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Karolinska Institutet, Stockholm, Sweden

**PERSISTENT ORGANIC POLLUTANTS
IN SWEDISH FIRST-TIME MOTHERS
AND EFFECTS ON INFANT HEALTH**

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Till min familj

ABSTRACT

Humans are exposed to a variety of persistent organic pollutants (POPs) that have been spread to the environment because of various human activities. Food is the main source of exposure to most POPs. Many POPs are lipid-soluble, accumulate in the human body and are easily transferred to the fetus and to breastfed infants through breast milk. POPs have been shown to cause a number of adverse effects in animals, including effects on reproduction, development and on endocrine, nervous and immune systems.

The overall aims of this thesis were to quantify body burdens of POPs in pregnant and nursing women in Sweden and to study whether current maternal, fetal or infant exposure to POPs is associated with birth weight or markers of thyroid function.

First-time mothers were recruited in Uppsala, Sweden between 1996 and 2010 (POPUP cohort). Samples (breast milk or blood) from the participating women were analysed for polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxin and dibenzofurans (PCDD/Fs) and polybrominated diphenyl ethers (PBDEs). Thyroid hormones were analysed in maternal and infant blood. Data on lifestyle factors and diet were collected by interviews and questionnaires.

The results showed that levels of PCBs and PCDD/Fs in breast milk decreased with 4-8% per year during the study period. Temporal trends for PBDEs varied depending on congener studied, with decreasing levels of BDE-47, -99 and -100 (5-10% per year) and slightly increasing levels of BDE-153 (1% per year). High maternal age and fast weight loss after delivery predicted higher levels of PCBs and PCDD/Fs in breast milk, whereas a high pre-pregnancy body mass index and large weight gain during pregnancy predicted lower levels. Women who were breastfed during infancy, grew up on the east coast of Sweden and had a high consumption of contaminated fatty Baltic fish during the year before pregnancy had higher levels of some POPs in breast milk.

Prenatal exposure to di-*ortho* PCBs (estimated by breast milk levels) was significantly associated with higher birth weight, whereas breast milk levels of PBDEs were associated with lower birth weight. The mean difference in birth weight between the 25th and 75th percentiles of exposure was approximately 100 g for di-*ortho* PCBs and -80 g for PBDEs.

Associations between exposure to PCBs, PCDD/Fs and PBDE and thyroid hormone levels in mothers during pregnancy and in infants after delivery were weak and non-significant in most cases. However, a higher maternal body burden of PCDD/Fs was associated with lower maternal levels of triiodothyronine (T3). This association was similar in early and late pregnancy, which strengthens its reliability.

Altogether, this thesis provides knowledge about exposure to POPs in Swedish first-time mothers that is useful in risk assessments of POPs in food. The margins between current body burdens of POPs and the levels tolerable from a health perspective are in some cases small or non-existent. In addition, the observed associations between POP exposure and birth weight and thyroid hormone status may be of importance for public health. Hence, it is desirable that body burdens of PCBs, PCDD/Fs and PBDEs in Swedish women continue to decrease. Efforts to reduce contamination of the environment and of the food chain should therefore be continued. Monitoring of POP levels in breast milk is an important tool to follow-up human POP exposure.

LIST OF PUBLICATIONS

- I. **Lignell S**, Aune M, Darnerud PO, Cnattingius S and Glynn A. 2009. Persistent organochlorine and organobromine compounds in mother's milk from Sweden 1996-2006: Compound-specific temporal trends. *Environ Res* 109(6): 760-767.
- II. **Lignell S**, Aune M, Darnerud PO, Soeria-Atmadja D, Hanberg A, Larsson S, Glynn A. 2011. Large variation in breast milk levels of organohalogenated compounds is dependent on mother's age, changes in body composition and exposures early in life. *J Environ Monit* 13(6): 1607-1616.
- III. **Lignell S**, Aune M, Darnerud PO, Hanberg A, Larsson SC, Glynn A. 2013. Prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) may influence birth weight among infants in a Swedish cohort with background exposure: a cross-sectional study. *Environ Health* 12: 44.
- IV. Darnerud PO, **Lignell S**, Glynn A, Aune M, Törnkvist A, Stridsberg M. 2010. POP levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from Uppsala, Sweden. *Environ Int* 36(2): 180-187.
- V. **Lignell S**, Aune M, Darnerud PO, Stridsberg M, Hanberg A, Larsson SC, Glynn A. Persistent organochlorine and organobromine compounds and serum levels of thyroid hormones in a Swedish mother-child cohort with background exposure. *Manuscript*.

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LIST OF ABBREVIATIONS

AhR	aryl hydrocarbon receptor
BFR	brominated flame retardant
BMD	benchmark dose
BMI	body mass index
CI	confidence interval
DDE	dichlorodiphenyl-dichloroethylene
DDT	dichlorodiphenyl-trichloroethane
DL	dioxin-like
EFSA	European Food Safety Authority
EU	European Union
HC	hierarchical clustering
IARC	International Agency for Research on Cancer
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
LOAEL	lowest observed adverse effect level
LOQ	limit of quantification
MOE	margin of exposure
NDL	non-dioxin-like
NFA	National Food Agency (Livsmedelsverket)
NOAEL	no observed adverse effect level
PBDE	polybrominated diphenyl ether
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo- <i>p</i> -dioxin
PCDF	polychlorinated dibenzofuran
POP	persistent organic pollutant
POPUP	persistent organic pollutants in Uppsala primiparas
RfD	reference dose
RIVM	National Institute of Public Health and the Environment
SCF	Scientific Committee on Food
SIM	single ion monitoring
T3	triiodothyronine
T4	thyroxine
TBG	thyroxine-binding globulin
TCDD	2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin
TDI	tolerable daily intake
TEF	toxic equivalency factor
TEQ	toxic equivalent
TRH	thyrotropin-releasing hormone
TSH	thyroid stimulating hormone
TTR	transthyretin
TWI	tolerable weekly intake
US-EPA	US Environmental Protection Agency
WHO	World Health Organization

1 BACKGROUND

1.1 INTRODUCTION

Persistent organic pollutants (POPs) are halogenated compounds that accumulate in the environment and in the human body because of their lipid solubility and resistance to chemical and biological degradation. They have been intentionally produced for different purposes or have been formed inadvertently during certain chemical processes. Examples of POPs are industrial chemicals (e.g., polychlorinated biphenyls, PCBs), chlorinated pesticides (e.g., dichlorodiphenyl-trichloroethane, DDT), brominated flame retardants (BFRs) (e.g., polybrominated diphenyl ethers, PBDEs) and by-products (e.g., dioxins (polychlorinated dibenzo-*p*-dioxins, PCDDs and polychlorinated dibenzofurans, PCDFs)) (Figure 1). Food is the main source of human exposure to most POPs. They are easily transferred to the fetus during pregnancy and breastfed infants are exposed to high levels from breast milk.

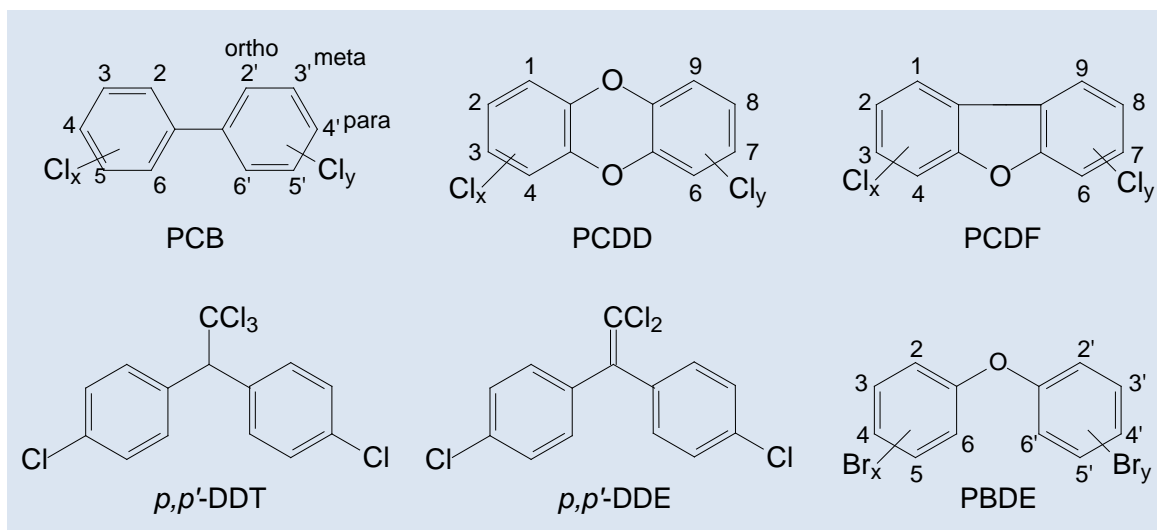


Figure 1. Chemical structures of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), *p,p'*-dichlorodiphenyl-trichloroethane (*p,p'*-DDT), the DDT-metabolite *p,p'*-dichlorodiphenyl-dichloroethylene (*p,p'*-DDE) and polybrominated diphenyl ethers (PBDEs).

In experimental studies in animals POPs have been shown to cause various effects on reproduction and development, as well as effects on the endocrine, nervous and immune systems (Darnerud 2003; Larsen 2006). The fetus is most sensitive to exposure, with children seemingly more sensitive than adults (Baccarelli et al. 2005). In humans, accidental exposure to high levels of PCBs and dioxins early in life causes, among other things, dermal lesions, retarded growth, delayed cognitive development and effects on reproduction (Aoki 2001; Guo et al. 2004). Similar but more subtle effects have been indicated in populations with background exposure to these substances (Langer 2008; Lundqvist et al. 2006; Schantz et al. 2003).

Because of their unwanted properties, the use, production and release of many POPs have been strongly controlled since the 1970s. Not surprisingly, the levels of many POPs have

decreased in the environment and in food (Bignert et al. 2012; Glynn et al. 2000; Ålander et al. 2012). However, POPs still persist in the environment and in the human body and therefore pose a possible threat to human health. In addition, for POPs that are still used by the industry or are still found in consumer products, the temporal trends may be increasing. For instance, the levels of some BFRs increased in the environment and in breast milk in Sweden from the 1970s to the 1990s (Bignert et al. 2012; Norén and Meironyte 2000).

To protect the Swedish consumers from high exposure to PCBs and dioxins the Swedish National Food Agency (NFA) has issued consumption advisories since the 1980s concerning certain fish species known to contain high levels of these chemicals (e.g. wild Baltic Sea salmon and Baltic Sea herring). The main objective of the advisories is to protect humans during their most sensitive periods of life, i.e. the fetal and infant periods and childhood. Accordingly, advisories regarding contaminated fish are directed to children and women in childbearing ages. By limiting the accumulation of PCBs and dioxins in women before they get pregnant, the fetus and infant are protected from high exposure to these substances. The most recent revision of the advisories for fish with high levels of dioxins and PCBs was conducted in 2007 (Ankarberg et al. 2007). The current recommended maximum consumption of such products as Baltic Sea herring and salmon for children and women in childbearing age is 2-3 times per year. For other groups, the recommended maximum consumption is once a week (NFA 2013).

The basis for this thesis is a study of POPs in Swedish first-time mothers that was initiated in 1996 at the NFA. The study was later given the name POPUP (Persistent Organic Pollutants in Uppsala Primiparas). In 1996, the scientific basis of consumption advisories regarding contaminated fish had just recently been revised. The conclusion reached by the NFA was that data on POP exposure of Swedish pregnant and nursing women were scarce. The POPUP study was thus started to improve basic data for future risk assessments of POPs in food.

The specific POPs studied in this thesis are PCBs, dioxins (PCDD/Fs), the DDT-metabolite *p,p'*-dichlorodiphenyl-dichloroethylene (*p,p'*-DDE) and PBDEs (Figure 1).

1.2 SOURCES OF POPs

1.2.1 PCB and PCDD/F

The industrial PCB production started in the late 1920s. At that time, PCBs were manufactured as chemical mixtures under different trade names (e.g., Aroclor and Clophen) (Bernes 1998). PCB products were highly appreciated because of their good insulating and plasticizing ability as well as their high temperature resistance. The majority of PCBs were used as dielectric fluids in transformers and capacitors, but they also had a number of other applications, such as ingredients in paints, sealants, lubricants and plastics (Bernes 1998; Erickson and Kaley 2011). In Sweden, the use of PCBs in new products has been restricted since 1972 and the use of old products containing PCBs was

prohibited in 1995 (Bernes 1998). PCB production and use have also been restricted in other parts of the world since the 1970s.

PCDDs and PCDFs (commonly called “dioxins”) are unintentionally formed as by-products during different industrial processes, such as production of chlorinated chemicals and bleaching of pulp and paper using chlorine and during incomplete combustion in for example waste incinerators (Rappe 1996). Although the emissions of dioxins have been greatly reduced since the 1980s, there are still diffuse sources of emission to the environment (e.g., uncontrolled backyard burning of waste).

1.2.1.1 The TEF-system

Depending on degree and pattern of chlorination, there are 75 possible PCDD congeners and 135 possible PCDF congeners (Figure 1). Seventeen of these congeners are chlorinated in 2,3,7,8-position and of toxicological concern. These congeners bind to and activate the aryl hydrocarbon receptor (AhR) and thereby elicit their toxic responses. Among the 209 possible PCB congeners, 12 non-*ortho*- and mono-*ortho*-substituted congeners also have the capability to bind to the AhR and are thus dioxin-like (DL). The remaining PCB congeners are named non-dioxin-like (NDL).

PCDD/Fs and PCBs are found as complex mixtures in almost all matrices. To facilitate comparisons of analytical data and risk assessment of 2,3,7,8-substituted PCDD/Fs and DL-PCBs the World Health Organization (WHO) has established the toxic equivalency factor (TEF) concept. To be included in the TEF system a congener must 1) show a structural relationship to the PCDDs and PCDFs, 2) bind to the AhR, 3) elicit AhR-mediated biochemical and toxic responses and 4) be persistent and accumulate in the food chain (Van den Berg et al. 2006). Each DL compound has been assigned a TEF based on its potency relative to the potency of the most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Table 1). By multiplying the concentration of each congener by its TEF and then adding the resulting concentrations, the toxic equivalent (TEQ) concentration of a mixture can be calculated. WHO TEF values were established in 1998 (Van den Berg et al. 1998) and reevaluated in 2006 (Van den Berg et al. 2006) (Table 1).

1.2.2 PBDE

PBDEs are included in a diverse group of chemicals called BFRs. They have been used in various materials to increase fire resistance since the beginning of the 1970s. Major uses have been in high-impact polystyrene, acrylonitrile butadiene styrene plastic, flexible polyurethane foam, textile coatings, wire and cable insulation and in electronics (WHO 1994). Some consumer products containing PBDEs include computers, home electronics, cars, furniture, building materials and textiles.

