption was addressed. A nutritionist carried out the 24-hour recall interviews. The mothers were asked to explain to the interviewer what they had fed to the child the last 24 hours.

2.2.3 Blood sampling

Non-fasting, venous blood was taken from the underarm of both mother and infant. The blood samples were collected in 3.5 ml serum-separating tubes and left to coagulate for 30-60 minutes. In the case of very difficult phlebotomy of the child, capillary blood

was collected through skin-rupture of the heel at 3 months and finger at 6- and 12 months. Blood samples were centrifuged for 10 minutes at 3000 revolutions per minute (rpm). Serum was transferred to cryo tubes and stored at -20 °C for 0-4 weeks before transportation on dry ice to storage at -80 °C at NIFES, until analysis.

2.2.4 Practice in the laboratory

All chemical analyses were performed at NIFES from October 2013 to December 2013. NIFES has internal routines methods for analysing human material. The samples were risk evaluated and all personnel involved in sampling and analysing had fulfilled theoretical and practical training. All personnel were offered and encouraged to vaccinate against hepatitis B. A fume hood in the Laboratory for contaminants was marked with yellow tape to signalize potential danger of infection. All surfaces were washed with Virkon or 70 % ethanol disinfectant after finishing the work.

2.3 Analysis of serum *p.p'*-DDE and PCB-153

NIFES sample protocol for *p.p'*-DDE and PCB-153 was based on a liquid-liquid extraction method developed by Lu and co-workers (2012) and further quantification by High Resolution Gas Chromatography - High Resolution Mass Spectroscopy (GC-HRMS). The main feature considered for selecting this particular method was the low amount of sample required for the analysis (20-100 µl of serum).

2.3.1 Internal and recovery standards

An internal standard (ISTD) was added at the start of the work-up of each sample to control for any potential sources of error during sample preparation and handling. Any methodological error had the same impact on the added ISTD as on the endogenous levels of *p.p'*-DDE and PCB-153 in the serum sample. A recovery standard (RSTD) was added at the end of the work-up to reclaim the ISTD. The ISTD and RSTD ingredients were purchased from Cambridge Isotope Laboratory (LGC Sweden) and mixed together at NIFES (Appendix III). The ISTD consisted of labelled analytes in a known concentration, corresponding to the analytes of interest, thus allowing us to find the unknown concentrations through isotope dilution-method (See equation 3 and 4 in section 2.6).

2.3.2 Serum sample preparation

Serum samples were kept at -80 °C before analysis. Samples were then placed in a freezer holding -4 °C before they were thawed at room temperature before preparation. An aliquot of 100 µl of serum sample was transferred to a polypropylene tube. Aliquots of ISTD (20 µl) and formic acid (HCOOH, 10 µl) were added and the system vortex-mixed for 1 min. Aliquots of distilled water (100 µl) and acetone (50 µl) were added and the system was vortex-mixed for 1 min. A mixture of dichloromethane/isohexane 1:4 (1000 µl) was added and the system vortex-mixed for 1 min followed and centrifuged at 3200 rpm for 2 minutes. The supernatant was separated from the final residue by transferring it to a plastic test tube. The final residue was washed one more time by adding 1000 µl of isohexane and thereafter vortex-mixed for 1 min. The supernatant was collected and gathered together with the original supernatant. The tube containing the supernatant was placed on a block heater (35 °C) and damped until the volume reached 1 ml. The 1 ml aliquot got transferred to tampered vials and damped again until about 500µl. RSTD (10 µl) and sulphuric acid (H_2SO_4, 30 µl) was added and vortex-mixed for 1 min. The samples were set to rest for 10 minutes while new tampered vials were filled with nonane (C_9H_2O, 20 µl). The fluid border was marked. The samples were vortex-mixed for 30 seconds, before centrifugation (2 min.) at 1200-1400 rpm. The residues were evaporated to 20 µl and were ready for the instrument used for analysis.

Formic acid was added to denaturize proteins to contribute to better extraction efficiency for PCBs and pesticides. Acetone and DCM/isohexane are both extraction agents for PCB and pesticides. A 1:4 relationship gave the best result after testing. This result to a lower density in the organic phase compared to the water/acid phase and thereby making the extraction easier. Freezing also makes the separation after the centrifugation easier.

2.3.3 HRGC-HRMS

A High Resolution Gas Chromatography – High Resolution Mass Spectrometry (HRGC-HRMS) instrument was used for analysing the serum samples in this study. The HRGC-HRMS instrument combines the separation properties of GC with the detection and identification capabilities of MS. **Figure 2-2** shows a schematic of the GC-MC used in this study. The model in use is the Trace GC Ultra/DFS (Thermo Fisher Scientific, Massachusetts, USA) with a TR-DIOXIN-5MS column (30 m long, inner diameter 0.25 mm, film thickness 0.1 µm). The injection volume of the instrument is 1µl.

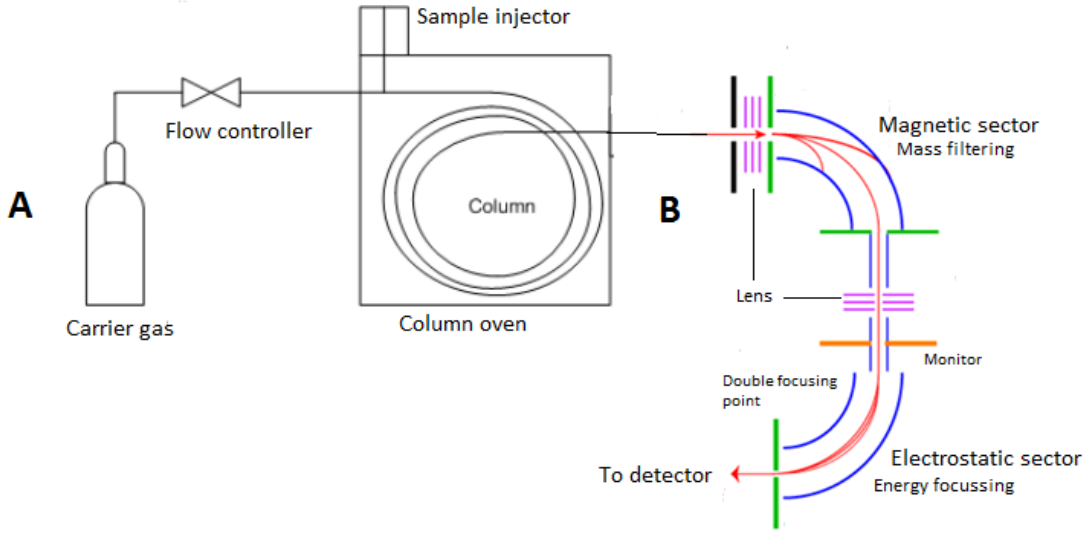

Figure 2-2: ***A****: Gas Chromatography: The sample solution is injected to the GC inlet and gets vaporized and swept onto the long column by the carrier gas. The different compounds are separated as a result of their relative interaction with the stationary phase and mobile phase. Compounds eluting from the column are converted to ions.* ***B****: Mass-spectrometry: The ions passes a lens to get to the magnetic sector where it is separated according to mass. Another lens is passed before it enters the electrostatic sector where it is separated according to kinetic energies. The results appear as a total ion chromatogram. Figure modified from (Openstax, 2014; University of Bristol, 2005)*

The capillary column in GC can be several meters long. The column has a stationary phase distributed as a coating over a substrate of an inert material. The mobile phase is called the carrier gas and is in this case helium. An absorptive interaction between the components in the gas stream and the coating leads to a differential separation of the components in the mixture. The components are transferred from the GC to the MS, which in this case is a sector instrument called Double Focusing System (DFS). This

kind of systems have a magnetic- and an electrostatic sector. The separation principle in this kind of instruments are based on the fact that all ions will be deflected in a magnetic field. The ions enter the MS through the source slit where they are deflected according to mass. The magnet is formed as a circular sector, thus the ions will have to follow a decisive curved orbit to reach the detector. Higher-mass ions are deflected less than lower-mass ions. Scanning the magnet enables ions of different masses to be focused on the monitor slit (University of Bristol, 2005). To obtain a spectrum of good resolution - i.e. where all ions with the same mass to charge (*m/z*) ratio appear coincident as one peak in the spectrum, ions have to be filtered by their kinetic energies (University of Bristol, 2005). The ions enter the electrostatic sector where ions of the same *m/z* have their energy distributions corrected for and are focused at the double focusing point on the detector slit. A multiplier will make the results detectable, and soon appear as a mass chromatogram (usually called Total Ion Chromatogram) (**Figure 2-3**). A greater specificity, accompanied by a marked increase in detection sensitivity are two of the benefits of using HRGC-HRMS compared to a simplified GC-MS (Cattabeni et al., 1986).

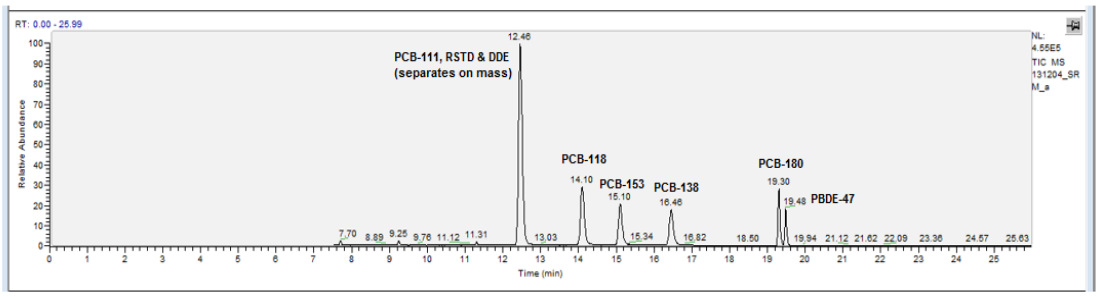

***Figure 2-3**: Total ion chromatogram of CRM (intensity versus mass/charge) obtained from the Trace GC Ultra/DFS.*

2.4 Quality Control

Laboratory quality control is important to detect, and thereby reduce and correct deficiencies in the analytical process. There are different validation parameters that enable judging the correct application of a specific analytical method. The parameters evaluated in the present thesis were: selectivity, accuracy, precision, recovery, sensitivity and limit of quantification (LOQ). Selectivity is the ability of an analytical method to detect the analyte without the interference of other sample components. The

selectivity is generally evaluated by spiking blank samples with and without the analytes. The accuracy describes how close the mean test results are to the true concentration of the analyte. The precision describes the closeness between individual measurements of the same analyte (degree of scatter or dispersion). The precision of a method also includes reproducibility of the analysis, which can involve different analysts and precision over time. Standard Reference Material® (SRM 1957, National Institute of Standards & Technology) was used to see how good the accuracy and the precision of the method were. 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 does not need to be 100 %, its range of variation should be specified in advance. The recovery in the present thesis was set to vary between 30-130 %. The range of variation was based on the recovery from other contaminant analysis at NIFES. The sensitivity of a method was defined as the incremental increase in measured signal per incremental increase in analyte concentration. To establish the sensitivity of a method a calibration curve describing the instrument response as a function of the analytical concentration should be constructed. The lowest concentration that a calibration curve can measure with acceptable degree of accuracy and precision is termed LOQ. Considering that the analytical method used in the present thesis have not been validated yet, a preliminary LOQ was established based on preliminary results during method development (**Table 2-1**). It is important to mention that values below the LOQ levels are referred in the present thesis 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. There are different methods used to deal with detected values below LOQ. In the present thesis it was decided to set values below LOQ to the LOQ-value (as this is a common way of doing it at NIFES).