There are 209 possible PBDE congeners with a different number and pattern of bromine (Figure 1). The three commercial PBDE mixtures mostly used are named after the number of bromines in the molecules of the dominating components: pentaBDE, octaBDE and decaBDE. The pentaBDE and octaBDE mixtures have been banned with-

in the EU since 2004 (EU 2003b) and decaBDE was banned in electric and electronic equipment in 2006 (EU 2003a).

Table 1. Summary of WHO 1998 and 2006 TEF values for dioxin-like compounds. Bold values indicate a change in TEF value between 1998 and 2006.

Compound	WHO 1998 TEF ^a	WHO 2006 TEF ^b
Chlorinated dibenzo- <i>p</i> -dioxins		
2,3,7,8-TCDD	1	1
1,2,3,7,8-PeCDD	1	1
1,2,3,4,7,8-HxCDD	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01
OCDD	0.0001	0.0003
Chlorinated dibenzofurans		
2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDF	0.05	0.03
2,3,4,7,8-PeCDF	0.5	0.3
1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDF	0.1	0.1
2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDF	0.01	0.01
1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDF	0.0001	0.0003
Non- <i>ortho</i> -substituted PCBs		
3,3',4,4'-tetraCB (PCB 77)	0.0001	0.0001
3,4,4',5'-tetraCB (PCB 81)	0.0001	0.0003
3,3',4,4',5'-pentaCB (PCB 126)	0.1	0.1
3,3',4,4',5,5'-hexaCB (PCB 169)	0.01	0.03
Mono- <i>ortho</i> substituted PCBs		
2,3,3',4,4'-pentaCB (PCB 105)	0.0001	0.00003
2,3,4,4',5'-pentaCB (PCB 114)	0.0005	0.00003
2,3',4,4',5'-pentaCB (PCB 118)	0.0001	0.00003
2',3,4,4',5'-pentaCB (PCB 123)	0.0001	0.00003
2,3,3',4,4',5'-hexaCB (PCB 156)	0.0005	0.00003
2,3,3',4,4',5'-hexaCB (PCB 157)	0.0005	0.00003
2,3',4,4',5,5'-hexaCB (PCB 167)	0.00001	0.00003
2,3,3',4,4',5,5'-heptaCB (PCB 189)	0.0001	0.00003

^aVan den Berg et al., 1998. ^bVan den Berg et al., 2006.

1.2.3 DDT

In the 1940s to 1960s, the insecticide DDT was widely used in agriculture and for malaria control. Most of the developed countries banned or restricted the use of DDT in the 1970s. The main reason for banning DDT was the observed adverse effects on reproductive success in wild animals (Beard 2006). In Sweden, the relatively low use of DDT was banned in agriculture in 1970 though spraying of forests was allowed for another five years (Bernes 1998). DDT is still used in malaria-infested areas of the world where human exposure remains high. In the environment and in humans DDT is mainly found as the extremely stable metabolite *p,p'*-DDE (Figure 1). Because of their persistence, DDT and its metabolites are found in biological samples throughout the world.

1.3 HUMAN EXPOSURE TO POPs

There are several cases of accidents in which certain human populations have been exposed to high levels of POPs. For example, an accident in a trichlorophenol manufacturing plant near the Italian town of Seveso in 1976 exposed the population in the vicinity to very high levels of TCDD (Bertazzi et al. 1998). Accidents have also occurred in which food items have been contaminated. The most well-known cases are the severe PCB and dioxin poisonings in Yusho (Japan, 1968) and Yu-Cheng (Taiwan, 1979), both caused by accidental contamination of rice oil (Aoki 2001).

For the general population, food is the main source of exposure to most POPs. Because of their lipid solubility and persistence, POPs bioaccumulate throughout the food webs and food of animal origin contain the highest levels. For instance, more than 90% of the human exposure to dioxins are estimated to come from food, and of this, at least 75% normally come from food of animal origin (Liem et al. 2000). For BFRs, indoor air and dust are other important sources of exposure (Johnson-Restrepo and Kannan 2009; Lorber 2008).

1.3.1 POP levels in food and intake levels

Many POPs that are spread to the environment finally end up in aquatic environments. Hence, investigations of PCDD/Fs, PCBs, *p,p'*-DDE and PBDEs in food on the Swedish market show that fatty fish contain the highest levels (Ankarberg et al. 2007; Törnkvist et al. 2011). The levels in other food of animal origin are generally lower, with fruit and vegetables containing the lowest concentrations (Ankarberg et al. 2007). Fatty wild-caught fish from the Baltic Sea and from the Swedish lakes Vänern and Vättern (e.g., herring, salmon and brown trout) contain the highest levels; specific levels, however, vary depending on location and size of the fish (Ankarberg et al. 2007; Cantillana and Aune 2012). The levels of PCDD/Fs, PCBs and *p,p'*-DDE in the marine environment have decreased since the 1970s, and declining trends in food of animal origin have been observed for the period 1990-2010 (Bignert et al. 2012; Glynn et al. 2000; Ålander et al. 2012). However, indications show that the levels of PCDFs in marine biota have stabilized at the levels around 1990 (Bignert et al. 2012). The levels of PBDEs in the marine environment increased from the late 1960s until the early 1990s. Though the trends are inconsistent, they have mainly decreased thereafter (Bignert et al. 2012).

The NFA has performed several estimations of the Swedish population's POP intake from food. These estimations have either been based on food consumption surveys or on market basket studies (trade statistics). The studies have shown decreasing intakes of PCDD/Fs, PCBs, *p,p'*-DDE and PBDEs from the late 1990s up to 2010 (Darnerud et al. 2006; NFA 2011; NFA 2012; Törnkvist et al. 2011). The median daily intake of total TEQ (sum of PCDD/F TEQs and DL-PCB TEQs) in adults decreased from 1.1 to 0.5 pg/kg body weight between 1998-1999 and 2010 (Figure 2). Fish and shellfish contributed to the largest part of the average dietary intake of total TEQ (69%), followed by dairy products (17%) and meat (7%) (NFA 2011). For NDL-PCBs, PBDEs

and *p,p'*-DDE, fish and shellfish were also the largest contributors to the average dietary intake (about 40-70%) as calculated from a market basket study in 2010 (NFA 2012).

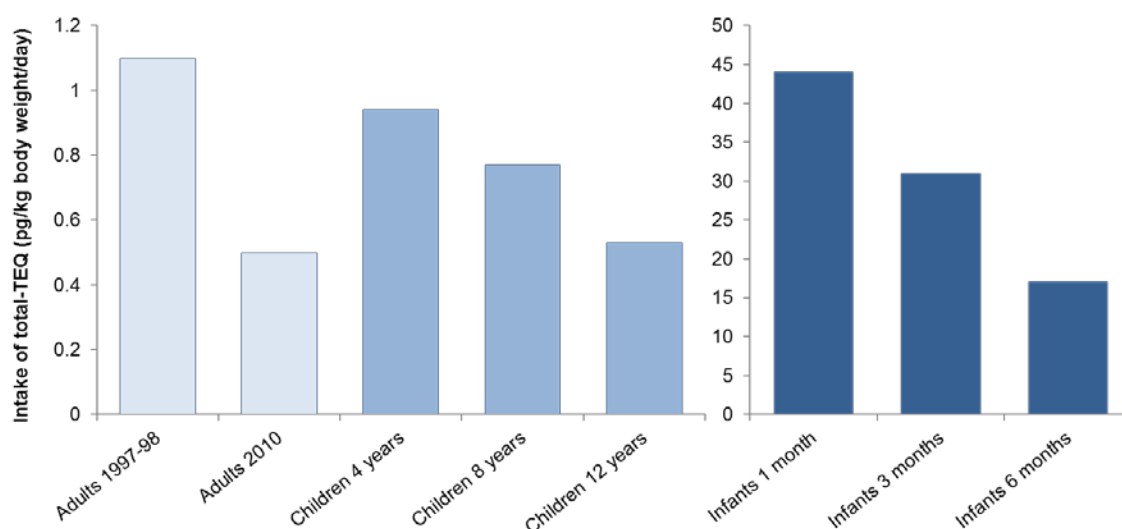


Figure 2. Calculated median total TEQ (sum of PCDD/F TEQs and DL-PCBs TEQ) intakes from Swedish food consumption surveys conducted in 1997-1998 (adults) (Ankarberg and Petersson-Grawé 2005), 2010 (adults) (NFA 2011) and 2003 (children) (NFA 2011). Intakes in infants are based on breast milk levels from first-time mothers in Uppsala (Bergkvist et al. 2010). TEF values from 1998 (Van den Berg et al. 1998) and data on PCDD/Fs and PCBs in food from 1990-2000 were used in the intake calculations for adults in 1997-1998. TEF values from 2006 (Van den Berg et al. 2006) and data on PCDD/Fs and PCBs in food from 2000-2010 were used in calculations for adults in 2010 and children in 2003. TEF values from 2006 were used in the calculations of intakes in infants.

Children are generally exposed to higher levels of POPs from food than adults, mainly because they eat more per kg body weight (Figure 2). Because of a relatively high lipid content, breast milk contains high levels of POPs. Exposure of breastfed infants is thus considerably higher than dietary exposure of children and adults (Figure 2). Based on levels of dioxins and PCBs in breast milk from the POPUP study, the mean intake of total TEQ in newborn infants can be estimated to be about 40-80 times higher than the intake from food in Swedish children (Bergkvist et al. 2010). The intakes decrease during the breastfeeding period because of a decrease in milk consumption per kg body weight by the infants. In addition, a monthly reduction in the levels of dioxins and PCBs in breast milk is assumed because lactation is an elimination pathway for POPs for women.

1.3.2 Body burdens of POPs in humans

POPs are generally well absorbed from the gastrointestinal tract. The absorption of PCDD/Fs and PCBs is dependent on degree of chlorination, but has been shown to be nearly complete in breastfed infants and between 50 and 90% in adults (Dahl et al. 1995; Larsen 2006). Because of their high lipid solubility and resistance to metabolism, POPs accumulate in the body during an individual's lifetime, chiefly in adipose tissue. Therefore, measured POP levels in body lipids are the result of long-term exposure before the sampling occasion. During pregnancy, the compounds are easily distributed from the mother to the fetus via the placenta, and after delivery from the maternal blood to the

milk. The half-lives of PCDD/Fs, PCBs and PBDEs in the human body are dependent on the degree of chlorination or bromination and on the position of the halogen atoms in the molecule. Reported half-lives range from a few months up to several decades (Geyer et al. 2004; Milbrath et al. 2009). The transfer from maternal blood to milk generally decreases with increasing chlorination or bromination (Mannetje et al. 2012; Wittsiepe et al. 2007).

Body burdens of POPs are often measured in lipids in different tissues and organs since there is equilibrium between levels in the lipids in different compartments. In pregnant and nursing women POPs may be analysed in maternal blood, cord blood, placenta or breast milk. High correlations between lipid-adjusted levels of both chlorinated and brominated POPs in these different matrices have been reported (Ayotte et al. 2003; Mannetje et al. 2012; Needham et al. 2011; Wittsiepe et al. 2007). This finding indicates that POP levels in any of the matrices give a good estimate of the maternal body burden during pregnancy and thus of the exposure of the infant during the fetal and nursing periods. Breast milk is a good matrix for POP analysis because it estimates maternal body burden and because the sampling of breast milk is non-invasive and relatively simple.

1.3.2.1 Determinants of POP body burdens

There are large interindividual differences in body burdens of POPs. Several personal characteristics and lifestyle factors (determinants) may contribute to these differences. One of the strongest determinants is age, higher body burdens of PCBs, dioxins and *p,p'*-DDE in older individuals have been reported in several studies (Hardell et al. 2010; Herbstman et al. 2007; Schade and Heinzow 1998; Wittsiepe et al. 2007). In other lifestyle factors, a high body mass index (BMI) at sampling has been shown to predict lower breast milk levels of PCBs in nursing women (Albers et al. 1996) and weight loss in obese individuals has been associated with higher blood levels of PCBs and *p,p'*-DDE (Chevrier et al. 2000). Blood levels of PCBs and *p,p'*-DDE in children have also been shown to decrease with increasing body weight and BMI, and blood levels of PCBs, dioxins and *p,p'*-DDE are higher in males than in females (Jonsson et al. 2005; Karmaus et al. 2001; Link et al. 2005; Nawrot et al. 2002). Smoking generally predicts lower breast milk or blood levels of PCBs and dioxins (Garabrant et al. 2009; Hedley et al. 2006; Uehara et al. 2007), possibly because of an increased metabolism and elimination rate in smokers (Flesch-Janys et al. 1996).

Pregnancy and breastfeeding are two important elimination routes for POPs in women. Indeed, inverse associations between parity/breastfeeding and POP levels in breast milk and blood have been repeatedly demonstrated (Hardell et al. 2010; Schade and Heinzow 1998; Vaz et al. 1993). Breastfed infants are exposed to high levels of POPs from breast milk. In this respect, several studies have shown that nursing affects serum levels of PCBs, dioxins and *p,p'*-DDE in children from infancy up to adolescence (Karmaus et al. 2001; Lackmann et al. 2004; Link et al. 2005; Nawrot et al. 2002; Patandin et al. 1997).

Several studies have reported positive associations between fish consumption and body burdens of PCBs, dioxins and *p,p'*-DDE, both in Sweden (Jonsson et al. 2005; Rylander et al. 2009) and in other countries (Agudo et al. 2009; Schade and Heinzow 1998). Fish consumption has also been associated with serum levels of PBDEs (Thomsen et al. 2008),

but exposure from the indoor environment is also supported by a positive association between breast milk and dust levels of PBDEs (Karlsson et al. 2007; Wu et al. 2007).

1.3.2.2 Temporal trends

Risk management decisions with the aim to reduce environmental contamination and human exposure to POPs have been followed up by investigations of temporal trends of POP exposure in the Swedish population. In accordance with the decreasing levels in the environment and in food, levels of PCBs, dioxins and *p,p'*-DDE in breast milk from Swedish mothers have decreased by at least 70% from the beginning of the 1970s up to about 2000 (Meironyté Guvenius 2002; Norén and Meironyte 2000). Decreasing trends of PCBs and *p,p'*-DDE have also been found in blood and adipose tissue from middle-aged Swedish men and women during the period 1993-2007 (Hardell et al. 2010). In contrast, levels of PBDEs in breast milk have increased since the 1970s, at least up to the end of the 1990s when the levels seem to have stabilized or even declined (Fangstrom et al. 2008; Meironyte et al. 1999; Meironyté Guvenius 2002). It is important to continue following the levels of POPs in breast milk to determine whether measures taken to reduce environmental and human exposure will also result in decreasing levels in the future.

Although there are differences between countries, levels of PCBs, dioxins and chlorinated pesticides in breast milk from Sweden are in the same range as those found in other European countries and in North America and Asia (Glynn et al. 2012; van Leeuwen and Malisch 2002). Regarding PBDEs, Swedish breast milk levels are in the same range as those found in other European countries. Human levels of PBDEs in North America are considerably higher, probably because of the extensive use of PBDEs in a variety of consumer products (Glynn et al. 2012; Schecter et al. 2005).