***Table 2-1**: Preliminary LOQ's for the two contaminants (ng/ml).*

	***p.p'*-DDE (ng/ml)**	**PCB-153 (ng/ml)**
LOQ	0.05	0.02

The reported mean values was based on duplicate serum samples analyses. A particular standard (Standard 3, Appendix III + IV) with a known concentration 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. Stability of the analytes under different conditions encountered during the procedure should be established. This will ensure that the loss of analyte due to unnecessary handling was avoided.

2.5 Calculations

On the basis of information on height and weight, self-reported by the participant in the Seafood-FFQ, body mass index (BMI) was calculated.

$$BMI = \frac{weight\ (kg)}{height\ (m2)} \quad (1)$$

Effect size (r) was calculated using the Standardized Test Statistic value (z) and the square root of the total number of cases (n).

$$r = \frac{z}{\sqrt{n}} \quad (2)$$

To calculate the concentrations of the contaminants in each serum sample, a relative response factor (RRF) was calculated from the calibration curve (3). This value is later put into the formula used to calculate the amount of PCB-153 and *p.p'*-DDE in the sample (4). RRF, the ratio of the slopes, compensates for differences in instrument response and analytical gain. ISTD is denoted $_{is}$, while the analyte is denoted $_{a}$.

$$RRF = \frac{C_{is}\ x\ A_a}{A_{is}\ x\ C_a} \quad (3)$$

$$C_a = \frac{\left(\frac{A_a\ x\ Ais\ x\ M_{is}}{RRF}\right)}{V\ (ml)} \quad (4)$$

A= area under peak, C= concentration, M= mass of ISTD and analyte, V= volume

2.6 Statistical methods

All statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS, Statistics 21, IBM Corporation, USA) or GraphPad (Prism 6). Figures and tables were made using SPSS, GraphPad and Microsoft Office Excel 2007.

Descriptive (age and anthropometric) variables, obtained from the questionnaires and interviews, were normally distributed. Normality was tested with the parametric Shapiro Wilks test and the non-parametric Kolmogorov-Smirnov test. Results obtained from the Seafood-FFQ and serum samples were skewed and therefore further analysis were performed by using non-parametric tests. Distribution and outliers were examined with scatter plots, box plots and a ROUT-test provided by GraphPad. The data set without outliers proved to have little changes in case of the mean and median, hence the full data set was decided to use for further analysis. Values were expressed as median values plus the sample size's minimum and maximum value. All p-values were two-tailed and values below 0.05 ($p < 0.05$) were considered significant. Spearman's rank order (rho) correlation coefficient was used to determine the relationship between variables. The Friedman test was used to compare the same sample of participants or cases across three or more points in time. Test statistics (χ^2) and degrees of freedom (df) are denoted together as $\chi^2(df)$. The Mann-Whitney U test was used to compare differences between two independent groups, and the Wilcoxon signed rank test was used to compare differences between paired data and repeated measurements at the different time points. Data was first analysed as a whole and later stratified into groups such as primiparous/multiparous, breastfed/not breastfed and liver consumers/not liver consumers.

When using some of the statistical non-parametric techniques effect size can be calculated (not provided by SPSS). Effect size is often represented by *r,* which is a correlation coefficient that range from 0 to 1, with higher values indicating a stronger association between the two variables (equation 2, section 2.6). Cohen's criteria is used to categorize the effect; 0.1 = small effect, 0.3 = medium effect, 0.5 = large effect, respectively. As Spearman's rho also is expressed as values between 0 and 1 the same criteria can be used.

3 Results

3.1 Recruitment

A total of 72 women were recruited during pregnancy (**Figure 2-1**), 55 of these completed the Seafood-FFQ and 69 of them provided blood serum samples. Additional women were recruited postpartum, and at three months a total of 92 women provided the Seafood-FFQ, and 90 provided the serum samples. At this point the recruitment was completed, and due to a few drop-outs along the way, the results were 87 Seafood-FFQs and 90 serum samples at 6 months, and 82 Seafood-FFQs and 78 serum samples at 12 months. 18 of the women recruited during pregnancy did not participate postpartum. The children followed the same pattern. Some of the blood samples collected was empty, as an analysis on vitamin D was prioritized. **Table 3-1** gives an overview of the data material used in this thesis. In general there were less serum samples analysed for the children compared to the mothers. This is because it is not possible to draw as much blood from a small baby compared to a fully grown and the blood drawing process is more difficult. This is especially evident at three months.

***Table 3-1**: Data material used in this thesis at the different times; in pregnancy week 28 and at 3, 6 and 12 months postpartum.*

Data material	28 weeks		3 months		6 months		12 months	
	Mother	*Child*	*Mother*	*Child*	*Mother*	*Child*	*Mother*	*Child*
Seafood-FFQ	55	-	92	-	87	94	82	83
24h interview	-	-	-	97	-	-	-	-
Serum sample	61	-	82	47	85	61	73	52

3.2 Descriptive characteristics of the study population

This is a longitudinal study that follows a group of pregnant women from their pregnancy week 28 to 12 months after birth. There were variations in the mean of the anthropometric measures, social and behavioural characteristics of the studied population, as a result of two different time sets for recruitment (in pregnancy and at 3 months postpartum), and because the women were allowed to drop out at any time during the study.

The mothers

The maternal characteristics are presented in **Table 3-2**. BMI was calculated (Equation 1, section 2.6) on the basis of the mothers' self-reported weight and height. There was a statistically significant increase in the BMI reported before pregnancy and the BMI at 3 and 6 months ($n=38$, $p<0.05$). The descriptive (age and anthropometric) variables were normally distributed among the women. There was a significant change in frequency of exercise across the times with women exercising more frequently postpartum (at 3 and 6 months) than in pregnancy ($n= 33$, $p<0.05$). When comparing the participant's focus on a healthy diet, there were no significant differences ($p>0.05$) across the different times. The percentage of mothers with higher education levels was well above 60 %. The income of the mothers reflect the high educational level with a mean income of 300 000-399 000 NOK yearly. The fathers of the studied children had on average a yearly mean income of 400 000-499 000 NOK.

Of the women recruited in pregnancy, only one had diabetes. At the other three times there was no one with the disease so it is assumed that she was one of the 18 drop-outs. At both recruitment stages (in pregnancy and at 3 months postpartum), none of the participants reported to be vegetarian. However, at the last two time periods one person answered yes in that particular question, which implies a recent lifestyle change. Between 4 and 7 per cent of the study population stated that they had some sort of food allergies (one stated to be allergic to shellfish). Of the women recruited in pregnancy, three (5 %) did not have Norwegian ethnicity. After the second, and last, recruitment a total of six women (7 %) answered that they were not of Norwegian origin.

***Table 3-2**: Descriptive statistics of mothers taking part in the study. Data were collected from women at 28 weeks pregnant and 3, 6 and 12 months postpartum. Data is presented either as mean ± SD or counts as dictated by the level of measurement*

Characteristics	28 weeks	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)	31 ± 5 (19,42)	31 ± 6 (19,42)	32 ± 6 (20,42)
Weight (kg)[a]	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 ± 13 (153,181)
BMI (kg/m2)[b]	24 ± 4 (17,35)	25 ± 5 (17,43)	25 ± 4 (17,40)	24 ± 4 (16,37)
	Count n (%)	*Count n (%)*	*Count n (%)*	*Count n (%)*
BMI category[c]				
Underweight (16-18.5)	3 (6)	2 (2)	2 (2)	3 (4)
Normal (18.5-25)	32 (59)	48 (52)	43 (51)	43 (52)
Overweight (25-30)	15 (27)	27 (31)	31 (37)	30 (37)
Obese class 1 (30-35)	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)
Primiparous[d]	24 (44)	34 (37)	30 (37)	36 (44)
Education				
Lower secondary school	0 (0)	4 (4)	5 (6)	6 (7)
Higher secondary school	16 (29)	28 (31)	29 (33)	25 (30)
< 4 years of university[e]	24 (44)	34 (37)	37 (43)	39 (48)
> 4 years of university[e]	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 (6)	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 (6)	6 (7)	7 (8)
Caucasian descent[f]	52 (95)	86 (93)	81 (93)	76 (93)

[a] *Weight reported when submitting the seafood-FFQ*
[b] *BMI presented in the column for pregnancy is based on weight before pregnancy.*
[c] *The BMI categorization is done by the WHO. Women were placed in the lowest possible category.*
[d] *Primiparous; a woman who is pregnant for the first time.*
[e] *University or university college*
[f] *Caucasian descent; white skin colour.*

The children

The characteristics of the children that participated in the study are presented in **Table 3-3**. Mean birth week, weight and length at birth were the same for the study group at every time set even though there were 14 less participants one year after birth. The descriptive variables were normally distributed, except for the birth week, length and height at 12 months, which was negatively skewed. There was no significant difference ($p>0.05$) between the girls and the boys regarding birth weight.

***Table 3-3**: Descriptive of the children in the study group. Data were collected from the children at 3, 6 and 12 months after birth. Data is presented either as mean ± SD or counts as dictated by the level of measurement. Min- and maximum levels of the anthropometric measures are also presented.*

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)
Twin pairs	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 (34,42)
Weight at birth (kg)	3.6 ± 0.6 (1.6,5.1)	3.6 ± 0.6 (1.6,5.1)	3.6 ± 0.6 (1.6,5.1)
Length at birth (cm)	50 ± 3 (43,57)	50 ± 3 (43,57)	50 ± 3 (43,57)
Current weight (kg)	6.4 ± 0.8 (4.5,9.0)	8.1 ± 0.9 (6.3,11.5)	10.1 ± 1.2 (7.9,13.5)
Current length (cm)	62 ± 3 (53,68)	68 ± 3 (57,75)	77 ± 3 (68,85)

Table 3-4 presents the eating habits of the children participating in the study. The percentage of exclusively and daily breast fed children is highest at 3 months postpartum and is less frequent as the child gets older. Porridge, fruit, bread and dinner was introduced after the 3 months check-up. At 12 months, 89 % of the children ate seafood for dinner. Breastfeeding prevalence was reported to decrease from 81 % at three months to 70 % at 6 months and to 33 % at 12 months.

Table 3-4: *Children's eating habits at 3, 6 and 12 months.*

	3 months n (%)	6 months n (%)	12 months 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 as dinner	-	14 (15)	74 (89)

[a] Other: fruit, bread, dinner

3.3 Mother's seafood intake

There was a significant correlation between the intake of seafood and the reported focus on a healthy diet ($p<0.05$) at 28 weeks and 3 and 6 months, whereas seafood intake and age, BMI, education and exercise did not correlate.

Most women claimed to have eaten seafood for dinner 1-2 times per week. This frequency was consistent at all four times (**Figure 3-1**). Less women eat seafood for dinner more than 3 times a week during pregnancy (4 %) than postpartum (12-15 %) (**Figure 3-1**).

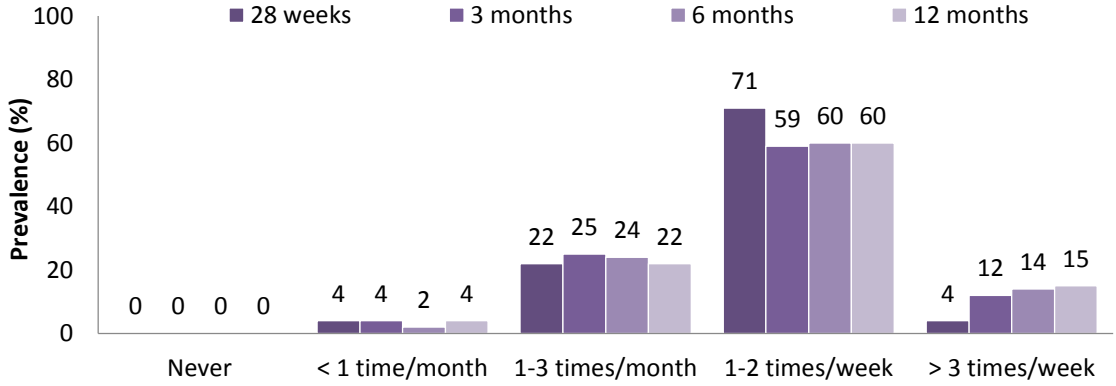

Figure 3-1: *Seafood intake as dinner at 28 weeks pregnant, and at 3, 6 and 12 months postpartum.*

On a weekly basis the majority of the women (~64 %) in the study reported eating one portion (150 g) of seafood for dinner. Some (~33 %) reported eating 1 ½ portion (225 g), while a very small number of women reported eating only half a portion (75 g), two (300 g) or three (450 g) portions (3 % in total). Median intake of seafood for dinner (calculated from question q1) was 32 g/day both at 28 weeks pregnant and at 3, 6 and

12 months. This corresponds to 224 g/week. Estimated average intake of seafood per day based on summation of all seafood categories (q5, appendix) were 34 g/day during pregnancy and 37 g/day, 41 g/day, 44 g/day at 3, 6 and 12 months postpartum, respectively (**Table 3-5**). These indexes have been used to calculate the percentile of the different seafood categories, which is deliberated further in the discussion part of the thesis. Processed and lean fish (total ~60 %) represented a large part of the consumption, while freshwater fish made an insignificant contribution to total seafood intake.

Table 3-5: *Intake of seafood for dinner (g/day), based on categories of seafood and contribution (%) to total seafood intake. Women at 28 weeks pregnant and 3, 6 and 12 months postpartum.*

	28 weeks n=55	**3 months n=92**	**6 months n=87**	**12 months n=82**
Seafood category	**g/day (%)**	**g/day (%)**	**g/day (%)**	**g/day (%)**
Oily	8 (24)	8 (22)	11 (27)	11 (25)
Lean	11 (32)	11 (30)	11 (27)	13 (30)
Processed	11 (32)	13 (35)	13 (32)	13 (30)
Shellfish	4 (12)	5 (14)	6 (15)	7 (16)
Total[a]	34 (100)	37 (100)	41 (100)	44 (100)

[a] Total seafood dinner intake: Oily, lean, processed and shellfish.

A histogram of the participants eating seafood as spread (**Figure 3-2**), revealed that the highest consume percentages (more than once a week) were 47 % and 50 % at 28 weeks of pregnancy and 12 months postpartum, respectively. The intake of seafood as spread showed no significant difference across the different times (p>0.05).

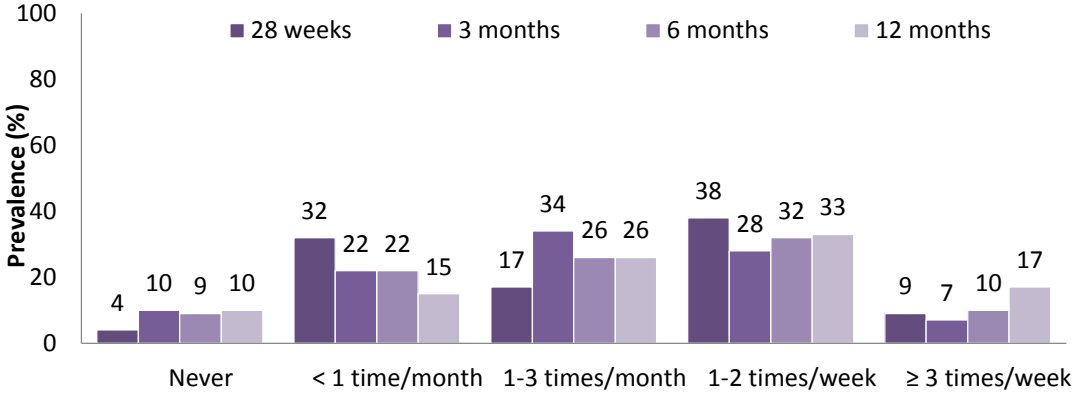

Figure 3-2*: Seafood intake as spread at 28 weeks pregnant, and at 3, 6 and 12 months postpartum.*

3.4 Determinations of serum *p.p'*-DDE and PCB-153

Mean values, standard deviation (SD), minimum and maximum serum levels for *p.p'*-DDE and PCB-153 are shown in **Table 3-6**. Only a few values that fell below the LOQ (**Table 2-1**). Median contaminant concentration (ng/ml) is shown in **Figure 3-3** for the mothers and in **Figure 3-4** for the children.

***Table 3-6**: Mean values, standard deviation (SD), minimum- and maximum concentrations are presented for the mothers and children at the different time points. The number of significant figures varies according to the associated uncertainty. $LOQ_{p.p'\text{-}DDE}$= 0.05 ng/ml, $LOQ_{PCB\text{-}153}$= 0.02 ng/ml.*

***p.p'*-DDE**	*n*	*n < LOQ*	*% < LOQ*	*Mean*	*SD*	Min	Max
Mother 28 weeks	61	0	0	0.3	0.1	0.1	1.0
Mother 3 months	82	4	5	0.2	0.4	LOQ	2.8
Mother 6 months	85	1	1	0.3	0.4	LOQ	2.6
Mother 12 months	73	3	4	0.3	0.4	LOQ	2.7
Child 3 months	47	2	4	0.2	0.2	LOQ	0.9
Child 6 months	61	6	10	0.3	0.4	LOQ	1.9
Child 12 months	52	2	4	0.4	0.7	LOQ	5.2
PCB-153							
Mother 28 weeks	61	0	0	0.14	0.08	0.02	0.05
Mother 3 months	82	2	2	0.09	0.06	LOQ	0.48
Mother 6 months	85	3	4	0.10	0.09	LOQ	0.70
Mother 12 months	73	2	3	0.10	0.07	LOQ	0.34
Child 3 months	47	2	4	0.12	0.09	LOQ	0.36
Child 6 months	61	5	8	0.12	0.09	LOQ	0.44
Child 12 months	52	4	8	0.1	0.1	LOQ	0.4

Statistics showed a significant difference between the *p.p'*-DDE levels in the mothers' serum across the four time points ($\chi^2(3)=30.1$, n= 27, $p < 0.001$) (**Figure 3-3**). The reduction in serum *p.p'*-DDE levels occurred from week 28 in pregnancy to 3 months postpartum ($p=0.019$), and were stable for the following recordings ($p < 0.001$). When considering the serum PCB-153 scores across the four time points statistics showed a significant difference also here ($\chi^2(3)=24.6$, n= 27, $p < 0.001$) (**Figure 3-3**). The reduction in serum PCB-153 levels was seen from the samples collected at 28 weeks to 3 months ($p=0.013$), and also these were stable for the following recordings ($p < 0.001$).

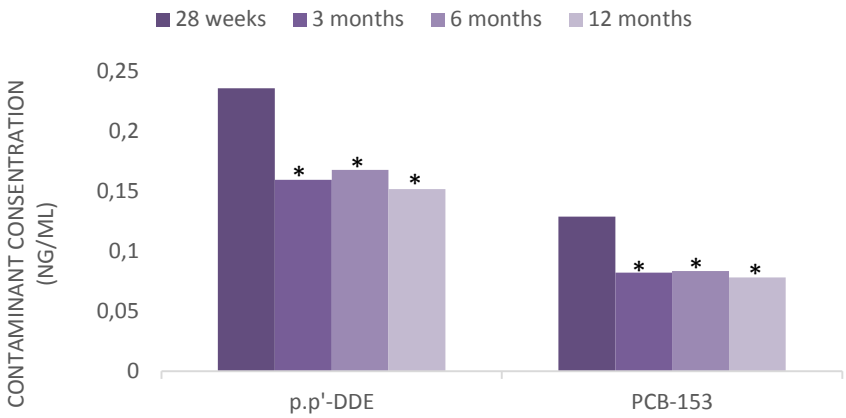

Figure 3-3: *Median contaminant concentration (ng/ml) for women in pregnancy, at 3, 6 and 12 months (n=27). Asterisk (*) denotes a significant difference between serum contaminant concentration in pregnancy (28 w) and postpartum (3, 6 and 12 months).*

The children's serum *p.p'*-DDE levels did not change significantly ($p>0.05$) during the 9 month period ($\chi^2(2)=1.6$, n=20, $p=0.449$). Neither did the PCB-153 serum levels ($\chi^2(2)=2.6$, n=20, $p=0.271$) (**Figure 3-4**).

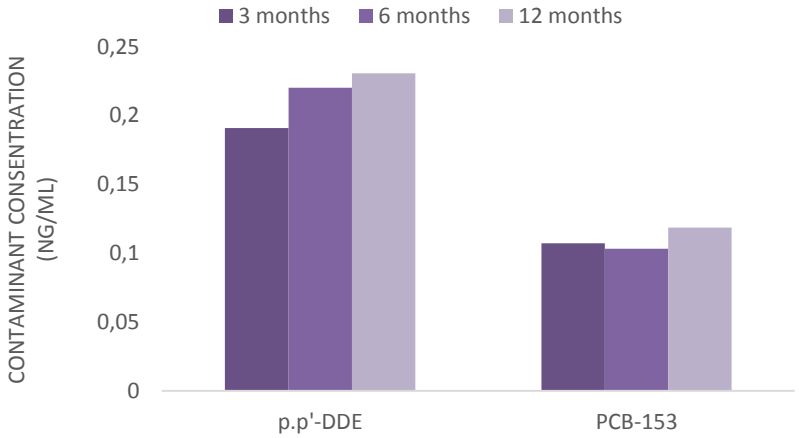

Figure 3-4: *Median contaminant concentration (ng/ml) for the children at 3, 6 and 12 months after birth (n=20).*

The number of observations included in the results shown above are based on approximately one third of the whole study population. This is because only a limited number of the participants provided serum samples at every time point. A Mann-Whitney U test confirmed that the observations were representative for the whole group. This seemed reasonable as the statistics showed no significant differences between the 27 mothers that had provided all the samples and the group as a whole. The same was the case for the children (n=20).