1.4 HUMAN HEALTH EFFECTS

1.4.1 Health risk assessments

1.4.1.1 PCDD/Fs and DL-PCBs

Several international expert groups have conducted risk assessments of PCDD/Fs and DL-PCBs in food (JECFA 2002; SCF 2000; SCF 2001; WHO 2000a). These risk assessments identified the fetal stage as the most sensitive period, and health-based tolerable intake levels for humans are based on animal studies of effects after *in utero* exposure.

In 2001, EU's expert group (Scientific Committee on Food, SCF) determined a tolerable weekly intake (TWI) of 14 pg TEQ/kg body weight, corresponding to a tolerable daily intake (TDI) of 2 pg TEQ/kg body weight for TCDD (SCF 2001). The TWI was extended to include all PCDD/F and PCB congeners with assigned TEF values (Van den Berg et al. 1998) and was based on the effects on sperm production and sexual behavior in male rats after exposure during the fetal stage. An uncertainty factor of 9.6 was applied. WHO's

expert group for food additives and contaminants (JECFA) used the same critical study as SCF in a risk assessment conducted in 2001 and reached a provisional tolerable monthly intake of 70 pg TEQ/kg body weight, which can be converted to a TDI of 2.3 pg TEQ/kg body weight (JECFA 2002).

The above-mentioned tolerable intake levels relate to the body burden in women of childbearing ages and are set to protect fetuses from too high exposures by limiting the long-term accumulation of dioxins and DL-PCBs in women before pregnancy. Body burden during pregnancy is the result of dioxin exposure (mainly from food) over an individual's lifetime; short-term intakes above the tolerable probably do not have any health consequences. SCF and WHO conclude that exposure in breastfed infants will exceed the tolerable levels, but because the breastfeeding period is short and associated with beneficial effects, breastfeeding should be promoted (SCF 2000; WHO 2000a).

Recently, the US Environmental Protection Agency (US-EPA) conducted a risk assessment of non-cancer effects of TCDD (US-EPA 2012). Two human epidemiological studies were chosen as a basis for deriving a reference dose (RfD) (Baccarelli et al. 2008; Mocarelli et al. 2008). Both studies include the human population that was exposed to high levels of TCDD after the industrial accident in Seveso (Italy) in 1976. Critical effects identified were increased levels of thyroid stimulating hormone (TSH) in newborns exposed to TCDD *in utero* (Baccarelli et al. 2008) and decreased sperm count and motility in men who were 1-9 years old at the time of the Seveso accident (Mocarelli et al. 2008). The estimated lowest observed adverse effect level (LOAEL) was 0.02 ng/kg body weight/day in both studies; after applying an uncertainty factor of 30, US-EPA proposed a RfD for TCDD of 0.7 pg TEQ/kg body weight/day (US-EPA 2012).

The Swedish NFA and the Institute of Environmental Medicine (Karolinska Institutet, Sweden) conducted a risk assessment of non-developmental exposure to PCDD/Fs and DL-PCBs (Hanberg et al. 2007). Based on cancer studies in rats, it was concluded that the risk for cancer in humans is probably very small or non-existent at a long-term daily exposure of 2-10 pg TEQ/kg body weight.

The International Agency for Research on Cancer (IARC) has evaluated information about carcinogenicity of PCDD/Fs and PCBs. From this evaluation, the IARC classified TCDD as a human carcinogen (Group 1) in 1997 (McGregor et al. 1998). Because of lack of human and animal data, it was not possible to classify the other PCDD/F congeners. In a later reevaluation 2,3,4,7,8-PeCDF and PCB 126 were also classified as carcinogenic to humans (Baan et al. 2009). In 2013, DL-PCBs and NDL-PCBs were classified as carcinogenic to humans (Lauby-Secretan et al. 2013). It was concluded that the carcinogenicity of PCBs cannot solely be attributed to the carcinogenicity of DL-PCBs.

1.4.1.2 NDL-PCBs

In 2005, the European Food Safety Authority (EFSA) conducted a risk assessment of the sum of six NDL-PCBs (PCB 28, 52, 101, 138, 153, 180) (EFSA 2005). No health-based tolerable intake level could be established because it was not possible to distinguish

between effects of NDL-PCBs from effects of DL-PCBs and dioxins in toxicological and epidemiological studies. The limited toxicological data on individual NDL-PCB congeners showed that effects on liver and thyroid were most sensitive. Based on these effects, the EFSA estimated that 500 µg/kg was a conservative NOAEL (no observed adverse effect level) body burden for total NDL-PCBs. NOAEL is the highest level that does not cause an adverse health effect in the most sensitive species. It was concluded that the median body burden in European countries (50 µg/kg body weight) was only about 10 times lower than the NOAEL body burden. From epidemiological studies, the EFSA concluded that there are indications of subtle neurodevelopmental effects in infants after fetal exposure to NDL-PCBs, DL-PCBs and dioxins, either separately or in combination (EFSA 2005). These effects may occur at maternal body burdens just slightly higher than those expected from the average daily intake in European countries.

1.4.1.3 PBDEs

In 2011, the EFSA assessed the human health risks with a dietary intake of PBDEs (EFSA 2011). Main targets for PBDE toxicity were the liver as well as the thyroid hormone, reproductive and nervous systems. Effects on neurodevelopment in mice were identified as the critical effect. The EFSA derived benchmark doses (BMDs) and their corresponding lower 95% confidence limits for a benchmark response (neurodevelopmental effect) of 10% (BMDL₁₀): 309 µg/kg body weight (BDE-47), 12 µg/kg body weight (BDE-99) and 83 µg/kg body weight (BDE-153). The BMDs were derived from studies in which PBDEs were administered to mouse pups on a single occasion in early postnatal life. Based on the calculated BMDL₁₀ values, and considering an oral absorption of 75% in rodents, body burdens at the BMDL₁₀ of 232 (BDE-47), 9 (BDE-99) and 62 (BDE-153) µg/kg body weight were derived. Human daily intake levels corresponding to the BMDL₁₀ body burdens were estimated to 172 (BDE-47), 4.2 (BDE-99) and 9.6 (BDE-153) ng/kg body weight. Because of the limited and uncertain database, the EFSA did not derive any health-based tolerable intake levels. Instead, margin of exposure (MOE) quotients were calculated by dividing the human intakes associated with the BMDL₁₀ body burdens by the estimated dietary intake in the population. The EFSA concluded that the MOEs for BDE-47 and BDE-153 did not indicate any health concern as regards current dietary exposure within the EU, whereas the MOEs for BDE-99 indicated a potential health concern, especially in young children (1-3 years) (EFSA 2011).

1.4.1.4 DDT

WHO's expert group on pesticide residues in food (JMPR) revised the provisional TDI for DDT compounds in 2000 to 10 µg/kg body weight (JMPR 2001). This provisional TDI was based on developmental effects (reproduction) in rats (an uncertainty factor of 100 was applied). In another WHO risk assessment of indoor residual spraying with DDT it was concluded that body burdens of DDT compounds below 1 µg/g lipids are safe from a human health perspective (carcinogenicity, developmental and reproductive effects) (WHO 2011).

DDT/DDE were evaluated by IARC in 1991, where enough evidence was gathered to classify the compounds as animal carcinogens (IARC 1991). Data on humans, however,

were insufficient, which led to DDT/DDE being classified as possibly carcinogenic to humans (Group 2B).

1.4.2 Influence of POPs on birth weight

The risk assessments of dioxins, PCBs, PBDEs and DDT described above are mainly based on other developmental effects than birth weight. However, birth weight is a commonly used variable in epidemiological studies of exposure to environmental contaminants and health, because it is easily measured, routinely recorded and a good marker for fetal and infant health.

1.4.2.1 Determinants of birth weight

Poor maternal nutrition and health are the main causes of low birth weight in developing countries with insufficient socio-economic conditions (UNICEF and WHO 2004). However, among substantially healthy and well-nourished mothers, gestational length is a very strong predictor of birth weight (Niklasson and Albertsson-Wikland 2008). Infants with low birth weight are often premature, but there are also small full-term infants, often called growth-retarded. Except for gestational length, several other factors are known to predict birth weight and thus should be considered as potential confounders in epidemiological studies. For example, offspring birth weight increases with increasing maternal pre-pregnancy BMI, weight gain during pregnancy, education and parity (Escartin et al. 2013; Mortensen et al. 2008; Stamnes Koepp et al. 2012). On the other hand, high or low maternal age, smoking during pregnancy and use of alcohol or drugs are associated with increased risk of having small infants, with female infants being smaller than male infants (Cnattingius 2004; Cnattingius et al. 1992; Goldenberg et al. 1997; Ko et al. 2002). Several medical conditions, both those existing before pregnancy (e.g., pre-existing hypertension) and those that emerge during pregnancy (e.g., gestational hypertension and preeclampsia), have been linked to an increased risk of having low birth weight babies (Delgado-Rodriguez et al. 1998; Easterling et al. 1991; Goldenberg et al. 1997). Among dietary habits, maternal seafood consumption has been positively associated with birth weight (Brantsaeter et al. 2012). It has been suggested that long-chain omega-3-fatty acids, abundant in fatty fish, may be responsible for this association, either by enhancing fetal growth or prolonging gestation (Larque et al. 2012; Olsen et al. 1993; Olsen et al. 1990; Olsen et al. 2006).

1.4.2.2 Epidemiological findings

Women accidentally exposed to very high levels of PCBs and PCDD/Fs before pregnancy delivered babies with decreased birth weights (Tsukimori et al. 2012). Studies have also shown an inverse association between background exposure to PCBs, PCDD/Fs and *p,p'*-DDE and birth weight (e.g., Fein et al. 1984; Halldorsson et al. 2008; Karmaus and Zhu 2004; Patandin et al. 1998). However, other studies have failed to find any significant associations (e.g., Gladen et al. 2003; Grandjean et al. 2001; Longnecker et al. 2005). In a recent meta-analysis that included 12 European birth cohorts, background exposure to PCB 153 (a marker of total PCB), but not *p,p'*-DDE, is suggested to be associated with decreased birth weight (Govarts et al. 2012). In Sweden, birth weight in relation to PCB exposure has been studied in a cohort of professional fishermen and their families

(Rylander et al. 1998). Maternal blood plasma levels of PCB 153 during the year of childbirth were modeled from actual levels measured in the mothers 4-22 years later. These levels were later used to estimate fetal exposure. Results differed depending on the particular model used, but an increased risk of giving birth to infants with low birth weight with increasing exposure level was indicated (Rylander et al. 1998). No Swedish studies are available on POP exposure in relation to birth weight where the levels of POPs have been measured in connection with pregnancy or delivery.

Few studies exist on the association between PBDE exposure and birth weight. No or inverse associations have actually been reported (Chao et al. 2007; Foster et al. 2011; Harley et al. 2011; Mazdai et al. 2003; Tan et al. 2009; Wu et al. 2010). Some of the studies are very small (N=12-41) (Chao et al. 2007; Mazdai et al. 2003; Tan et al. 2009) or report only simple correlations (Mazdai et al. 2003; Wu et al. 2010). However, two larger studies adjusting the results for possible confounding factors reported inverse associations. Foster et al. (2011) reported an inverse association between BDE-99 in umbilical cord serum and birth weight in a study of 97 Canadian mothers. In another study, Harley et al. (2011) reported inverse associations between BDE-47, BDE-99 and BDE-100 in maternal serum and birth weight in a cohort of 286 mothers from California.

1.4.2.3 Birth weight and infant and adult health

Birth weight is not only a marker for fetal and infant health, but also predicts health in adults. Low birth weight in relation to gestational length has been associated with increased risks of adult obesity, coronary heart disease, stroke, hypertension, type-2 diabetes, kidney failure and cancer (Calkins and Devaskar 2011). In addition, high birth weight has been related to obesity, insulin resistance, hypertension and cancer (Calkins and Devaskar 2011). In these cases prenatal experiences, as reflected in birth weight, are considered important for the development of the adult disease. This concept of “fetal origins of adult disease” was first hypothesized by David Barker, who observed associations between low birth weight and cardiovascular disease (Barker 1990; Barker 2006; Calkins and Devaskar 2011). It is thought that the fetus is able to adapt to the environment during development and that adaptations to malnutrition (in low birth weight fetuses) lead to persistent alterations in structure, physiology and metabolism that will affect susceptibility to disease later in life (Osmond and Barker 2000). This is also called “fetal programming”. Malnutrition is not the only stimulus that may result in such alterations during fetal life: infections, hypoxia, stress, toxins and endocrine status may also be important factors (Calkins and Devaskar 2011; Lau and Rogers 2004). Accordingly, effects of exposure to environmental contaminants during the fetal period, manifested as changes in birth weight, may cause permanent changes that are of importance for future health.

1.4.3 Influence of POPs on thyroid hormones

The complex thyroid hormone system in animals has been shown to be vulnerable to disruption by high exposure to a variety of POPs through changes in hormone production, transport or metabolism. Because the chemical structure of several POPs and their metabolites closely resembles that of thyroid hormones, attention has been paid to their

possible effects on thyroid hormone status (Brucker-Davis 1998). Effects of many POPs on thyroid function are well documented in experimental studies in laboratory animals. PCBs, dioxins and PBDEs have all been shown to reduce levels of circulating thyroid hormones (mainly thyroxine, T4) and increase the levels of TSH (Boas et al. 2009; Brucker-Davis 1998; Hallgren et al. 2001; Zhou et al. 2001). Some epidemiological studies have also reported associations between exposure to POPs and thyroid hormone status in humans (see section 1.4.3.2 below).

1.4.3.1 Thyroid hormone function in humans

The thyroid hormones, thyroxine (T4) and triiodothyronine (T3) (Figure 3), regulate a wide array of biological functions in the human body, including development, growth and metabolism. They are also essential for normal brain development during the fetal period, infancy and childhood (Williams 2008; Zoeller and Rovet 2004).

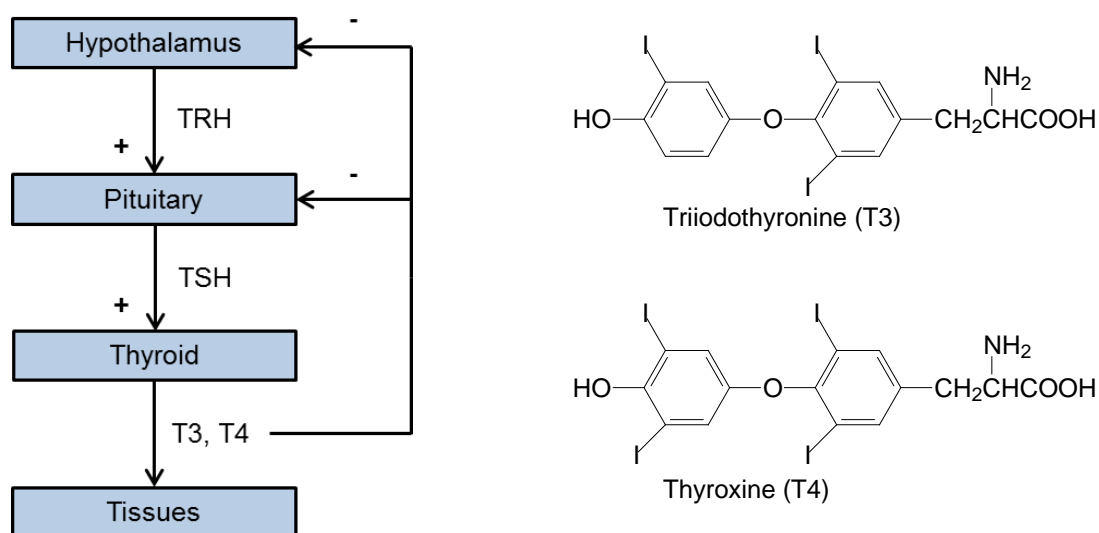


Figure 3. Left: Illustration of the thyroid hormone axis (simplified). TSH is produced in the pituitary gland through stimulation by hypothalamic TRH. Circulating TSH reaches the thyroid gland, where it stimulates release of T3 and T4. The system is balanced through negative feedback of T3 and T4. Right: Chemical structures of T3 and T4.

T4 and T3 are synthesized in the thyroid gland in response to pituitary TSH, which is formed following stimulation by a thyrotropin-releasing hormone (TRH), produced in the hypothalamus. The production of thyroid hormones is regulated by a negative feedback of T3 and T4 at both the hypothalamic and pituitary level (Figure 3). T4 and T3 contain four and three iodine atoms, respectively. Adequate dietary intake of iodine is therefore essential for normal thyroid hormone status. T4 is the major hormone produced by the thyroid. T3 is the biologically active hormone and is mostly derived from peripheral deiodination of T4. T3 and T4 in the blood are bound to transporter proteins, mainly to thyroxine-binding globulin (TBG) and, to a lesser extent, to transthyretin (TTR) and albumin.

Because the fetal production of thyroid hormones does not begin until gestational week 14-16, the fetus is initially dependent on the passage of maternal circulating thyroid hormones through placenta (Patel et al. 2011). This transfer, as well as other hormonal and metabolic changes during pregnancy, requires an increased production of thyroid hormones in the mother. Maternally derived T4 is converted to active T3 within the fetal brain, a conversion essential for early brain development (de Escobar et al. 2004). Severe maternal and congenital hypothyroidism (thyroid hormone deficit) caused by iodine deficiency is associated with impaired neurodevelopment. Studies have revealed that hypothyroid fetuses suffer various postnatal disorders, including mental retardation, deafness and spasticity (Chen and Hetzel 2010; Trumpff et al. 2013). There is also evidence that even mild or moderate reductions in maternal thyroid hormone levels in early pregnancy are associated with impaired brain development (Glinioer and Delange 2000; Haddow et al. 1999; Trumpff et al. 2013). European studies have shown that low blood levels of T4 during early pregnancy (defined as the 10th lowest percentile of free T4 of the study sample of pregnant women) are associated with impaired cognitive development in children (Trumpff et al. 2013). Adequate levels of thyroid hormones in newborns and young infants are also essential because this is a period of active brain development (Glinioer and Delange 2000). Elevated neonatal TSH levels at birth have been associated with impaired intellectual or psychomotor development in early childhood (Trumpff et al. 2013).

Studies on the impact of maternal thyroid status during pregnancy on thyroid function in the offspring in later life are scarce. However, a study of 16-year-old children showed that serum levels of TSH and free T4 were in accordance with maternal levels during pregnancy (Pakkila et al. 2013). Moreover, maternal hypo- and hyperthyroidism during pregnancy was associated with modified TSH levels in these children.

Concern about possible effects of POPs on thyroid hormone function has been raised, mainly because of the importance of thyroid hormones for neurodevelopment. PCBs and PBDEs effect behavior and learning in mice after neonatal exposure (Branchi et al. 2005; Eriksson et al. 2006; Viberg et al. 2003) and neurotoxic effects have also been suggested in epidemiological studies (Grandjean and Landrigan 2006). It can therefore be speculated that POPs exert their neurotoxic effects during development by interfering with thyroid hormone function.

1.4.3.2 Epidemiological findings

The association between background exposure to chlorinated POPs (particularly PCBs) and thyroid hormone levels in humans has been studied extensively. These studies have been reviewed in several recent publications (Boas et al. 2006; Boas et al. 2009; Goodman et al. 2010; Hagmar 2003; Jugan et al. 2010; Langer 2008; Salay and Garabrant 2009). Thyroid hormones in relation to PBDE exposure remains a considerably less well studied area.

Several studies in pregnant women have shown an inverse relation between exposure to chlorinated POPs (PCBs, dioxins or *p,p'*-DDE) and circulating levels of T3 or T4 (Alvarez-Pedrerol et al. 2009; Chevrier et al. 2008; Koopman-Esseboom et al. 1994;

Lopez-Espinosa et al. 2009; Takser et al. 2005). However, there are also studies reporting no significant association between maternal levels of PCBs, dioxins and *p,p'*-DDE and thyroid hormones (Dallaire et al. 2009; Lopez-Espinosa et al. 2009; Steuerwald et al. 2000; Wilhelm et al. 2008).

As mentioned earlier in this thesis, the US-EPA based their RfD for dioxins and DL-PCBs on an epidemiological study showing a positive association between maternal plasma levels of dioxins and DL-PCBs at delivery and neonatal blood levels of TSH (Baccarelli et al. 2008; US-EPA 2012). Included in the study were infants born in 1994-2005 by mothers who were exposed to high levels of TCDD after the 1976 industrial accident in Seveso (Italy). The exposure levels in the Seveso study were very high, but several other studies have investigated the association between background exposure to chlorinated POPs and thyroid hormone levels in infants and children. Studies on dioxins and DL-PCBs (N=23) are summarized in detail by Goodman et al. (2010). The authors conclude that there is no clear relation between background exposure to DL compounds and thyroid endpoints in infants and children. Results differ substantially in studies in which thyroid hormones were measured in cord blood or in infant blood within two weeks after birth. Reported associations between T4 and T3 and PCBs, dioxins and *p,p'*-DDE are mainly non-significant (e.g., Dallaire et al. 2008; Dallaire et al. 2009; Longnecker et al. 2000; Sandau et al. 2002; Steuerwald et al. 2000; Takser et al. 2005), although some studies found significant inverse associations (Asawasinsopon et al. 2006; Herbstman et al. 2008; Koopman-Esseboom et al. 1994; Maervoet et al. 2007; Wang et al. 2005). Non-significant associations with TSH are also commonly reported (e.g., Dallaire et al. 2009; Herbstman et al. 2008; Longnecker et al. 2000; Lopez-Espinosa et al. 2010; Maervoet et al. 2007; Ribas-Fito et al. 2003) although significant positive associations were found in a few investigations (Alvarez-Pedrerol et al. 2008; Chevrier et al. 2007; Koopman-Esseboom et al. 1994; Sauer et al. 1994).

Only a limited number of studies of PBDE exposure and thyroid hormones in pregnant women could be found. Some of the studies included a very small number of participants (N≤25) (Kim et al. 2012; Mazdai et al. 2003; Zota et al. 2011). However, one larger study (N=270) reported inverse associations between tri- to hexa-brominated PBDEs and TSH in maternal serum sampled about the 27th week of gestation (Chevrier et al. 2010). No significant association was observed with T4. In contrast, maternal late pregnancy serum levels of tetra- to hexa-brominated PBDEs in 137 women were positively associated with T4, but not related to TSH (Stapleton et al. 2011).

As in the case of pregnant women, the number of studies of PBDE exposure in relation to thyroid hormones in infants is limited. Five larger studies (N>50) that also adjusted the results for potential confounders were found. Two of these studies only analysed TSH in infant serum soon after birth and found no significant associations with maternal body burdens of tetra- to hexa-brominated PBDEs (Chevrier et al. 2011; Eggesbo et al. 2011). In three studies thyroid hormones were analysed in cord blood (Herbstman et al. 2008; Lin et al. 2011; Shy et al. 2012). In a US study, some evidence of inverse associations was noted between tetra- to hexa-brominated PBDEs in cord serum and levels of T4 (Herbstman et al. 2008). In contrast, a Taiwanese study found a positive association

between hexa-brominated BDE-154 in breast milk and cord T4, but an inverse association with penta-brominated BDE-99 (Shy et al. 2012). In the same Taiwanese cohort, Lin et al. (2011) found decreasing levels of cord T3 with increasing levels of hexa- and hepta-brominated PBDE congeners in cord blood.

Taken together, studies on associations between background POP exposure and thyroid hormones in pregnant women and infants are clearly inconsistent. Yet, several studies suggest inverse associations between chlorinated POPs and T3 or T4 in pregnant women. The number of studies on PBDEs is small and the results often point in different directions. Differences in results cannot be easily explained by differences in exposure levels. Instead, large differences in study design such as matrices used for analysis of POPs (maternal blood, cord blood, infant blood, breast milk) and thyroid hormones (maternal blood, cord blood, infant blood), sampling time, specific substances and thyroid hormones chosen for analysis and statistical models (e.g., selection of confounders) may be at least partly responsible for the discrepancies. No Swedish studies have been conducted on the association between POP exposure and thyroid hormone status in pregnant women or infants.

1.5 AIMS

The overall aims of this thesis were to quantify body burdens of POPs in pregnant and nursing women in Sweden (POPUP cohort) and to study whether maternal, fetal or infant exposure to POPs is associated with birth outcomes and markers of thyroid function.

Specific objectives were to:

- quantify individual breast milk levels of POPs in nursing women in Sweden to improve risk assessments (Paper I).
- follow up if risk management measures to decrease human exposure still have an effect on breast milk levels of POPs in Swedish nursing women by studying how these levels have changed during the study period (1996-2010) (Paper I).
- investigate whether personal characteristics and lifestyle factors known to be important determinants of breast milk levels of POPs (Paper II) may be used to advise women about how to reduce their body burdens. Such determinants are also important because they may confound the association between exposure and health effects.
- study whether prenatal exposure to chlorinated and brominated POPs is associated with birth weight in the POPUP cohort and if possible associations between POP exposure and birth weight are modified by maternal fish consumption (Paper III).
- determine whether exposure to chlorinated and brominated POPs is associated with thyroid hormone status in mothers and infants from the POPUP cohort (Paper IV&V).

2 SUBJECTS AND METHODS

2.1 STUDY POPULATION AND SAMPLING

This thesis is based on the POPUP cohort (Persistent Organic Pollutants in Uppsala Primiparas), a population of first-time mothers recruited in Uppsala County from 1996 and onwards. The recruitment is still ongoing, but the work in this thesis is based on the period 1996-2010. The study can be divided into two parts (Figure 4). During the first part of the study (1996-1999), pregnant women were recruited among controls in a case-control study of risk factors for early miscarriages in women born in Uppsala County (Cnattingius et al. 2000). To increase the number of participants from the coastal regions a complementary recruitment was conducted at the antenatal care clinic in Östhammar on the East Coast of Uppsala County. A total of 325 women donated blood samples in early (week 6-12) and late (week 32-34) pregnancy. After delivery, the participating women were asked whether they were willing to donate a breast milk sample and whether a blood sample could be taken from their infants at 3 weeks and 3 months of age. In all, 211 mothers agreed to donate breast milk and blood was sampled from 160 infants at 3 weeks and 115 infants at 3 months.

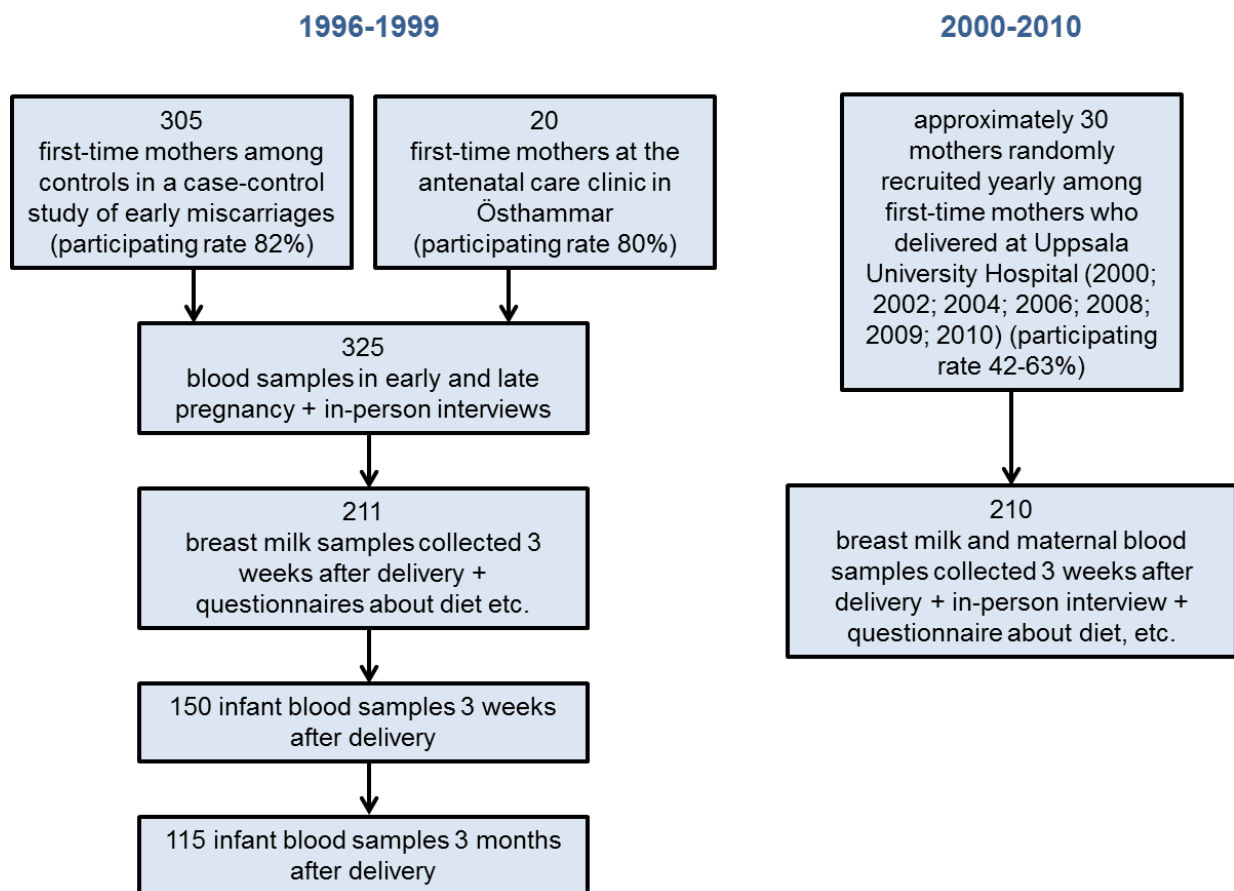


Figure 4. Flow-chart over the POPUP cohort.