Correlation between mother and child

A Spearman's correlation was conducted to examine the relationship between serum concentration levels in mother and child (**Figure 3-5**). A Spearman correlation was chosen over a Pearson correlation, due to violation of Pearson's assumptions. A significant correlation is found between the mothers and children's content of *p.p'*-DDE (n=42, p=0.02) and PCB-153 (n=42, p=0.02) in the serum samples at 3 months. Analysis showed significant correlations for *p.p'*-DDE (n= 57, p= 0.01), but not for PCB-153 (n=57, p=0.67) at 6 months. At 12 months analysis showed that neither *p.p'*-DDE nor PCB-153 had a significant correlation between mother and child (data not shown). To control for biased correlation the data set were tested without the particular higher values measured for *p.p'*-DDE at 3 and 6 months. Even after these values were excluded there was a significant correlation (p>0.05), implying that the observed correlation was not driven by the extreme values.

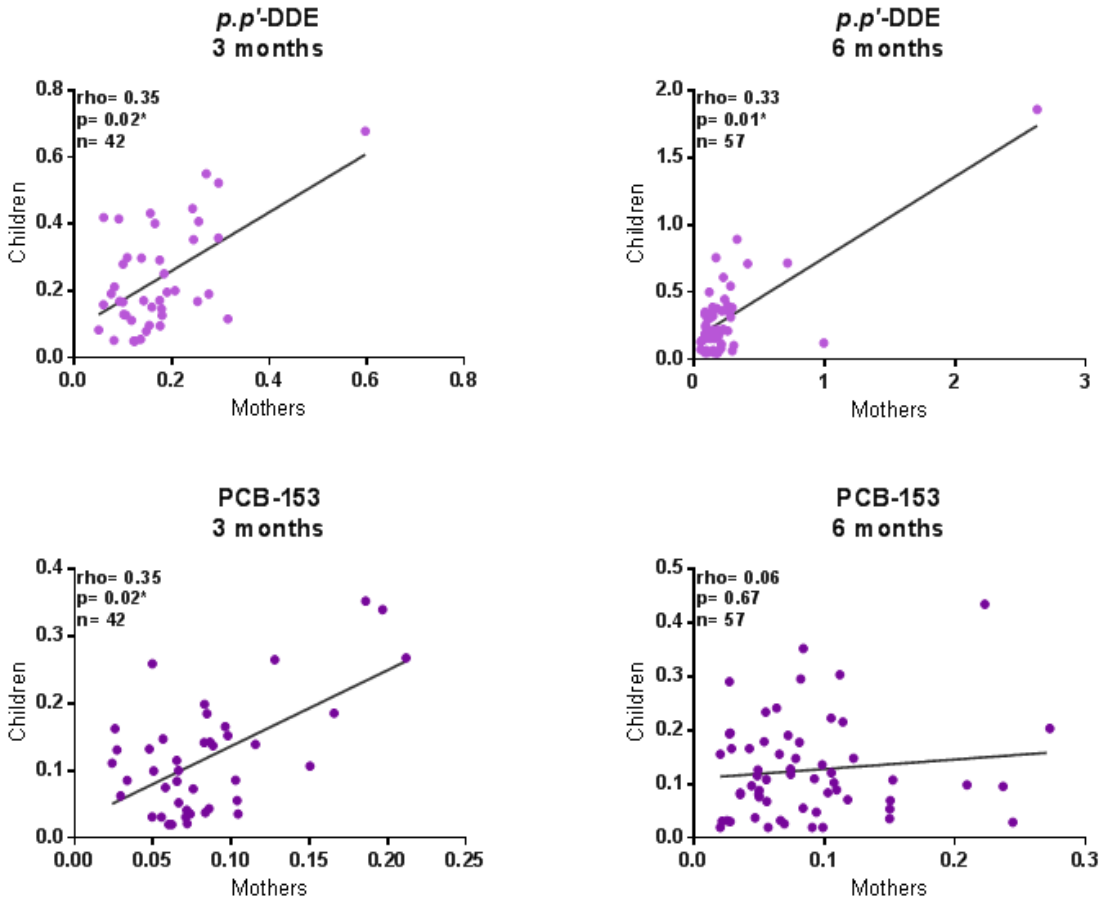

Figure 3-5: *Spearman's correlation coefficient (rho) for serum p.p'-DDE and PCB-153 in mother and child. A significant correlation is denoted *.*

Biomarker correlation

The correlation coefficients (rho) between serum concentrations of PCB-153 and *p.p'*-DDE at the different times varied between 0.57 and 0.86, with the highest value found in children at 12 months and the lowest value found in mothers at 3 months (**Table 3-7**). In general, children revealed a higher correlation coefficient than the mothers.

Table 3-7: *Correlations between serum concentrations of PCB-153 and p.p'-DDE*

	Mother 3 months	Mother 3 months	Mother 6 months	Mother 12 months	Children 3 months	Children 6 months	Children 12 months
rho [a]	0.66**	0.57**	0.75**	0.59**	0.82**	0.69**	0.86**

[a] Spearman's correlation coefficient

** Correlation is significant at the 0.01 level (2-tailed)

3.4.1 Confounding factors

As this is not a large randomized trial, confounding factors have to be taken into account (Kamangar, 2012). The following sections presents confounding factors that other studies have shown to affect the interpretation of the results (Govarts et al., 2012; Jonsson et al., 2005; Papadopoulou et al., 2013; Polder et al., 2009; Rylander et al., 1997, 2012; Verner et al., 2013).

Seafood intake

The statistics showed no significant ($p>0.05$) correlation between the mothers' total seafood intake at 28 weeks and their serum contaminant levels at the same time (**Table 3-8**). A significant correlation was however seen between serum levels of PCB-153 and the intake of shellfish ($p=0.04$). This significans proved to be applicable even after fish liver consumers were removed from the statistics. The study population was divided into two groups based on their seafood consumption to see if there could be spotted a significant difference in serum contaminant levels. Statistics showed that mothers denoted as high consumers (total seafood intake >450g/week or oily fish intake >300g) did not have significant higher ($p>0.05$) serum concentrations of contaminants than the low consumers. No significant difference ($p>0.05$) in serum contaminant level was seen when comparing children of high consumers with children of low consumers.

When separating the study population with regards to consumption of fish liver (> 1 times per year) it was evident that there was significant higher ($p>0.05$) *p.p'*-DDE and PCB-153 serum concentrations in the mothers who had eaten fish liver compared

to those who had not (**Table 3-9**). However, the children of the mothers who had eaten fish liver did not have significantly higher serum *p.p'*-DDE and PCB-153 concentrations than the children of the mothers who had not eaten liver. The significance for the mothers eating fish liver proved to be applicable even after the extremes of *p.p'*-DDE and PCB-153 were removed.

***Table 3-8**: Spearman's correlation coefficient (rho) for Seafood-FFQ variables and serum contaminant concentrations (ng/ml) at 28 weeks pregnant. The n shows how many of the women who have answered the question/variable in the Seafood-FFQ.*

	28 weeks		
	n	*p.p'-DDE*	*PCB-153*
Seafood as dinner			
All	50	0.037	-0.219
Oily	50	0.246	-0.080
Lean[a]	48	0.023	-0.087
Shellfish	49	0.059	0.297*
Processed	45	0.047	-0.047
Seafood as spread	48	-0.111	-0.013
Supplements[b]	50	-0.100	-0.098
Total seafood intake[c]	48	-0.033	-0.097

* Correlation is significant at the 0.05 level (2-tailed)
[a] Lean: Lean and intermediate oily fish (< 5 g fat/100 g)
[b] Supplements: ω-3 supplementation both as capsules or liquid
[c] Total seafood intake: Dinner, spread and supplements

***Table 3-9:** Probability (p) and total n for liver consumption obtained from Seafood-FFQ and the mothers' serum p.p'-DDE and PCB-153 concentrations (ng/ml). Asterisk (*) denotes a significant higher concentration in the serum in the mothers that have eaten liver compared to the mothers that have not at 28 weeks, 3, 6 and 12 months..*

Liver consumption > 1/year	**p**	**Total n (liver consumed/liver not consumed)**	**r[a]**
p.p'-DDE 28 weeks	0.041*	61 (7/54)	0.26
PCB-153 28 weeks	0.002*		0.38
p.p'-DDE 3 months	0.008**	82 (9/73)	0.29
PCB-153 3 months	0.005**		0.31
p.p'-DDE 6 months	<0.001**	85 (14/71)	0.40
PCB-153 6 months	0.003**		0.33
p.p'-DDE 12 months	<0.001**	73 (12/61)	0.45
PCB-153 12 months	0.003**		0.35

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)
[a] Effect size

Breastfeeding prevalence

Children who had been breastfed had higher serum levels of *p.p'*-DDE and PCB-153 than children who were given formula (**Table 3-10**). **Figure 3-6** shows the median contaminant levels at the different times. The largest difference between breastfed and formula fed children seems to be at 6 months.

***Table 3-10**: Probability (p) and total n for breastfeeding variables obtained from 24h interview and Seafood-FFQ and the children's serum p.p'-DDE and PCB-153 concentrations (ng/ml). Asterisk (*) denotes significantly higher concentrations in the serum in the children that have been breastfed compared to the children that have not been breastfed at the different times.*

Breastfeeding	p	Total n (Breastfed/ not breastfed)	r[c]
Breastfed[a] at 3 months			
p.p'-DDE 3 months	0.04*	46 (29/17)	0.31
PCB-153 3 months	< 0.01**		0.47
p.p'-DDE 6 months	< 0.01**	58 (35/23)	0.43
PCB-153 6 months	< 0.01**		0.62
p.p'-DDE 12 months	< 0.01**	48 (29/19)	0.59
PCB-153 12 months	< 0.01**		0.61
Breastfed[b] at 6 months			
p.p'-DDE 6 months	< 0.01**	61 (42/19)	0.51
PCB-153 6 months	< 0.01**		0.63
p.p'-DDE 12 months	< 0.01**	52 (34/18)	0.59
PCB-153 12 months	< 0.01**		0.67
Breastfed[b] at 12 months			
p.p'-DDE 12 months	< 0.01**	52 (17/35)	0.54
PCB-153 12 months	< 0.01**		0.44

* Correlation is significant at the 0.05 level (2-tailed)
** Correlation is significant at the 0.01 level (2-tailed)