In 2000-2010, 210 mothers were randomly recruited (after delivery) among first-time mothers who had a normal delivery at Uppsala University Hospital and were Swedish by birth (Figure 4). These mothers donated breast milk and blood 3 weeks after delivery.

During the whole study period (1996-2010), breast milk was sampled at home during the third week after delivery (day 14-21 post-partum) using a manual breast milk pump or a passive breast milk sampler. The women were instructed to sample milk both in the beginning and in the end of the breastfeeding sessions. The goal was to sample 500 ml milk from each mother during seven days of sampling. The milk was kept in the home freezer in acetone-washed glass bottles and newly sampled milk was poured on top of the frozen milk.

The POPUP cohort was restricted to first-time mothers because nursing is an important elimination route for POPs and thus an important determinant of the body burden of POPs in women. In the second part of the study (2000-2010) women who were born in non-Nordic countries were also excluded because they may differ from women born in the Nordic countries regarding exposure and body burdens. In both parts of the study data on lifestyle factors and diet were collected by in-person interviews and by self-administered questionnaires (Figure 4). Data were also obtained from the Swedish Medical Birth Register at the National Board of Health and Welfare. The study was approved by the local ethics committee of Uppsala University and participants gave their informed consent before inclusion in the study.

2.2 ANALYSES OF POPs IN BREAST MILK AND SERUM

POPs were analysed in maternal serum and breast milk collected from the participants. A short summary of the analytical methods follows below. In some cases different methods and laboratories have been used during the study period. Calibration studies have been performed to compare the results from different methods/laboratories.

Individual breast milk samples were analysed for NDL-PCBs (PCB 28, 52, 101 and the *di-ortho* substituted congeners: PCB 138, 153 and 180), DL-PCBs (mono-*ortho* substituted congeners: PCB 105, 118, 156, 167 and non-*ortho* substituted congeners: PCB 77, 126, 169), seventeen 2,3,7,8-substituted PCDD/Fs, *p,p'*-DDE and five PBDE congeners (BDE-47, -99, -100, -153, -154). Late pregnancy serum samples collected during the first part of the study (1996-1999) were analysed for NDL-PCBs, mono-*ortho* PCBs and *p,p'*-DDE.

In most cases, PCBs, *p,p'*-DDE and PBDEs were analysed at the NFA according to a previously described method (Atuma et al. 2000; Atuma and Aune 1999; Aune et al. 1999; Lind et al. 2003). After extraction and separation, the final analyses of NDL-PCBs, mono-*ortho* PCBs and *p,p'*-DDE were performed on a gas chromatograph with dual capillary columns of different polarity and dual electron-capture detectors. Non-*ortho* PCBs (in samples from 2000-2004) and PBDEs were analysed by gas chromatography/low resolution mass spectroscopy/electron capture negative ionization and detected by the single ion monitoring (SIM) technique. In samples from 2008-2010, all PCBs were

analysed by gas chromatography coupled to high-resolution mass spectrometry (Aune et al. 2012).

PCDD/Fs (all samples) and non-*ortho* PCBs (samples from 1996-1999 and 2006-2010) were analysed at the National Institute of Public Health and the Environment (RIVM), the Netherlands, at the Department of Chemistry at Umeå University or at the NFA. The samples were analysed according to validated procedures consisting of milk sample extraction, clean-up and fractionation on a series of different chromatographic columns (e.g., multi-layer silica, alumina, Florisil® and active carbon), and identification and quantification of the analytes using gas chromatography with high-resolution mass spectrometry in SIM-mode using isotope dilution technique (Aune et al. 2012; Lignell et al. 2009).

The total lipid content of each sample was determined gravimetrically after the extraction. All samples were fortified with internal standards before extraction to correct for analytical losses and ensure quality control. A number of control samples were analysed together with the samples to verify the accuracy and precision of the measurements. The laboratory at the NFA is accredited and has successfully participated in several international proficiency tests.

2.3 ANALYSES OF THYROID HORMONES IN SERUM

Free thyroxine (free T4), total triiodothyronine (total T3) and TSH were analysed in early and late pregnancy serum samples from the mothers (N= 220 and 281, respectively) and in infant serum samples collected 3 weeks and 3 months after delivery (N=150 and 115, respectively). Thyroid hormones were measured on an automatic immunoassay system (Autodelphia, Wallac Oy, Turku, Finland). The analyses were performed at the routine laboratory of the Department of Clinical Chemistry at the Uppsala University Hospital. The laboratory is certified by a Swedish government authority (Swedac).

2.4 DATA ANALYSIS

Lipid-adjusted breast milk and serum levels of POPs were used in all statistical analyses because these levels give a good estimate of the maternal body burden during pregnancy and, consequently, of the exposure of the fetus and breastfed infant (Ayotte et al. 2003; Needham et al. 2011). In addition, POP levels were logarithmically transformed because the distribution of data closely followed a log-normal distribution.

In **Paper I**, temporal trends of PCBs, PCDD/Fs and PBDEs in breast milk were investigated by simple and multiple regression analysis. In the multiple models the trends were adjusted for maternal age, pre-pregnancy BMI, weight gain during pregnancy and weight loss from delivery to breast milk sampling. Because of the logarithmic transformation of the POP-levels, the associations were presented as percent change of concentration per year, and not as change in absolute levels. PCDD/F TEQs and PCB TEQs were calculated using both 1998 and 2006 TEFs (Van den Berg et al. 1998, Van

den Berg et al. 2006) and the results obtained with the different systems were subsequently compared.

In **Paper II**, hierarchical clustering (HC) was used to identify compounds with similar patterns in breast milk levels based on correlations between levels of single compounds. Multiple linear regression was performed to analyse associations between levels of PCBs, PCDD/Fs and PBDEs in breast milk and independent variables (personal characteristics) suspected to be determinants of breast milk levels. A three-step procedure was implemented. In a “basic model” (step 1) six independent variables that have been shown to be associated with serum and breast milk levels of POPs were included as explanatory variables (Glynn et al. 2007; Lignell et al. 2009; Schade and Heinzow 1998). These variables were maternal age, sampling year, pre-pregnancy BMI, weight gain during pregnancy, weight loss after delivery and education. In step 2 additional possible predictor variables (see Table 1 in Paper II) were added one by one to the basic model. Finally, all variables significantly associated with breast milk levels of POPs in step 1 and step 2 were included in “total models” (step 3).

In **Paper III**, breast milk levels of di-*ortho* PCBs and PBDEs were employed to estimate prenatal exposure. Associations between prenatal exposure and birth weight were investigated by simple and multiple linear regression models. In the crude analyses the associations between di-*ortho* PCBs or PBDEs and birth weight were examined in separate models. In the adjusted model di-*ortho* PCBs and PBDEs were included simultaneously. In addition, covariates included in the adjusted model were maternal age, pre-pregnancy BMI, weight gain during pregnancy, education, smoking during pregnancy and sex of the child. These variables have all been associated with birth weight (Bailey and Byrom 2007; Brynhildsen et al. 2009; Chattingius et al. 1992; Goldenberg et al. 1997; Mortensen et al. 2008; Newburn-Cook and Onyskiw 2005). The influence of including gestational length and maternal fish consumption to the statistical models was also investigated. To study possible sex differences in the relation of birth weight to exposure, all models were run for male and female infants separately.

In **Paper IV and V**, associations between POP exposure (PCBs, PCDD/Fs, *p,p'*-DDE, PBDEs) and thyroid hormone status in mothers and infants were investigated using linear regression models.

In **Paper IV**, maternal body burdens and prenatal exposure to NDL-PCBs, mono-*ortho* PCBs, PCDD/Fs and *p,p'*-DDE were estimated from maternal serum levels in late pregnancy or from breast milk levels. Postnatal exposure of the infants was calculated from levels in breast milk (on fresh weight basis) and percentage of full breastfeeding (see Paper IV for a detailed description of the calculations of postnatal exposure). Associations between body burden/exposure and thyroid hormone status in late pregnancy and in infants (3 weeks and 3 months of age) were investigated (Table 2). In cases when statistically significant associations were found in simple regression analyses, multiple regression models were used to adjust the associations for potential confounders. Independent variables included in the adjusted models were maternal age, pre-pregnancy

BMI, education, smoking, alcohol consumption, breastfeeding, season of sampling, sex of the infant, birth weight and infant weight at 3 months.

Table 2. Associations between POP exposure and thyroid hormone levels in maternal and infant blood investigated in Paper IV and V. The table indicates in what paper (IV or V) the results are presented.

POP exposure basis	Maternal TH ^a in early pregnancy	Maternal TH ^a in late pregnancy	Infant TH ^a at 3 weeks	Infant TH ^a at 3 months
Maternal serum levels in late pregnancy^b				
LPCB	V	IV	IV	IV
Di- <i>ortho</i> PCB	V	IV	IV	IV
Mono- <i>ortho</i> PCB TEQ	V	IV	IV	IV
<i>p,p'</i> -DDE	V	IV	IV	IV
Maternal breast milk levels 3 weeks after delivery^b				
PCDD/F TEQ	V	IV	IV	IV
PBDE	V	V	V	V
Postnatal exposure^{b,c}				
LPCB				V
Di- <i>ortho</i> PCB				V
Mono- <i>ortho</i> PCB TEQ				V
PCDD/F TEQ				V
<i>p,p'</i> -DDE				V

^aAnalysed thyroid hormones in serum were free T4, total T3 and TSH. ^bLPCB=sum of the low-chlorinated PCB congeners PCB 28, 52 and 101; di-*ortho* PCB=sum of PCB 138, 153 and 180; mono-*ortho* PCB TEQ=sum of PCB 105, 118, 156 and 167 TEQ; PCDD/F TEQ=sum of TEQs for the 17 congeners with WHO TEFs; PBDE=PBDE congeners BDE-47, BDE-99, BDE-100 and BDE-153 and the sum of these congeners; ^cPostnatal exposure calculated from breast milk levels and % of full breastfeeding.

In **Paper V**, maternal late pregnancy serum levels or breast milk levels were used to estimate maternal body burdens of NDL-PCBs, mono-*ortho* PCBs, PCDD/Fs and *p,p'*-DDE during pregnancy as well as fetal and infant exposure (Table 2). Thyroid hormones were analysed in maternal early pregnancy serum. Thus, the associations between exposure to PCBs, PCDD/Fs and *p,p'*-DDE and late pregnancy thyroid hormones (studied in Paper IV) could be expanded by investigating early pregnancy thyroid hormones. In addition, associations between maternal and infant thyroid hormone levels and maternal PBDE body burdens were studied. After a crude analysis, a number of possible confounders were considered for inclusion in the model (maternal age, country of birth, pre-pregnancy BMI, education, smoking, alcohol consumption, maternal weight gain during pregnancy, season of sampling, gestational length, mode of delivery, sex of the infant, birth weight, number of days between delivery and sampling and infant weight at 3 months). Covariates that were associated with any of the maternal or infant outcomes ($p \leq 0.05$) in the bivariate models were included in the final models.

3 RESULTS AND DISCUSSION

This section summarizes and discusses the main results of this thesis. For a detailed description of the results, the reader is referred to the separate papers.

3.1 TEMPORAL TRENDS OF POPs IN BREAST MILK (PAPER I)

In **Paper I**, temporal trends of PCBs, PCDD/Fs and PBDEs in breast milk were investigated. Samples from 1996 to 2006 were included in the results and were analysed for NDL-PCBs (N=325), mono-*ortho* PCBs (N=325), non-*ortho* PCBs (N=220), PCDD/Fs (N=183) and PBDEs (N=276).

3.1.1 PCB and PCDD/F

Levels of all PCB congeners and PCDD/F TEQs decreased significantly during the study period. Results for some of the PCB congeners as well as PCDD/F TEQs are shown in Table 3. The mean decrease in PCB levels varied between 3 and 9% per year. PCDD TEQ levels decreased faster than PCDF TEQ levels, with estimated annual decreases of 7 and 5% per year, respectively. Differences in declining rates between congeners may be due to differences in human exposure, in persistence of different congeners but may also be due to uncertainties in results for congeners with many results below the limit of quantification (LOQ).

Since the publication of Paper I, new data on POPs in breast milk from 2008 to 2010 has been generated within the POPUP study. Updated temporal trends have been estimated (Lignell et al. 2012) and are presented in Table 3 and Figure 5. Similar annual changes for the period 1996-2010 as for 1996-2006 indicate that the decreasing trends of PCBs and PCDD/Fs have continued after 2006.

Previous studies of PCBs and PCDD/Fs in pooled breast milk samples from Stockholm, Sweden have shown substantial decreasing levels from the beginning of the 1970s up to 1997 (Norén and Meironyte 2000; Vaz et al. 1993). Our results demonstrate that the downwards trends, although slower, have continued after 1997 (Figure 6).

Declining trends of chlorinated POPs in breast milk concur with observed decreasing levels in food on the Swedish market since the beginning of the 1990s (Glynn et al. 2000; Törnkvist et al. 2011; Ålander et al. 2012). Consequently, efforts to reduce contamination of the environment and of food have been successful, resulting in reduced human exposure. However, levels of PCDD/Fs in guillemot eggs and in herring from some areas in the Baltic Sea region have not decreased during the last decades, indicating that the levels of PCDD/Fs may no longer decrease in the Baltic Sea environment (Bignert et al. 2012). Continuous measurements of dioxins in breast milk will show if human exposure, and consequently levels in breast milk, will also stabilize at the current level.

Because we sampled breast milk from individual mothers, it was possible to statistically adjust the temporal trends for personal characteristics (maternal age, pre-pregnancy BMI,

weight gain during pregnancy and weight loss after delivery). In most cases this adjustment resulted in slightly faster declining rates. Maternal age was the most important covariate. There were positive associations between maternal age and breast milk levels of PCBs and PCDD/Fs in the POPUP cohort. In addition, maternal age increased during the study period and the increasing age of the mothers thus counteracted the decreasing temporal trends in the unadjusted regression models. This observation highlights the importance of age adjustment in studies of temporal trends of POPs in humans.

A comparison of TEQ levels calculated with the TEFs from 1998 and 2006 (Van den Berg et al. 1998; Van den Berg et al. 2006), revealed that the new TEFs resulted in some changes in median TEQ levels (see Table 1, Paper I). However, similar temporal trends for PCDD/F TEQs, mono-*ortho* PCB TEQs and non-*ortho* PCB TEQs were observed regardless of the TEF system used.

Table 3. Annual changes (% per year) in concentrations of POPs in breast milk from first-time mothers in the POPUP cohort. Data for 1996-2006 from Paper I (Lignell et al. 2009). New data are included in the 1996-2010 trends (Lignell et al. 2012).