[a] Breastfed exclusively
[b] Breastfed daily
[c] Effect size

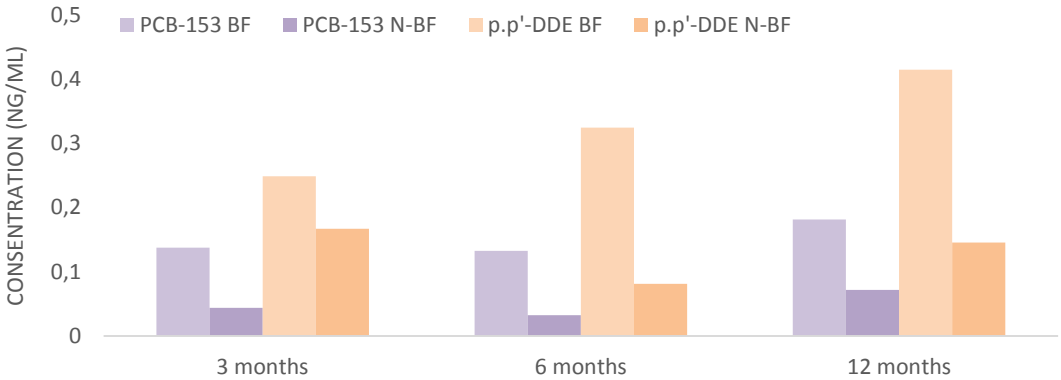

***Figure 3-6:** Median contaminant concentration in children's serum at 3, 6 and 12 months. BF=Breastfed, N-BF= Non-Breastfed. Median values at 3 months is separated by exclusively breastfeeding while at 6 and 12 months they are separated on account of daily breastfeeding.*

Parity

Multiparous mothers did not have significant lower serum concentration of *p.p'*-DDE or PCB-153 than the primiparous mothers at 28 weeks pregnant. Significantly higher levels of *p.p'*-DDE were observed in serum of primiparous mothers at 3 months (n=71, p=0.03, z= 2.1) compared to multiparous women. This difference was not seen for PCB-153. The children born by mothers who had given birth before did not have significant lower levels of the contaminants than the first-born children did.

BMI and age

When separating the women in accordance to BMI the women that were considered overweight (BMI>25) had significant higher serum *p.p'*-DDE concentration than the normal and underweight women at 28 weeks (n=49, p=0.026, z=2.219). This was not the case for PCB-153. When comparing the women in accordance to age it was evident that the women 30 years and older had a significant higher level of PCB-153 in their serum at 28 weeks (n=49, p=0.05, z=1.96) and 3 months (n=72, p= 0.009, z=2.61) than the younger women. On the other hand, *p.p'*-DDE did not show any difference with regards to serum contaminant level and age.

4 Discussion

The aims of this study was to study the contaminant concentration of *p.p'*-DDE and PCB-153 in mothers at 28 weeks pregnant, and in both mothers and children at 3, 6 and 12 months postpartum. Factors such as seafood consumption, BMI, age, breastfeeding prevalence and parity were taken into account to see if it had a significant impact on the interpretation of the results. In the following, the results from the study are discussed before an overview of methodological considerations is given. The latter sections present a summary, a conclusion and future perspectives.

4.1 Recruitment and descriptive characteristics

The fact that the recruitment is done at the local health centre made the study available to a large proportion of relevant participants. This may also be favourable in regard to inclusion rates (Lande et al., 2000). It was desirable to include roughly 150 families in the project. The target population consisted of a total of 489 women, while data material for the present study was collected from 115 of them (23 %).

Maternal age was in accordance with the average age (30.3) of all women (both primiparous and multiparous) giving birth in Norway in 2010 (Statistics Norway, 2014). This age also resembled the age (29.6 ± 4.6, n= 39 375) of the participants in the Norwegian Mother and Child Cohort Study (MoBa) (Brantsaeter et al., 2008). The mean BMI was 24 ± 4 kg/m^2 before pregnancy. This was comparable to the BMI for the participants in the MoBa study. As expected, the BMI was a little higher three months postpartum (25 ± 5). Over 60 % of the study population had higher education. This is considerably higher compared to national statistics (32.5 %) (Statistics Norway, 2013). Women with a lower education have shown to be less concerned about their health and will less likely participate in a surveillance program (Schade & Heinzow, 1998). The whole study population was pregnant and living on the west coast of Norway. Women living in the coastal areas of Norway appears to eat seafood on average 6 grams a day more than women living in the inland do (Meltzer et al., 2002). Because of the factors just mentioned (education, pregnant, residence), the findings presented here may not be representative for the Norwegian non-pregnant population and direct comparisons must therefore be interpreted with caution. The children's

height and length median was within 2 SD from the WHO Child growth standards at 3, 6 and 12 months (WHO, 2009).

4.2 Discussion of results

4.2.1 Seafood intake status among the mothers

The majority (~ 60 %) of women reported eating seafood for dinner once or twice a week, while few (12-15 %) women reported eating seafood more than three times a week, particularly not at 28 weeks pregnant (4 %) (**Figure 3-1**). Results from the present study show no significant differences between the frequencies of seafood across the four different times. The median seafood consumption was found to be 224 g/week, a value far below the recommendations that states 300-450 g of fish weekly (Directorate of Health, 2013). These recommendations were provided after data collection in the present study was started, and the frequency categories in the Seafood-FFQ are therefore not compatible with the recommendations. It was not possible to estimate the quantitative intake (g) of seafood as spread. Because of this, it is safe to assume that the total seafood intake (g/week) in the study population is slightly higher than the calculated median intake of 224 g/week.

Oily fish constituted 24-27 % of total seafood intake as dinner among the women in the study (**Table 3-5**). This corresponds to approximately 65 g/week, an amount that is well below the recommended intake of 200 g of oily fish per week. As good as all seafood spread consists of oily fish, i.e. mackerel in tomato sauce and smoked salmon/trout, so this percent can safely be revised upwards. It is however unlikely that these women will reach the recommendations since the average reported frequency of seafood as spread was approximately 25 g once or twice a week. The consumption of oily fish is in agreement with the fish intake among women of reproductive age in Norway, whom eat oily fish in amounts equivalent to less than half a portion per week (Johansson, 1997; Totland, 2012). The majority of women consumed more lean fish for dinner than oily fish (**Table 3-5**). The intake of lean fish, including processed fish, from the present study (~66 %) are in accordance with national reports (VKM, 2006) and other previous studies (Brantsaeter et al., 2012) stating that lean fish is the major contributor to total seafood intake.

It has been demonstrated that there is an increased risk of not gaining the positive health effects of the marine ω-3 PUFAs when consumption is less than twice per week (Hibbeln et al., 2007). This indicates that the intake of oily fish among the participant in the study was too low with regards to obtaining the beneficial effects from the ω-3 PUFAs. With a low intake of oily fish one can expect low levels of POPs as oily fish is one of the biggest contributors to POPs in the Norwegian diet (Norwegian Institute of Public Health, 2010). Even though oily fish is considered a major source to POPs it is important to emphasize that oily fish does not give the same increase in blood levels as intake of fish liver or seagull eggs (Kvalem et al., 2009). When it comes to oily fish consumption it is a question on quantity, and therefore, after careful consideration, the Norwegian Directorate of Health (2013a) conducted the clarification of the dietary advice on fish intake. This states that even if a person eats more than the recommended amount of oily fish, it does not necessarily comprise a health risk, but reduces the wide safety margins.

The seafood consumption in Norway is despite of the clarification too low and pregnant and lactating women should therefore be encouraged to increase their seafood intake (even though it consist of POPs). One way of doing this might be to increase the intake of seafood as spread. Most of the spread available is oily, with high amounts of the marine ω-3 PUFAs, and an increased intake can be enough to avoid negative neurodevelopmental outcomes in the child (Lauritzen et al., 2001) and postpartum depression in mother (Markhus et al., 2013). The use of spread to increase the total seafood intake might be particularly convenient for pregnant and lactating women, as spread is an individual meal and will not change the dietary habits for the whole family, although this also probably would be of interest.

4.2.2 Contaminant status

This section addresses both mother and children's contaminant levels and to which extent these levels are affected by confounding factors such as seafood intake, breastfeeding prevalence, parity age and BMI.

Mean and median values

Both *p.p'*-DDE and PCB-153 was detected in the serum of corresponding mother and child, not surprisingly as they are the most abundant two POPs in human serum (**Figure 1-6**). In the present study, the serum median values were considered for examining the

concentration of contaminants. The median values were compared to the reference values given by the The German Human Biomonitoring Commission (Schulz et al., 2011) (**Table 1-2**).

***p.p'*-DDE**

The median values were 0.2 ng/ml for both mother and child at all times, after adjusting for the associated uncertainty (**Figure 3-3**). This is well below the reference value (RV_{95}) from Germany (**Table 1-2**), and comparable with values gathered from Danish school children (0.2 ng/ml) and their mothers (0.3 ng/ml) (Morck et al., 2014). Examining the maximum levels at the different times, it is evident that the mothers having the highest concentrations were all above 30 years old, hence within the RV_{95} for their age group. The women with an origin outside Norway had among the highest serum *p.p'*-DDE concentrations of the women in the study population. This is in accordance with other studies (Polder et al., 2009; Skaare et al., 1988). A possible reason for the observed high contaminant levels in non-Norwegian mothers compared to Norwegian mothers might be the continuous use of DDT in countries battling malaria, or that DDT was banned at a later stage compared to Norway. The fact that DDT is still in use causes the surroundings to always get a refill of this persistent pollutant. Even though multiple longitudinal studies show a decline in POPs concentration in both environment (EEA, 2013) and in human serum samples (Hagmar et al., 2006; Nost, et al., 2013; Vo et al., 2008) over the last decade(s) one can assume that the concentrations in the polar regions would be even lower if there was a global ban (Skaare et al., 1988). The children's maximum levels shows that at 3 months (1 child, value: 0.88) and 6 months (6 children, values: 0.71-1.86) and in particular 12 months (3 children, values: 0.71-5.22) there are samples that exceeds the RV_{95} value (0.7 ng/ml). Four reasons reoccurred as factors for high serum *p.p'*-DDE concentrations in the children; mothers origin, age, BMI and length of breastfeeding; factors that will be addressed in upcoming sections.

PCB-153

Median values of PCB-153 for mother (0.08 – 0.13 ng/ml) and child (0.10-0.11 ng/ml) was way below the German RV_{95} value at all times (Schulz et al., 2011), but also lower than the concentrations measured in Danish mothers (0.18 ng/ml) and children (0.12 ng/ml) (Morck et al., 2014). PCB-153 can be used as a biomarker for total PCB

exposure because concentration of this congener are relatively high and highly correlated to the total molar concentration of PCBs (Govarts et al., 2012). The ΣPCB RV_{95} is set to 1.0 ng/ml for children (ages 7-14), 2.0 ng/ml and 3.2 ng/ml for adults 20-29 years and 30-39 years, respectively. As mentioned earlier the total serum PCB concentration consist of approximately 15 % PCB-153 (Lin et al., 2013), and by multiplication one can estimate the ΣPCB quantity in a serum sample. After this estimation, it is evident that neither the mothers nor the children's median values come close to reaching the RV_{95} for ΣPCB.

Biomarker correlation and presentation

The degree of correlation between the two biomarkers, PCB-153 and *p.p'*-DDE, were relatively high for both mothers and their children at every time (ranging from 0.57 to 0.86) (**Table 3-9**). This supports a common exposure source for both contaminants, namely through the diet. The same high correlation coefficient is not possible to spot in studies were different countries/populations have been compared and where PCB and pesticides have been used to various extents (Jonsson et al., 2005; Skaare et al., 1988).

The majority of the studies done on this topic have presented the contaminant levels as ng/g serum lipid. The present study did not analyse the study population's serum lipid level and this adjustment was therefore not done. Studies performed by Swedish scientists (Glynn et al., 2011; Rylander et al., 2012) presents results that show very high correlations between fresh weight and lipid adjusted values for serum PCB-153, thereby indicating that the effect of not using lipid adjustment in epidemiological studies of PCB exposure would be of minor concern. A high and very similar correlation between fresh weight and lipid-adjusted values in both fasting and non-fasting individuals was also seen, suggesting data from non-fasting subject could be used even if lipid data is missing (Rylander et al., 2012).

Time trends of *p.p'*-DDE and PCB-153

There was a significant higher *p.p'*-DDE and PCB-153 concentration in pregnancy (28 weeks) compared to 3, 6 and 12 months postpartum (**Figure 3-3**). This is a result of excretion over the placenta and through breast milk (Verner et al., 2013; Vizcaino et al., 2014). Not all of the mothers breastfeed, but everyone will transmit some of their

own body burden onto the child over the placenta. This rate will increase as the child gets bigger and it's therefore believed to be greatest the last three months of pregnancy (after the 28 week samples was collected). This was not reflected in the children's serum levels, and may come because of the inconsistency of breastfeeding (**Figure 3-4**). Some of the children are breastfed and will increase their serum contaminant level, while other children are not breastfed and will therefore experience a drop in serum contaminant level due to quick growth rate the first year of life (**Figure 3-6**).

Confounding factors

There were no correlation between the mothers' contaminant level at 28 weeks and the children's birth weight and length. The findings on this topic is very inconsistent. Multiple studies report of no correlation (Gladen et al., 2003; Longnecker et al., 2005; Wojtyniak et al., 2010), while a recently published meta-analysis reports that PCB-153 causes a small for gestational length (Govarts et al., 2012). Since the study population only consisted of women, and very few of them were smoking, test performed on gender differences and smoking relations to serum concentrations of the selected POPs were not performed.

Seafood intake

The present study shows that at 28 weeks there is no correlation between seafood intake and serum contaminant levels, besides from the consumption of shellfish and PCB-153 (**Table 3-7**). High consumers, and children of high consumers, did not show significant higher serum contaminant levels than the mother having lower seafood intake. The body burden and toxicity of POPs depend on their bioaccumulation resulting from long-term exposure and are not directly linked with the amount consumed at a given time (Papadopoulou et al., 2013). Therefore, contaminants accumulated in mother prior to pregnancy also play an important role. There are several publications on the positive relationship between dietary intake and blood levels of POPs either assessed as food consumption (Gasull et al., 2011; Jacobson & Jacobson, 1996), or as calculated intakes (pg TEQ/kg bw/day or ng/kg bw/day) of the certain POPs (Darnerud et al., 2006; Kvalem et al., 2009; Papadopoulou et al., 2013).

A reason for the correlation between PCB-153 and shellfish may be the fact that crab, especially the brown meat, is high in PCBs (Directorate of Health, 2009; NIFES, 2010). This study showed that the women eating fish liver had significant higher concentrations of PCB-153 and *p.p'*-DDE at all times. Fish liver was kept as a separate category because of its relative high content of POPs such as PCBs, dioxins and pesticides (Kvalem et al., 2009; NIFES, 2010; VKM, 2006). Consumption is, because of this, discouraged during pregnancy by the Norwegian Directorate of Health (Directorate of Health, 2009). Children's seafood consumption at 12 months had little relations to measured *p.p'*-DDE and PCB-153 levels suggesting that the primary predictor of serum contaminant levels is breastfeeding (Barr et al., 2006).

POP transfer from mother to child

A correlation in the serum contaminant levels were seen between mother and child at 3 months for both *p.p'*-DDE and PCB-153 and only in *p.p'*-DDE at 6 months (**Figure 3-5**). At 12 months none of the congeners showed a correlation. These results are in agreement with previous observations where strong maternal-infant correlations for the concentrations of these contaminants were found (LaKind et al., 2004). This correlation is probably a result of both placental transmission (Vizcaino et al., 2014) and breastfeeding (Verner et al., 2013). PCB-153 has shown one of the highest partition rates into breast milk (and across the placental barrier) of the PCB congeners while *p.p'*-DDE, having fewer chlorine substitutions, may have an even higher partition rate (EFSA, 2005b; Needham et al., 2011; Vizcaino et al., 2014). This may be one explanation for the observed correlation between serum concentration in mother and child at 6 months for *p.p'*-DDE, and not PCB-153.

Results from **Table 3-10** indicate that breastfed children have significantly higher serum contaminant levels than formula-fed children. This is in accordance with studies conducted in Germany by Lackmann and co-workers (2004, 2005) which found that the infant's that had been breast fed had an approximately 10 fold higher serum concentration of *p.p'*-DDE and PCB-153 at 6 weeks old than the infant's that had been given formula. A reason for this significant difference is that the infants not exposed to breast milk will experience a drop in contaminant level after birth as a result of fast growth and thereby a dilution of the body burden, whereas breastfed children will experience a steady increase as long as they are breastfed (Verner et al., 2013). Even

though breast milk is thought to be the primary determinant of children's serum contaminant levels until at least 7 years of age (Barr et al., 2006) it is important to emphasize that the Norwegian Scientific Committee on Food Safety (VKM, 2013) states that "the benefits associated with breast milk and breast feeding clearly outweigh the risk presented by the current levels of contaminants in the breast milk". Whether or not the child is exclusively or partially breastfed up to 6 months, or partially breastfed up to 12 months does not affect this conclusion (VKM, 2013).

Parity

Multiparous women did not have significant lower serum levels of the selected contaminants in their serum samples at 28 weeks pregnant than the primiparous women. This contradict reports (EFSA, 2005b; WHO, 2003) stating that multiparous women should have a significant lower level of these contaminants because of previous breast feeding and placental transfer. Reason for this may be that this study have a smaller sample size than what the bigger reports are based on, and that this study have not corrected for the mothers' life-long length of breastfeeding as this information was not asked for in the Seafood-FFQ. At three months, multiparous women showed a significant lower *p.p'*-DDE concentration compared to primiparous women. This sudden difference between the two groups may be explained by various transmission rates as a result of the previous birth(s) or by properties related to the steric conformation of *p.p'*-DDE. However, to our knowledge, no studies have been carried out on this topic, and it is therefore hard to conclude with any possible reasons.

Age

Women over 30 years of age had significant higher PCB-153 concentrations than their younger fellow participants at both 28 weeks pregnant and 3 months postpartum. This is in accordance with earlier findings, and can be caused by both an age-dependent bioaccumulation of the persistent and lipophilic contaminants and that elderly individuals have been exposed to higher doses of the pollutants early in life compared to the younger subjects (Polder et al., 2009; Rylander et al., 1997). When exploring the impact of age on the serum concentration in women it is important to keep in mind that the age is associated with both parity and total length of breastfeeding, as they work against an accumulation in the mother's serum (Jonsson et al., 2005). The statistical model conducted in this study only accounted for parity, as total length of breastfeeding

was not given, and might be the reason that *p.p'*-DDE did not show the same significant difference with regards to serum concentration and age.

BMI

The women that were considered overweight (BMI>25) had a significant higher serum *p.p'*-DDE concentration at 28 weeks than the women with a lower BMI (BMI<25). The same trend was not seen for PCB-153. This can be associated with the findings of a German study who found that the post-pregnancy BMI was positively associated with the DDT concentrations in breast milk, but negatively associated with PCB concentrations (Schade & Heinzow, 1998). Another study (Jonsson et al., 2005) stated the importance of timing when looking at the relations between BMI and serum contaminant level. Jonsson and co-workers (2005) found that if the substantial exposure was years ago and the subject was old enough to have reached a steady state of their body burden of POPs, it should be possible to see a positive association between POPs in serum and an elevated BMI.

4.3 Methodological considerations

4.3.1 Seafood-FFQ

To assess the habitual seafood intake among the women a Seafood-FFQ was used. This was self-administrated via e-mails at 28 weeks and 3 months, and done manually at 6 and 12 months. Electronically administration may increase the response rates (Johansson, 1997). A difference in response rate was, however, not observed. A drawback with the questionnaire is that the participants did not have the opportunity to consult anyone if they had any uncertainties about the questionnaire either with electronic or manually completion. The fact that it was the pregnant and lactating mothers that was scrutinized in this study makes the measurements of dietary habits extra challenging. This is a group that has large individual variations, were some experience periods of nausea, vomiting, constipation and bed rest that may influence eating habits (Meltzer et al., 2008). Multiple forms, and the fact that the first form was set to describe the diet in pregnancy from the 3rd month onwards accounted for this. The first trimester of pregnancy have shown to be the most vulnerable to nausea and vomiting caused by pregnancy (Kramer et al., 2013).

4.3.2 Analytical quality of the HRGC-HRMS method

The analytical method used in the present thesis has proved to be a reliable and robust approach for quantifying POPs. However, it must be mentioned that the method in question is under evaluation and will be validated and accreditated in the near future. Validation is needed to assure that the quantitative measurements of the compounds (in this case *p.p'*-DDE and PCB-153) are reliable and possible to reproduce at a later stage. Validation occurs through documenting the selectivity, accuracy, precision, recovery, sensitivity, and stability of the method (FDA, 2001), all described in Section 2.4 of this thesis. When the analytical quality of the method were to be assessed CRM, blank samples and std3 had to be taken into account (Appendix IV).

Two samples of CRM were run every day. For *p.p'*-DDE all of the samples was inside the desired concentrations (**Figure A3**). This was not the case for PCB-153, where there were some samples that did not passed the t-test (bars marked with red) (**Figure A4**). The t-test was conducted to see which samples that did not stay inside the recovery range of 30-130 %. In general, it was observed a lack of agreement between CRM parallels, only one of the two CRM samples prepared at specific days showed a value that was inside the recovery variation range. The decision was therefore made not to exclude any samples that were analysed that particular day. The same was seen in regards to the blank (**Figure A1 and A2**) and the parallel samples where one was inside the desired range, while the other was outside. There are several lurking factors responsible for the observed behaviour among them. Sample preparation and instrumental variability seem the most obvious. It was decided to evaluate the sample preparation factor, considering that previous publications have demonstrated that HRGC-HRMS is a reliable technique for quantifying POPs. To this aim, new samples were prepared to check whether the lack of agreement was still present between parallels. Unfortunately, due to some technical problems the HRGC-HRMS was shutdown. Despite the inconvenience, the consistency between the conclusions derived from the present and previous reported studies gives enough credence to confirm the reliability of the results of the present thesis.