Substance	Simple regression ^a	Multiple regression ^b			
	1996-2006	1996-2006		1996-2010	
	Mean (SE)	Mean (SE)	Half-time ^c	Mean (SE)	Half-time ^c
PCB 28	-4.3* (1.4)	-3.9* (1.4)	17	-3.6* (0.7)	19
PCB 138	-6.2* (0.7)	-6.8* (0.5)	10	-6.5* (0.3)	10
PCB 153	-7.3* (0.7)	-8.0* (0.5)	8	-7.5* (0.3)	9
Mono- <i>ortho</i> PCB TEQ ^{d,f}	-6.9* (0.7)	-7.4* (0.5)	9	-6.8* (0.3)	10
Non- <i>ortho</i> PCB TEQ ^{e,f}	-6.1* (0.9)	-6.5* (0.7)	10	-7.4* (0.4)	9
PCDD TEQ ^f	-6.0* (0.7)	-6.7* (0.5)	10	-8.2* (0.3)	8
PCDF TEQ ^f	-3.7* (0.8)	-4.6* (0.6)	15	-5.4* (0.4)	12
BDE-47	-4.2* (1.1)	-4.3* (1.1)	16	-8.6* (0.7)	8
BDE-99	-7.6* (1.2)	-7.5* (1.2)	9	-9.7* (0.7)	7
BDE-100	-0.9 (1.3)	-1.2 (1.2)	-	-4.8* (0.7)	14
BDE-153	+4.9* (0.8)	+4.9* (0.7)	-15	+1.1* (0.5)	-64
SumPBDE ^g	-1.7 (1.0)	-2.0* (0.9)	34	-5.3* (0.5)	13

^aResults of simple linear regression of the association between ln-transformed POP levels and the year of sampling. ^bResults of multiple linear regression of the association between ln-transformed POP levels and year of sampling. Variables adjusted for maternal age, pre-pregnancy BMI and body weight change during pregnancy and after delivery. ^cEstimated time (years) for the concentrations to be halved in the population. Negative values indicate the estimated time (years) for the concentrations to be doubled in the population. ^dSum of PCB 105, PCB 118, PCB 156 and PCB 167 TEQs. ^eSum of PCB 77, PCB 126 and PCB 169 TEQs. ^fTEQs calculated with TEFs from 2006 (Van den Berg et al. 2006). ^gSum of BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154. SE, standard error. *p≤0.05

3.1.2 PBDEs

Dissimilar temporal trends were observed for the different PBDE congeners during the study period. The concentrations of BDE-47 and BDE-99 decreased, whereas the levels of BDE-153 increased; no significant trend was observed for BDE-100 (Table 3). Overall, this resulted in a small but significant decrease in the concentration of sumPBDE (sum of BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154) between 1996 and 2006 (2% per

year). When data from 2008-2010 were added (Lignell et al. 2012), the declining rates for BDE-47, BDE-99 and sumPBDE increased and the decreasing trend for BDE-100 became significant (Table 3, Figure 5). In addition, the increase in levels of BDE-153 seems to have leveled out. Consequently, risk management measures taken to reduce the emissions of PBDEs to the environment in the beginning of the 2000s have already resulted in decreased human exposure levels.

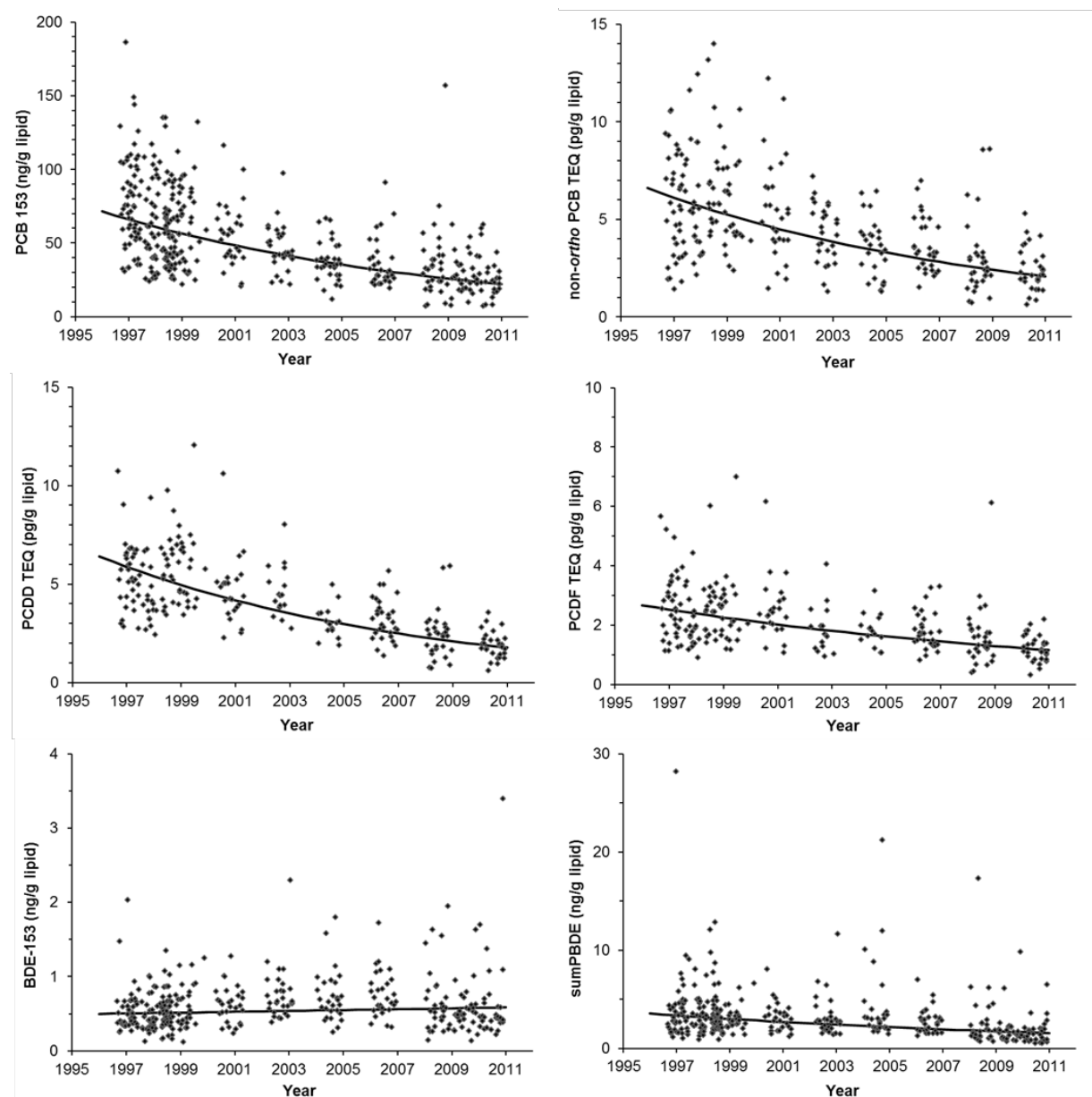


Figure 5. Temporal trends (1996-2010) of PCB 153 (N=398), non-*ortho* PCB TEQs (sum of PCB 77, 126, 169 TEQs) (N=269), PCDD TEQs (N=235), PCDF TEQs (N=235), BDE-153 (N=353) and sumPBDE (sum of BDE-47, -99, -100, -153 and -154.) (N=353) in breast milk from first-time mothers in the POPUP cohort. TEQs have been calculated using TEFs from 2006 (Van den Berg et al. 2006). Each point corresponds to the contaminant level in an individual milk sample. The lines represent regression lines from analyses that included maternal age, pre-pregnancy BMI, weight gain during pregnancy and weight loss after delivery as explanatory variables.

Different technical mixtures as sources of PBDEs may explain the observed differences in temporal trends between congeners. The use of mixtures containing lower brominated congeners (e.g., BDE-47 and BDE-99) has been reduced voluntarily since the 1990s, resulting in decreasing exposure of humans. However, the use of mixtures with higher brominated congeners (e.g., BDE-153) was probably not reduced until the restriction by law in the beginning of 2000. Because BDE-153-containing products are probably still present on the market and in homes/workplaces, the reduced use is still not possible to detect in the human body. Another explanation to account for the differences in temporal trends between lower brominated congeners and BDE-153 may be a higher persistence of higher brominated congeners (Geyer et al. 2004).

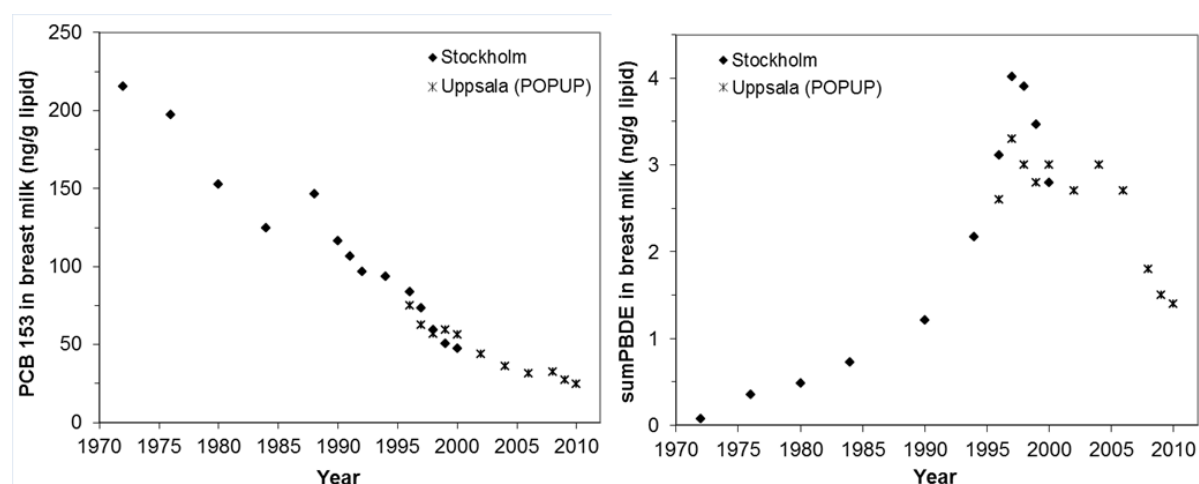


Figure 6. Levels of PCB 153 and sumPBDE in breast milk from Swedish mothers from 1972 to 2010. Results from Stockholm represent analysed levels in pooled breast milk samples (one sample per year) (Meironyte et al. 1999; Meironyté Guvenius 2002; Norén and Meironyte 2000), and results from Uppsala are yearly median levels from the POPUP study.

Consistent with our results, a study of pooled breast milk samples from Stockholm showed that the levels of PBDEs in human milk have decreased since the middle of the 1990s after an increase during the 1970s and 1980s (Figure 6). Also in agreement with our results, Swedish market basket studies performed in 1999 and 2010 showed that exposure to BDE-47 and BDE-99 from food was significantly lower in 2010 than in 1999 (NFA 2012).

Inclusion of lifestyle factors in the regression models did not affect the temporal trends for PBDEs (Table 3). BDE-153 was the only congener that showed a significant association with maternal age. This finding supports the hypothesis that BDE-153 is more persistent in the human body than the lower brominated congeners.

3.2 DETERMINANTS OF BODY BURDENS OF POPs (PAPER II)

In **Paper II**, we identified factors that are important determinants of body burdens (breast milk levels) of PCBs, PCDD/Fs and PBDEs in mothers from the POPUP cohort. Mothers who were recruited in 1996-2006 (N=325) were included.

3.2.1 Clustering analysis and grouping of substances

Based on correlations between levels of single compounds/congeners in breast milk, the cluster analysis formed distinctly separate groups of compounds (see Figure 1, Paper II). These groups/clusters were most probably dependent on routes of exposure and structural similarities of the molecules. More specifically, PBDEs were separated from the chlorinated compounds at the highest hierarchical level, probably because of differences in routes of exposure. Food is the major exposure pathway for PCDD/Fs and PCBs, whereas both food and indoor dust are important contributors to the total exposure to PBDEs (Johnson-Restrepo and Kannan 2009; Liem et al. 2000). Among the chlorinated compounds, PCB 28 was organized as an outlier with low similarity to the other PCBs, which may be because of a faster metabolism of this low-chlorinated congener and because of differences in exposure routes. Building materials may give a significant contribution to the exposure to PCB 28 among individuals living in buildings with materials containing PCBs (Schwenk et al. 2002). The PCB and PCDD/F congeners formed clusters depending largely on the number and positions of the chlorines on the molecules, indicating that differences in toxicokinetics in the human body affects concentration patterns.

3.2.2 Determinants of POP levels in breast milk

The basic regression model showed that breast milk levels of PCBs and PCDD/Fs were significantly associated with maternal age, sampling year, pre-pregnancy BMI, weight gain during pregnancy and weight loss after delivery (Figure 7). PCB 28, however, was not associated with age and BMI. A higher maternal age and a larger weight loss during the period from delivery to milk sampling predicted higher breast milk levels of PCBs and PCDD/F TEQs. These levels decreased with increasing sampling year, pre-pregnancy BMI and weight gain during pregnancy. Higher level of education was associated with higher breast milk levels of non-*ortho* PCB TEQs and PCDF TEQs.

Sampling year and maternal age explained the largest part of the variation in breast milk levels (17-33%). Temporal trends are discussed in Paper I (see section 3.1). Higher body burdens of PCBs and PCDD/Fs in older women are expected because of the accumulation of these substances in the body during the lifetime of the individual. The lack of association between PCB 28 levels and age in the POPUP cohort indicates that PCB 28 is less persistent than other PCBs and PCDD/Fs. It also points to the importance of recent exposure from, for instance, building materials in the environment. High pre-pregnancy BMI, probably an indication of recent weight gain, and a large weight gain during pregnancy may have caused a dilution of POPs because of an increased amount of body fat. On the other hand, large weight loss after delivery could have caused a mobilization of POPs from lost body fat. It can be speculated that women with different levels of education have different dietary habits, leading to differences in exposure to some POPs.

Similarly, as with most PCBs and PCDD/Fs, BDE-153 showed significant associations with all independent variables in the basic model. The resemblance between BDE-153 and the chlorinated POPs could at least partly be due to a longer half-life of BDE-153 in the body than of the lower brominated PBDEs studied (BDE-47, -99 and -100).

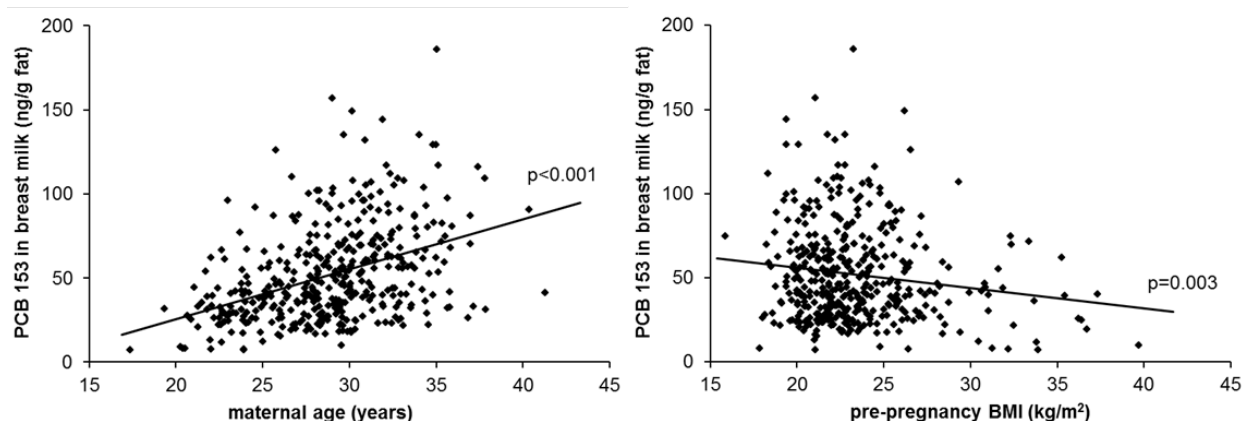


Figure 7. PCB 153 in breast milk versus maternal age (left panel) and pre-pregnancy BMI (right panel) in women from the POPUP cohort. Data from 2008-2010 has been added to the dataset that was used for Paper II, total N=413. Trend lines obtained from simple linear regression analyses are shown.