4.4 Summary & conclusions

As Norway is a coastal country, seafood is an integrated part of the Norwegian diet, and an important source of several essential nutrients. However, result from the present study suggest that average maternal seafood consumption is lower than recommended by the Norwegian Directorate of Health, and an increase should therefore be encouraged.

The serum contaminant level of the study population was generally low, and beside shellfish and liver there were not seen a correlation between seafood intake and serum *p.p'*-DDE and PCB-153. The consumption of fish liver was strongly correlated with elevated serum levels of *p.p'*-DDE and PCB-153, and demonstrates the importance of communicating relevant dietary advice for pregnant and lactating women. The mothers' serum *p.p'*-DDE and PCB-153 concentration was significant lower at 3, 6, and 12 months postpartum compared to measurements done in pregnancy. This is probably due to the excretion over the placenta and in breast milk. This study did not find significant lower serum *p.p'*-DDE and PCB-153 concentrations in the multiparous women compared to the primiparous although bigger reports have shown this correlation. PCB-153 and *p.p'*-DDE increased with age and BMI, respectively. The children's serum levels were affected by their mothers' contaminant level as their mothers' provide them with all the nutrients from life in the utero until they are introduced to other foods than breast milk. The children given breast milk had higher serum concentrations of PCB-153 and *p.p'*-DDE than the children who were formula fed from an early stage.

The study population have their residency on the western coast of Norway, have a higher median educational level and a high focus on a healthy diet. Hence, a comparison to the general population needs to be done with caution. The current study indicate that the present content of the environmental contaminants in oily fish does not support a limit of two meals of oily fish a week for young- and pregnant women.

Briefly summarized:

- The seafood consumption (especially that of oily fish) among pregnant and lactating women is too low compared to the national recommendations.
- In general, the serum concentrations of *p.p'*-DDE and PCB-153 in mother and child in this study population is low.
- Breastfed children have higher serum *p.p'*-DDE and PCB-153 concentrations compared to children who are not breastfed.
- The women who eat fish liver more than once a year have significant higher serum *p.p'*-DDE and PCB-153 levels than the mothers who never eats fish liver.

Main conclusion: Serum *p.p'*-DDE and PCB-153 levels in mother and child does not correlate with maternal fish intake prior to- and during pregnancy.

4.5 Potential improvements of the study design

There are several measurements that can be done to improve the study design. The most important improvement is to change the categories in the first question regarding seafood as dinner. This should be converted to "2-3 times/week" to facilitate identification of respondents who are ranked below and above the recommended weekly intake of seafood. The question on portion size of seafood as spread should also be evaluated. Today there is no possible way to calculate weekly intake as there were no closed response categories. A question on the weight right after birth should be incorporated in the FFQ. If both weight at birth and at 3 and 6 months is present one can calculate the BMI change for the mothers postpartum. This change can be associated with contaminant levels transferred from mother to child. One last aspect regarding the Seafood-FFQ is to get information on life-long length of breastfeeding, as this may play a part in the transmission rate of POPs. The analytical methodology needs to go through more samples so that parameters such as LOD and a LOQ value can be established, and the method can be validated.

4.6 Future perspectives

This type of monitoring studies are essential to ensure that the degree of contamination is minimized, although more advanced statistical models should be used in order to avoid misintrepertations and misleading conclusions. Multilevel linear models is a good tool in longitudinal studies in which the problems of participant drop-out and other forms of missing measurements within individuals are often encountered (Field, 2013). These multilevel linear models can be used to explore the causal links between the linkages of predictors and changes in outcome variables across time. It is more powerful than other methods (e.g. ANOVA, Friedman Tests and Mann Whitney U Tests used in this study) in examining the effects associated with repeated measures. This because it models the covariance matrix (i.e., fitting the true covariance structure to the data) rather than imposing a certain type of structure as commonly used in traditional univariate and multivariate approaches (Field, 2013). While a second round using such tools is planned to be done in the future, it was due to its complexity, beyond the scope of this master's project.

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6 Appendix

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Appendix I: Seafood-FFQ

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
☐	☐	☐	☐	☐

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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 <u>som middag</u>?

	3 ganger eller mer/uke	1-2 ganger/uke	1-3 ganger /mnd	Sjeldnere enn 1 gang/mnd	Aldri
Laks, ørret	☐	☐	☐	☐	☐
Makrell	☐	☐	☐	☐	☐
Sild	☐	☐	☐	☐	☐
Kveite	☐	☐	☐	☐	☐

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Uer	☐	☐	☐	☐	☐
Steinbit	☐	☐	☐	☐	☐
Flyndre, rødspette	☐	☐	☐	☐	☐
Torsk	☐	☐	☐	☐	☐
Sei	☐	☐	☐	☐	☐
Hyse	☐	☐	☐	☐	☐
Abbor, gjedde (ferskvann)	☐	☐	☐	☐	☐
Røye, sik (ferskvann)	☐	☐	☐	☐	☐
Reker	☐	☐	☐	☐	☐
Krabbe	☐	☐	☐	☐	☐
Hummer	☐	☐	☐	☐	☐
Blåskjell	☐	☐	☐	☐	☐
Kamskjell	☐	☐	☐	☐	☐
Fiskekaker	☐	☐	☐	☐	☐
Fiskeboller	☐	☐	☐	☐	☐
Fiskepudding	☐	☐	☐	☐	☐
Fiskegrateng	☐	☐	☐	☐	☐
Fiskepinner	☐	☐	☐	☐	☐

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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	☐	☐	☐	☐	☐

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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)

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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	☐	☐	☐	☐	☐

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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) ☐

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1 spiseskje (11 ml) ☐

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

1-2 kapsler ☐ 5 eller flere kapsler ☐

3-4 kapsler ☐

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	☐	☐	☐	☐	☐

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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:

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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

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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
☐	☐	☐	☐	☐

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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

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☐ Bruker medisiner Spesifiser gjerne produktnavn:

_____

_____

TAKK FOR INNSATSEN!

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Appendix II: Interviewer administrated FFQ

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? _____

Appendix III: ISTD, RSTD, STD3 & List of chemicals

***Table A 1**: Content of the different compounds in ISTD and RSTD*

	Art.nr	Content		ng/ml
	CLM-1627	13C12 pp-DDE		50
	EC-1435	13C12 PCB-118		20
ISTD	EC-1436	13C12 PCB-138		20
	EC-1406	13C12 PCB-153	Diluted with isopropanol to	20
	EC-1407	13C12 PCB-180		20
	EO-4982	13C12 PBDE-47		25
	Art.nr	**Content**		**ng/ml**
RSTD	EC-1415	13C12 PCB-111 Rec	Diluted with nonane to	40

***Table A 2**: Standards used in the calibration curve. Std 3 is used to confirm the validity of the calibration curve and consequently the reliability of the computed concentrations at spesific days. Control card Std3 is found in Appendix IV.*

	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

***Table A 3**: List of chemicals (name, supplier) used in the preparation of the samples.*

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

APPENDIX III

Appendix IV: Analytical quality

BLANK

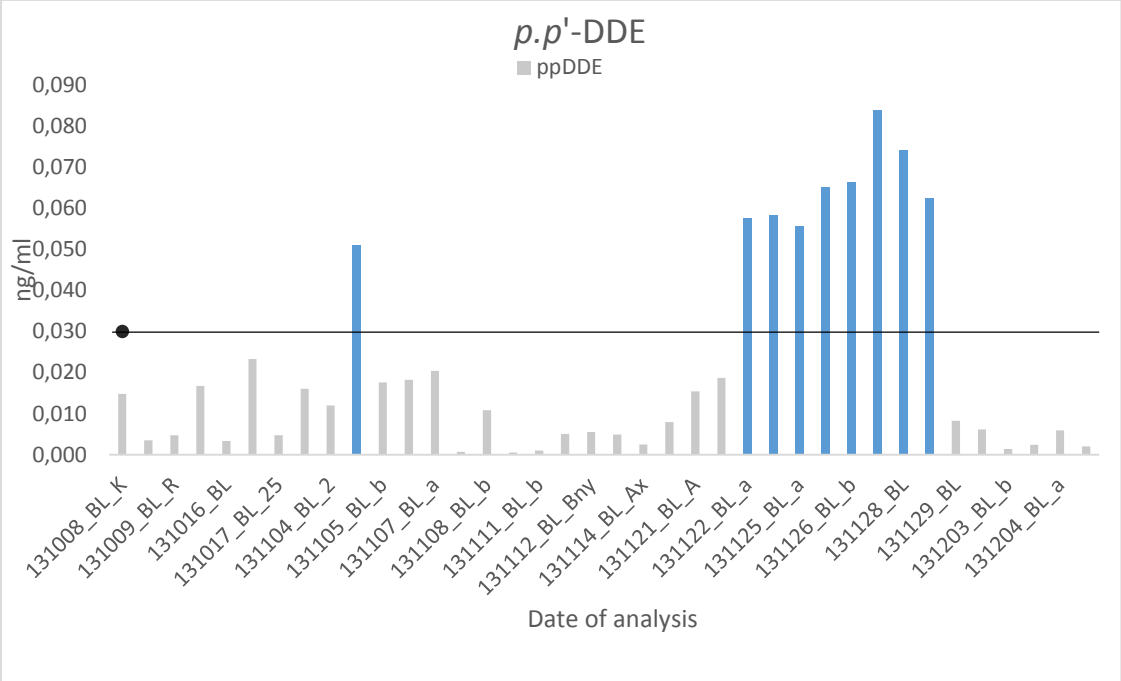

Figure A 1: Blank values for *p.p'*-DDE (ng/ml) at the different preparation days. Blue bars indicate values above 30 % that of CRM.

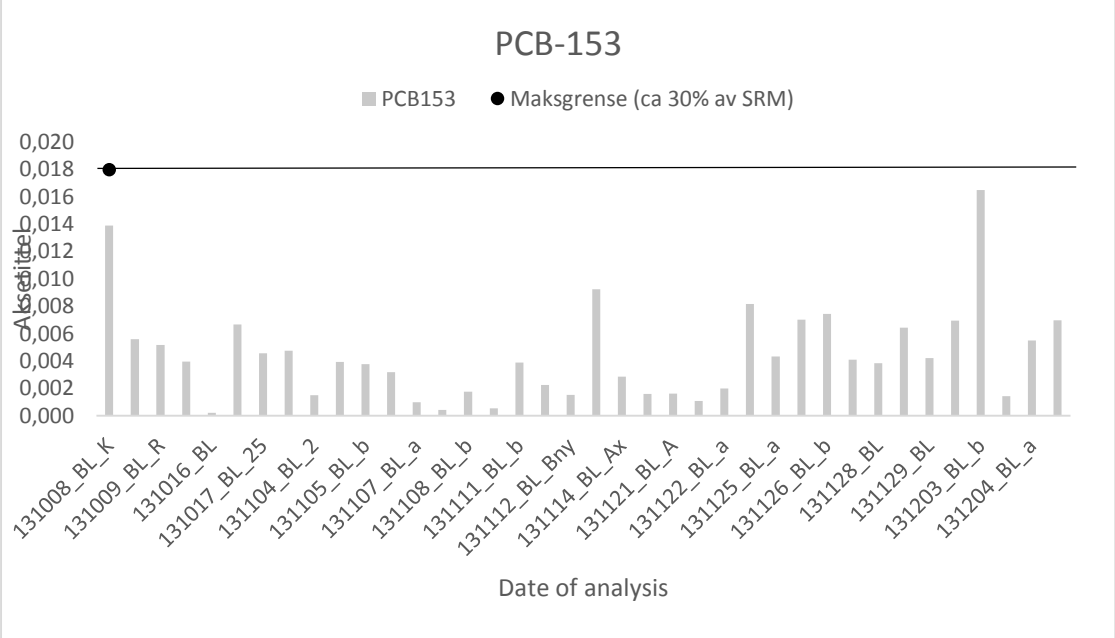

Figure A 2: Blank values for PCB-153 (ng/ml) at the different preparation days.

CRM

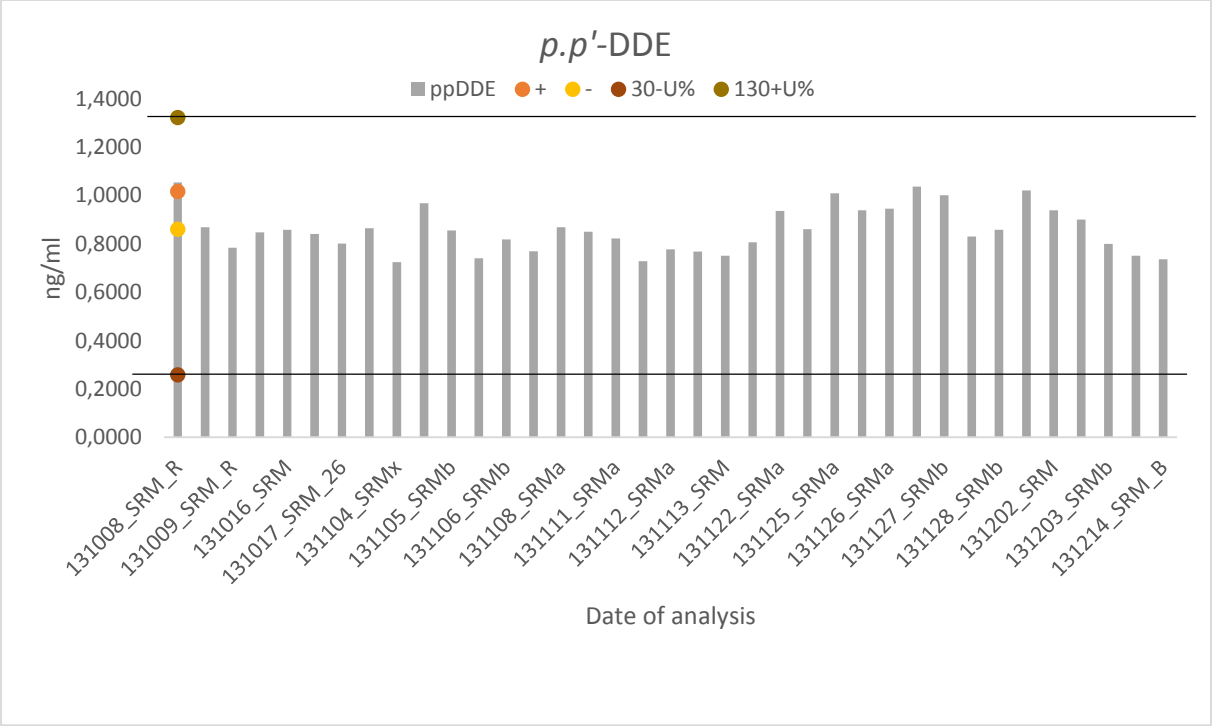

Figure A 3: CRM values for *p.p'*-DDE (ng/ml) at the different preparation days.

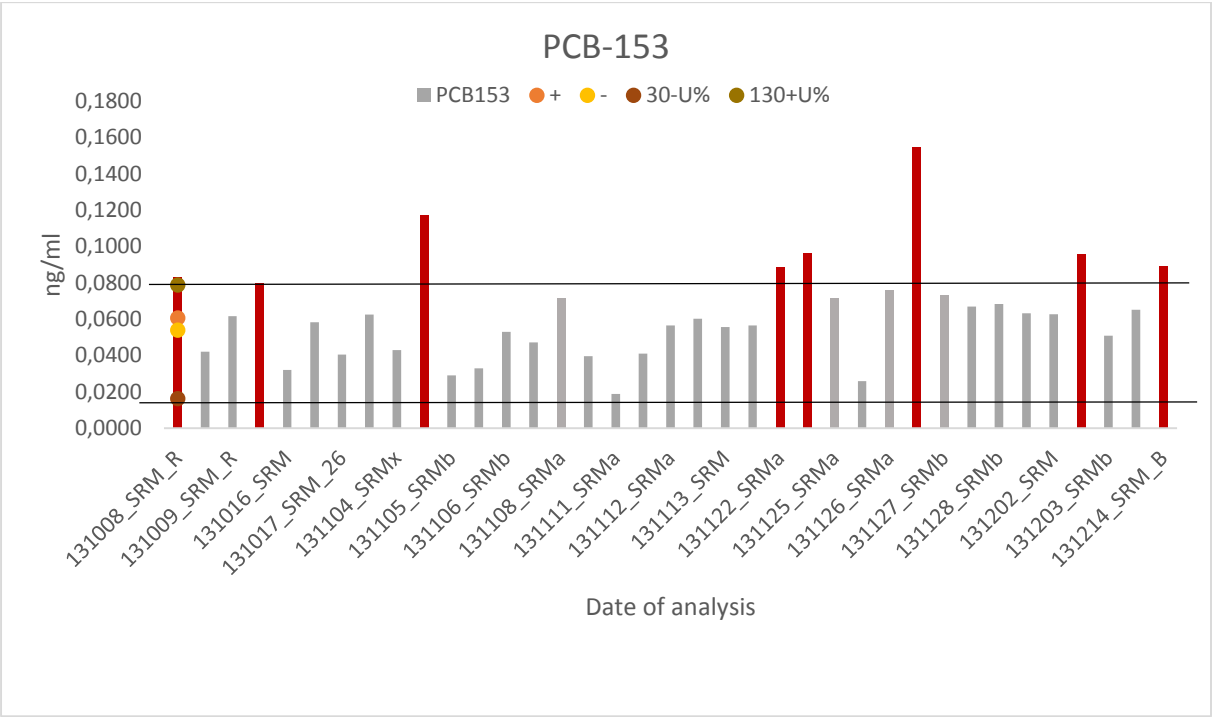

Figure A 4: CRM values for PCB-153 (ng/ml) at the different preparation days. Red bars indicate values above 130 % recovery.

Std3

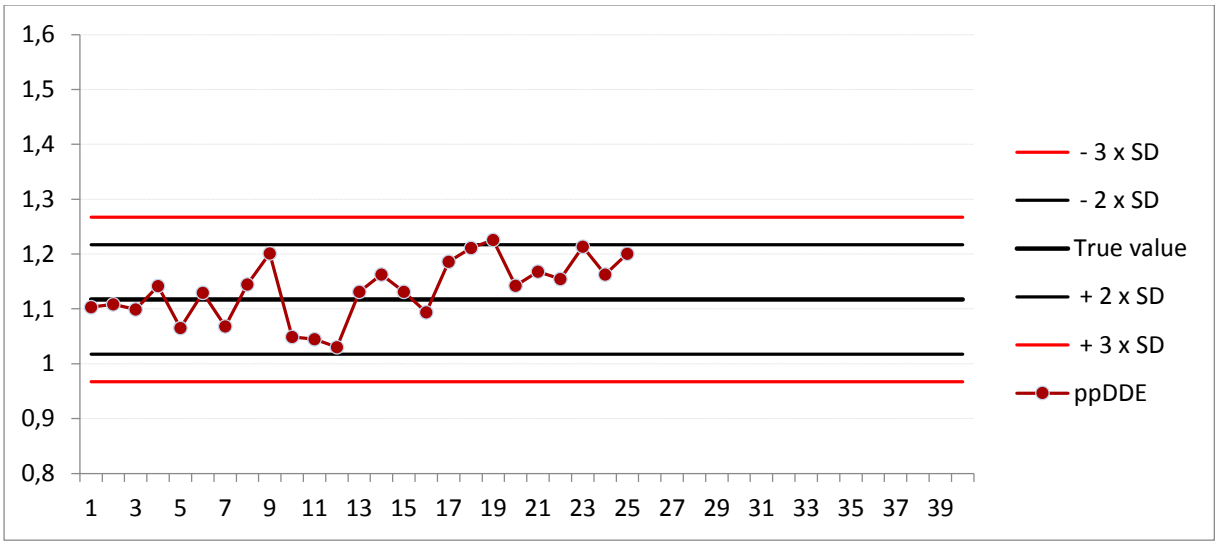

Figure A 5: *Std3 for p.p'-DDE.*

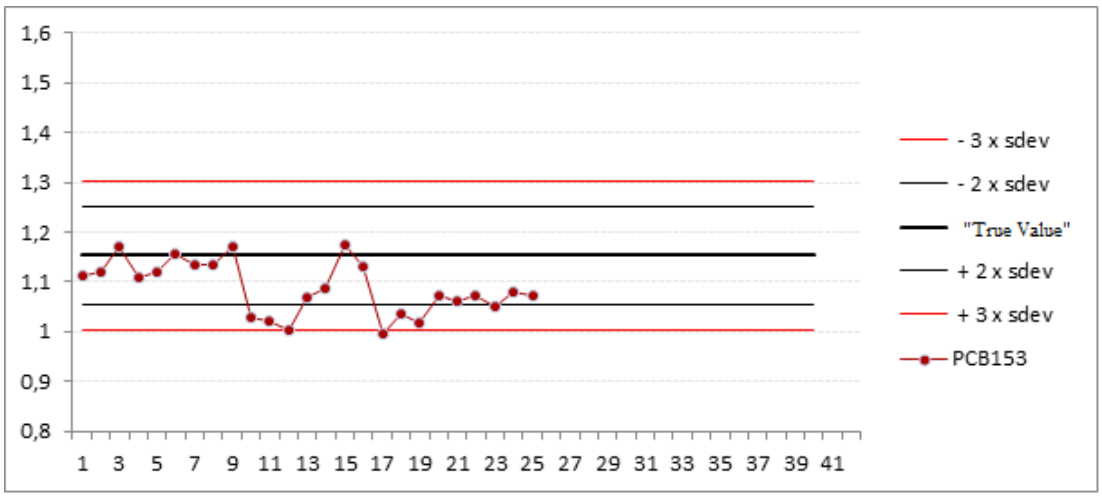

Figure A 6*: Std3 for PCB-153*