Other factors examined additional to the variables in the basic regression model were smoking and alcohol consumption during pregnancy, breastfeeding during infancy, childhood on the east coast or in a fisherman family, physical activity, computer usage and consumption of food of animal origin. Some of the observed significant associations obtained are discussed below. In most cases they were weaker and less consistent than the associations found with the variables in the basic model.

Breastfeeding is an important source of early POP exposure. In this respect, our results showed that women who had been breastfed during infancy had 12-22% higher levels of some PCBs and PCDF TEQs in their milk than women who had not been breastfed (Figure 8). Most of the participating mothers were born in the 1960s and 1970s when the levels of PCBs and PCDD/Fs in breast milk were about 10 times higher than today. Our results indicate that exposure that the women got from breastfeeding can still be detected in the body at the approximate age of 30. PCBs and PCDD/Fs may thus be persistent enough to be transferred between three generations, from grandmother to grandchild, through the breastfeeding pathway.

Women who lived on the east coast of Sweden during their childhood had higher levels of non-*ortho* PCB TEQ and PCDD/F TEQ in their milk than women who grew up elsewhere in Sweden. This may be explained by a higher consumption of contaminated fish early in life owing to the higher availability on the east coast of fatty fish from the Baltic Sea (Rylander et al. 1997). Consequently, food habits during childhood are important for the body burden of PCBs and PCDD/Fs decades later in life.

Investigating associations between dietary habits and POP levels in breast milk, we found that women with the highest consumption of fatty Baltic fish during the year before

pregnancy had 11-16% higher levels of mono-*ortho* PCB TEQ, PCDF TEQ and BDE-153 in their milk than women who did not consume such fish (Figure 8). These results confirm that contaminated fish from the Baltic Sea may be an important source of exposure. The consumption of fatty Baltic fish in the women with the highest consumption was 2-21 g/day, which corresponds to 6-61 portions (125 g fish/portion) per year. These women exceeded the current advice to women in childbearing age regarding consumption of contaminated Baltic Sea fish (not more than 2-3 times per year). Several other studies have reported positive associations between body burdens of PCBs and PCDD/Fs and consumption of fatty Baltic fish (Jonsson et al. 2005; Rylander et al. 2009; Svensson et al. 1995) or fish in general (e.g., Agudo et al. 2009; Schade and Heinzow 1998).

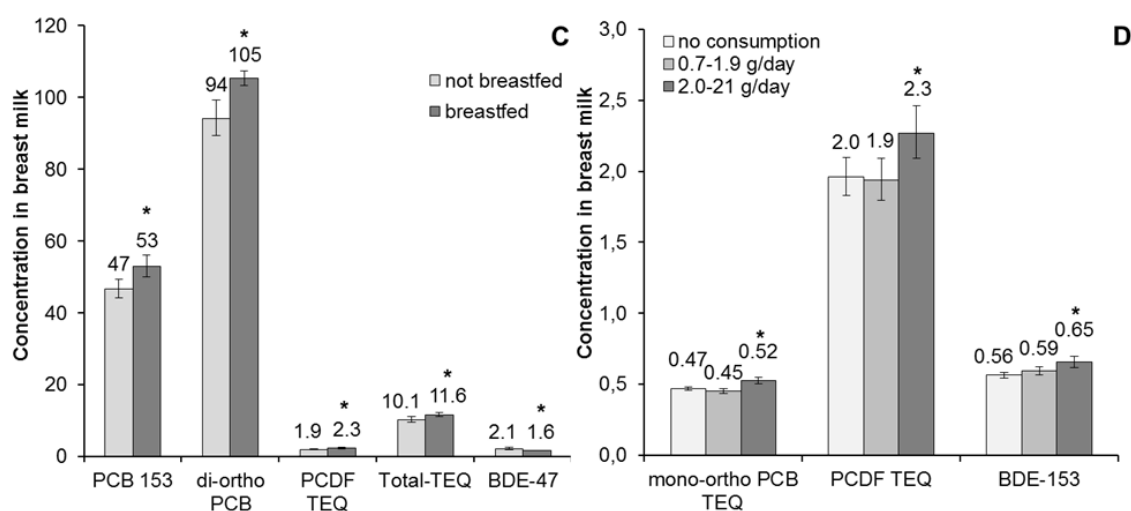


Figure 8. POP concentrations in breast milk (adjusted geometrical mean \pm standard error) from women who were breastfed or were not breastfed during infancy (N=263 and 29, respectively) (left) and from women with different consumption rates of fatty fish from the Baltic Sea (no consumption, N=171; 0.7-1.9 g/day, N=69; 2.0-21 g/day, N=53) (right). The results are adjusted for all variables that were significantly associated with milk concentrations of POPs in step 1 and 2 in the regression analyses. The concentrations are given in ng/g milk fat (PCB 153, di-*ortho* PCB, BDE-47, BDE-153) or pg/g milk fat (mono-*ortho* PCB TEQ, PCDF TEQ, total TEQ). * $p < 0.05$ in a general linear model compared with the reference group (1st category). The figure is obtained from Paper II (Lignell et al. 2011) and reproduced by permission of The Royal Society of Chemistry.

Collectively, the results suggest that a woman who grew up on the east coast of Sweden and had a small weight increase during pregnancy (25th percentile), a fast weight loss shortly after delivery (75th percentile) and a high current consumption of fatty Baltic fish (2-21 g/day) would have 40-50% higher levels of dioxins in her breast milk compared with a woman who did not grow up on the east coast of Sweden with an average weight gain during pregnancy, an average weight loss after delivery and no consumption of fatty Baltic fish. Following current recommendations for the consumption of contaminated fish from the Baltic Sea, having a normal weight gain during pregnancy and avoiding fast weight loss soon after delivery are feasible advice to women in order to limit dioxin levels in breast milk.

3.3 PRENATAL EXPOSURE TO POPs AND BIRTH WEIGHT (PAPER III)

In **Paper III**, associations between prenatal exposure (i.e. breast milk levels) to di-*ortho* PCBs and sumPBDE (sum of BDE-47, BDE-99, BDE-100 and BDE-153) and birth weight were investigated. Participants who were recruited in 1996 to 2010 were included. Breast milk levels of di-*ortho* PCBs and PBDEs were available for 416 and 364 mothers, respectively.

3.3.1 PCBs and birth weight

The multivariate regression model showed a weak but significant positive association between prenatal exposure to di-*ortho* PCBs and birth weight (Table 4). The mean increase in birth weight was 137 g (95% confidence interval, CI: 17-257) for every 1-unit increase in ln-transformed di-*ortho* PCB concentration, corresponding to a mean difference in birth weight between the 25th and 75th percentile of exposure of approximately 100 g.

Table 4. Associations between exposure to di-*ortho* PCBs and PBDEs and birth weight. Results obtained from Paper III (Lignell et al. 2013a).

	All participants			Female infants			Male infants		
	β^a	SE ^b	N	β^a	SE ^b	N	β^a	SE ^b	N
<i>Multivariate model^c</i>									
di- <i>ortho</i> PCBs ^d	137*	61	346	42	99	161	200*	77	185
sumPBDE ^e	-54	46	346	32	70	161	-126*	62	185
<i>Multivariate model including gestational length^f</i>									
di- <i>ortho</i> PCBs ^d	143*	65	254	149	123	110	141 [#]	77	144
sumPBDE ^e	-106*	52	254	-47	93	110	-139*	64	144

^aRegression coefficient, i.e. change in birth weight per 1-unit increase in ln-transformed contaminant concentration in breast milk. ^bStandard error. ^cDi-*ortho* PCB and sumPBDE were included in the same model. Other covariates included were age of the mother, pre-pregnancy BMI, weight gain during pregnancy, education, smoking and infant sex. Infant sex was not included in the separate analyses for female and male infants. ^dSum of PCB 138, PCB 153 and PCB 180. ^eSum of BDE-47, BDE-99, BDE-100 and BDE-153. ^fGestational length was added to the covariates in the multivariate model. * $p \leq 0.05$ [#] $p \leq 0.10$

In contrast to our results, accidental exposures to very high levels of PCBs or PCDD/Fs have been shown to decrease birth weight (Tsukimori et al. 2012). Results from studies of background exposure to PCBs and birth weight are inconsistent, where some report inverse associations (e.g., Halldorsson et al. 2008; Patandin et al. 1998; Hertz-Picciotto et al. 2005; Rylander et al. 1998), while others report no significant associations (e.g., Gladen et al. 2003; Longnecker et al. 2005; Weisskopf et al. 2005). Generally, the PCB exposure levels in studies showing inverse associations were higher than in the POPUP cohort. One possible explanation for the discrepancies between studies is that the dose-effect curve is non-monotonic with a positive low-dose effect of di-*ortho* PCBs on birth weight and a negative effect at higher exposure levels. However, it can also be speculated that observed inverse associations in some studies may be partly confounded by maternal pre-pregnancy BMI and weight gain during pregnancy. The observed positive association

between di-*ortho* PCB exposure and birth weight in our study was only significant when both these covariates were included in the regression model. Increased pre-pregnancy BMI and maternal weight gain are both associated with increased birth weight as well as with decreased levels of PCBs in breast milk and maternal blood (Glynn et al. 2007; Lignell et al. 2011). Thus, these variables are important confounders. Few other studies on PCB exposure in relation to birth weight adjust their results for maternal weight gain, but we suggest that both BMI and weight gain should be considered in future studies. Using a pharmacokinetic model, Verner et al. (2013) found that previously reported inverse associations between exposure to PCB 153 and birth weight may be due to confounding by gestational weight gain.

In addition to maternal BMI and weight gain, concurrent exposure to sumPBDE was also important to include in the regression model in our study. It therefore seems as though PCBs and PBDEs influence birth weight via biochemical pathways in such way that exposure to PBDEs may partially mask the association between di-*ortho* PCBs and birth weight, and vice versa. Adding gestational length to the multivariate model did not significantly change the result for di-*ortho* PCB (Table 4), indicating that the association between di-*ortho* PCB exposure and birth weight was mediated by increased fetal growth rather than by increased gestational length. Further, the positive association was not appreciably changed when fish consumption during pregnancy was added to the regression model.

Because di-*ortho* PCB levels in breast milk are strongly correlated with levels of DL-PCBs, PCDD/Fs and *p,p'*-DDE in the POPUP cohort (see Paper III), di-*ortho* PCB could be a marker for exposure to other chlorinated POPs. It is therefore difficult to separate the influence of different chlorinated compounds on birth weight from each other, and if the observed associations are causal, it is possible that they are caused by the total mixture of compounds.

3.3.2 PBDEs and birth weight

Although not statistically significant, there was an inverse association between sumPBDE exposure and birth weight in both the unadjusted and multivariate models (Table 4). However, a significant association did emerge when gestational length was included in the regression model. The mean decrease in birth weight was 106 g (95% CI: -4 to -208) for every 1-unit increase in ln-transformed sumPBDE concentration, corresponding to a mean difference in birth weight between the 25th and 75th percentile of exposure of approximately 80 g. The inverse association was also significant in the multivariate model (without gestational length) when the analysis was restricted to individuals with data on gestational length (data on gestational length was missing for about one third of the participants) (see Table 3 in Paper III). Our data do not make it possible to draw firm conclusions about the reason behind the difference between the unrestricted and restricted analysis, but we feel it may be due to chance. However, the results suggest that the observed inverse association between sumPBDE and birth weight was not due to a PBDE influence on gestational length. If di-*ortho* PCB was excluded from the statistical model, the inverse association was not significant, again suggesting that exposure to these different classes of POPs may mask the effect of each other. Because of this reason, our

results strongly suggest that it is important to control for concurrent exposure to different contaminants (such as PCBs and PBDEs) in studies of POPs and birth weight.

There are few other studies on the association between PBDE exposure and birth weight showing diverging results. Only two larger studies that also adjusted their results for possible confounders were found (Foster et al. 2011; Harley et al. 2011). Both of these studies show inverse associations. However, in one of the studies the significance levels did not persist when controlling for maternal weight gain (Harley et al. 2011).

The association between sumPBDE and birth weight became stronger when fish consumption was included in the multivariate model: β decreased with 29% from -106 to -137. Fish consumption is the major contributor to the dietary intake of PBDEs. Indeed, maternal seafood consumption has been positively associated with birth weight in several observational studies (Brantsaeter et al. 2012). Our results indicate that fish consumption may be a confounder of the association between PBDE exposure and birth weight because of its positive association with both exposure and outcome.

3.3.3 Sex differences

When female and male infants were studied separately, the multivariate model showed a significant positive association between di-*ortho* PCB and birth weight in male infants, but not in females (Table 4). Similarly, the inverse association between sumPBDE and birth weight was only observed in male infants. These sex differences may be due to chance or to a smaller number of female infants in the study (161 females and 185 males). However, it is also possible that male fetuses are more vulnerable to PCB and PBDE exposure than females. Other studies have shown an inverse association between PCBs (or POPs in general) and birth weight restricted to male infants (Hertz-Picciotto et al. 2005; Rylander et al. 1995; Sonneborn et al. 2008).

In conclusion, the present relatively large study of background prenatal exposure to di-*ortho* PCBs and sumPBDE indicates that these substances may influence birth weight in different directions. Exposure to di-*ortho* PCBs tended to be associated with higher birth weight, whereas the association between sumPBDE and birth weight was inverse. Both high and low birth weight has been linked to such risk factors as overweight, hypertension and insulin resistance later in life (Calkins and Devaskar 2011; Parsons et al. 1999). One could therefore speculate that, if causal, the small PCB- and PBDE-induced shifts in birth weight distribution may influence future public health in populations with background exposure. This influence could be especially important in boys in the lower or upper ends of the birth weight distribution.

3.4 POP EXPOSURE AND THYROID HORMONES (PAPER IV AND V)

Several possible associations between POP exposure (maternal body burdens and estimated pre- and postnatal exposure of infants) and levels of thyroid hormones in mothers and infants were investigated and presented in **Paper IV** and **V** (Table 2). Women who donated blood during pregnancy (recruited in 1996-1999) were included. Blood was also sampled from their infants after birth. Most associations were weak and non-significant. Presented below are the significant results that I found worthy of discussion and further thought. For a more comprehensive presentation of the results, the reader is referred to Paper IV and V.

3.4.1 POP body burdens and thyroid hormones in pregnancy

Simple linear regression models showed significant inverse associations between maternal body burdens of di-*ortho* PCBs, mono-*ortho* PCB TEQs, PCDD/F TEQs and total T3 in early and late pregnancy (Figure 9). After adjusting for possible confounders, the only significant association was for PCDD/F TEQ. In early pregnancy the decrease in total T3 level was 0.54 ± 0.21 nmol/L serum (mean \pm standard error) for every 1-unit increase in maternal PCDD/F TEQ breast milk level (N=66) (Paper V). The corresponding value in late pregnancy was 0.39 ± 0.12 nmol/L serum (N=91) (Paper IV).

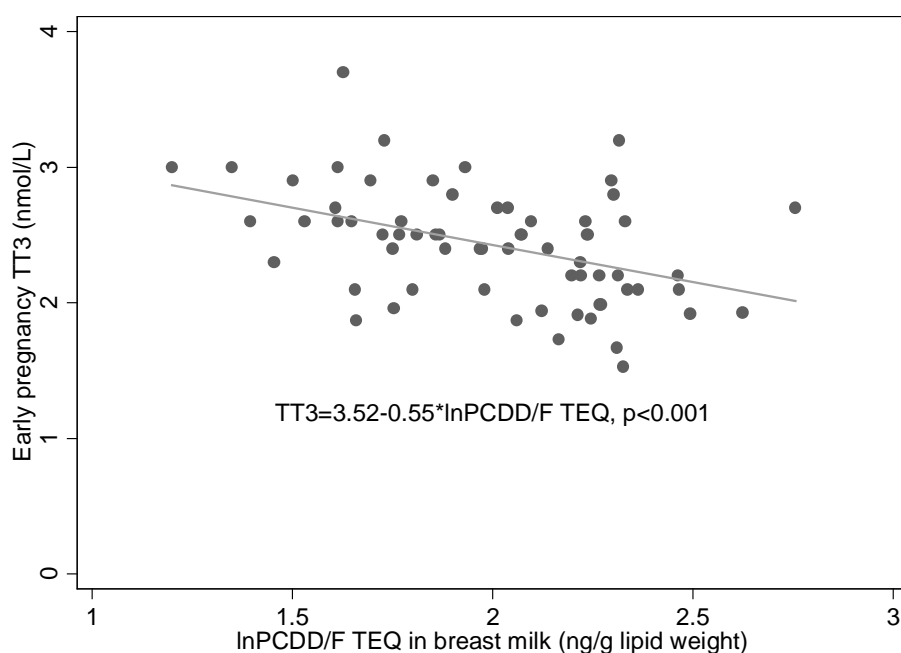


Figure 9. Crude associations between maternal breast milk levels of PCDD/F TEQ and maternal serum levels of total T3 (TT3) in early pregnancy in the POPUP cohort (N=67). Figure obtained from Paper V (manuscript).

The inverse association between breast milk levels of PCDD/F TEQ and maternal levels of total T3 was significant in both early and late pregnancy and persisted after covariate adjustment. These results are in accordance with studies in laboratory animals in which

PCBs and PCDD/Fs have been shown to reduce the levels of circulating thyroid hormones (Boas et al. 2009). Although results from epidemiological studies differ, an inverse relationship between background exposure to chlorinated POPs and levels of T3 or T4 has been reported in several publications (Alvarez-Pedrerol et al. 2009; Chevrier et al. 2008; Koopman-Esseboom et al. 1994; Takser et al. 2005).

Thyroid hormones are essential for normal brain development. The fetus is dependent on the maternal supply of thyroid hormones and even mild reductions in maternal hormone levels during critical periods of brain development may affect children's cognitive development (Patel et al. 2011; Trumpff et al. 2013). Although the observed association between PCDD/F TEQ and total T3 in our study was weak, our findings add to the literature that suggests that exposure to chlorinated POPs may decrease levels of thyroid hormones in pregnant women. If the observed inverse associations in our and other epidemiological studies are causal, they may be of importance for neurological development. Maternal thyroid hormone levels during pregnancy may also affect thyroid hormone status in children in later life (Pakkila et al. 2013).

For PBDEs, there were no associations with maternal thyroid hormone levels that were consistent in early and late pregnancy. In early pregnancy BDE-153 in breast milk was inversely associated with total T3 in the crude and adjusted regression models (Paper V). The decrease in the total T3 level in the adjusted model was 0.20 ± 0.08 nmol/L serum (mean \pm standard error) for every 1-unit increase in maternal BDE-153 breast milk level (N=126). In late pregnancy there was a significant inverse association between BDE-99 and TSH that persisted after covariate adjustment ($\beta = -0.17 \pm 0.09$, N=166) (Paper V). Taken together, our results on the association between PBDE exposure and thyroid hormone levels in pregnant women were weak and inconsistent.

3.4.2 POP exposure and thyroid hormones in infants

Associations between fetal and postnatal exposure to PCBs, PCDD/Fs, *p,p'*-DDE and PBDE and thyroid hormone levels in infants were non-significant in most cases. The only significant association that also persisted after covariate adjustment was an inverse association between maternal late pregnancy serum levels of LPCBs (sum of the low-chlorinated congeners CB-28, 52 and 101) and total T3 in infants at 3 weeks of age (Paper IV). Because the levels of LPCB were below LOQ in many women, this exposure variable was categorized in quartiles. The adjusted mean level of total T3 was 9% lower in infants in the highest exposure quartile compared with infants in the reference quartile with the lowest exposure.

In the US-EPA risk assessment of non-cancer effects of TCDD the RfD was based on increased levels of TSH in newborns exposed to high levels of TCDD *in utero* (US-EPA 2012). Results from other studies on the association between background POP exposure and thyroid hormone status in infants are inconclusive. Some studies found inverse associations between PCB exposure (mainly di-*ortho* congeners) and levels of thyroid hormones in cord blood (Herbstman et al. 2008; Maervoet et al. 2007). However, it is not possible to reach a conclusion about associations between background POP exposure and

thyroid hormone levels in infants. The exposure levels and the statistical power (i.e. number of participants) in the POPUP cohort may be too low to detect effects on thyroid hormone status in infants.

3.5 POP EXPOSURE VERSUS HEALTH-BASED GUIDANCE VALUES

In this section, exposure to DL compounds and PBDEs in mothers and infants in the POPUP cohort is compared with health-based guidance values from the risk assessments discussed in section 1.4.1.

3.5.1 PCBs and PCDD/Fs

The TWI for dioxins and DL-PCBs established by the European SCF (14 pg TEQ/kg body weight) was based on developmental effects in male rats after exposure *in utero* (SCF 2001). The TWI was set to protect fetuses from harmful exposure levels. The 1-compartment, first-order elimination pharmacokinetic model used by the SCF was applied to estimate the steady-state body burden in humans at the TWI level:

$$\text{Body burden (ng/kg bw)} = f * \text{intake (ng/kg bw/day)} * \text{half-life (days)} / \ln(2)$$

where f is the fraction of dose absorbed, which is assumed to be 50% for absorption of dioxins from food for humans. The estimated *half-life* of the elimination of dioxins is set to 2740 days (SCF 2001). This calculation resulted in an estimated TWI body burden of 4.0 ng TEQ/kg body weight, or, assuming 30% fat in women aged about 30 years (ICRP 2002) and a complete distribution of the compounds to this body fat, 13 ng/kg body fat. The median total TEQ level in breast milk from the POPUP cohort (1996-2010) is only slightly lower than the TWI body burden, i.e. 10 ng/kg lipids (range: 1.6-31). Thirty-one percent of the women exceeded the estimated TWI body burden during the study period. However, the levels of dioxins and DL-PCBs in breast milk decreased between 1996 and 2010. The median total TEQ level in breast milk sampled in 2008-2010 (5.9 ng/kg lipids) was about half of the TWI body burden. In addition, breast milk from 5% of the women had higher total TEQ levels than 13 ng/kg lipids. Using the RfD for TCDD as proposed by the US-EPA (0.7 pg/kg body weight/day) (US-EPA 2012) instead of the TWI, a RfD steady-state body burden can be estimated to 4.6 ng/kg body fat. The median breast milk level of total TEQ in the POPUP cohort in 2008-2010 exceeded this RfD body burden. Moreover, 75% of the women had higher total TEQ levels than 4.6 ng/kg lipids in their breast milk.

To estimate the intake of dioxins and DL-PCBs in breastfed infants in the POPUP cohort we used a mean consumption of 700 g breast milk per day in a one-month old infant of 4.5 kg (Niklasson and Albertsson-Wikland 2008; Sievers et al. 2002), a mean lipid level in breast milk of 3% (mean level in the POPUP cohort) and total TEQ levels in breast milk from the mothers recruited in 2008-2010. The median daily intake in breastfed infants is estimated to 28 pg TEQ/kg body weight (range: 7.5-102). This intake level far exceeds the RfD of 0.7 pg TEQ/kg body weight/day and also exceeds 20 pg/kg body weight/day which is the estimated intake at the LOAEL for decreased sperm count and

motility that was used by the US-EPA to derive the RfD (US-EPA 2012). The LOAEL was based on a study of men who were 1-9 years old at the time of exposure and it is not known if exposure to DL compounds during breastfeeding will cause similar effects. Exposure to DL compounds *in utero* seems to be most critical for health, but the US-EPA risk assessment shows that exposure after birth may also be of importance for later health development.

Because the breastfeeding period is short, the European SCF and the WHO have concluded that possible risks from high exposure to DL compounds from breast milk are outweighed by the beneficial effects of breastfeeding (SCF 2001; WHO 2000a). Breastfeeding clearly lowers the risk of death from infectious diseases (mostly gastrointestinal and respiratory tract infections) in both developed and developing countries (Hornell et al. 2013; Quigley et al. 2007; WHO 2000b). The evidence for long-term beneficial health effects of breastfeeding during childhood is weaker and sometimes controversial. However, decreased risks of overweight/obesity, type-2 diabetes and atopic diseases, reduced systolic blood pressure and total blood cholesterol, as well as beneficial effects on IQ in children and adolescents have been suggested (Hornell et al. 2013; Horta and Victora 2013).

3.5.2 PBDEs

Although available data did not allow for determination of health-based tolerable intake levels, BMDL₁₀-body burdens ($\mu\text{g/kg}$ body weight) for neurodevelopmental effects of PBDEs in mice were derived in the risk assessment performed by the EFSA (EFSA 2011). Assuming 30% fat in women aged approximately 30 years (ICRP 2002) and a total distribution of the compounds to this body fat, these BMDL₁₀-body burdens can be estimated to 770 (BDE-47), 30 (BDE-99) and 210 (BDE-153) $\mu\text{g/kg}$ body lipids. Median breast milk levels of BDE-47, BDE-99 and BDE-153 in the POPUP cohort (2008-2010) make up less than 0.5% of the estimated BMDL₁₀-body burdens.

The EFSA calculated that chronic human daily intake levels associated with the BMDL₁₀-body burdens were 172 (BDE-47), 4.2 (BDE-99) and 9.6 (BDE-153) ng/kg body weight (EFSA 2011). In breastfed infants in the POPUP cohort (2008-2010) the median daily intake of these congeners can be estimated to 2.5 ng/kg body weight for BDE-47 (range: 0.4-56), 0.5 ng/kg body weight for BDE-99 (range: 0.02-6.4) and 2.3 ng/kg body weight for BDE-153 (range: 0.6-22) (consumption of 700 g breast milk per day in a one-month old infant weighing 4.5 kg). Median exposure to PBDEs in breastfed infants in the POPUP cohort is thus lower than the estimated BMDL₁₀ intake levels, but the margins are small, especially for BDE-99 and BDE-153. Variation in breast milk levels between individuals is large and infants exposed to the highest levels will exceed the BMDL₁₀ intake levels for BDE-99 and BDE-153 established from studies of neonatally exposed mice.

3.5.3 Conclusions

Most women in the POPUP cohort have body burdens of DL compounds below the estimated body burden at the current TWI level adopted within the EU. However, the

margin to the estimated TWI body burden is small and most women exceed the estimated body burden at the RfD proposed by US-EPA. It is therefore desirable that body burdens of DL compounds continue to decrease to reduce fetal exposure. It is also desirable to reduce the exposure of breastfed infants to both DL compounds and PBDEs because exposure early in life may have important consequences for health. Based on reduced risk for infections, the WHO recommends exclusive breastfeeding during the first six months of life and extended complimentary breastfeeding until two years of age (Horta and Victora 2013; WHO and UNICEF 2003). In Sweden, the NFA has adopted the WHO recommendation of six months of exclusive breastfeeding. Lower levels of DL compounds and PBDEs would improve the quality of the breast milk and may also increase the beneficial effects associated with breastfeeding.

In order to decrease human exposure, efforts to reduce contamination of the environment and of food with DL compounds and PBDEs should be continued. Fish is the major dietary source of exposure. In addition, fish is the principal source of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) suggested to have a protective effect against cardiovascular disease and to improve neurodevelopment in infants and young children (FAO/WHO 2011). Accordingly, lower levels of DL compounds may increase the beneficial effects associated with nutrients present in fish.

Reduced levels of PBDEs in food are important, but a general reduction of PBDE levels in the human environment (i.e. in indoor air and dust) is of equal importance. As shown by correlations between levels of PBDEs in house dust and breast milk (Bjorklund et al. 2012; Wu et al. 2007), the indoor environment is an influential exposure route for PBDEs.

3.6 GENERAL METHODOLOGICAL CONSIDERATIONS

The study on the association between exposure to POPs and birth weight (Paper III) and parts of the study on thyroid hormones (Paper IV-V) presented in this thesis are cross-sectional, i.e. exposure and outcome were measured at the same time point. Most other studies in this field have also employed a cross-sectional design. An inherent drawback of the cross-sectional approach is that it is not possible to conclude whether the observed associations are causal. For instance, PCDD/F exposure may affect the level of total T3 in blood in pregnant women in the POPUP cohort, but reverse causality, i.e. an effect of thyroid hormone status on the levels of PCDD/F in the body, cannot be excluded. To be able to draw conclusions about causality exposure (maternal body burdens of POPs) could be measured before pregnancy in studies of associations between POP exposure and health outcomes in pregnant women and newborns. Such a study design would eliminate the risk that the large changes in metabolism and body composition that occur during pregnancy will affect the results.

In our study the exposure (POP levels in breast milk) was even measured after the outcome (birth weight, thyroid hormone levels during pregnancy). However, POP concentrations in breast milk and maternal blood are highly correlated, and breast milk levels give a good estimate of the maternal body burden during pregnancy and thus of the exposure of the infant during the fetal period. Samples of both breast milk and late

pregnancy blood serum were collected from some of the women in the POPUP study. Both sample types were analysed for PCBs and *p,p'*-DDE. Correlations between contaminant levels in serum and milk were strong (Figure 10). In the POPUP cohort strong correlations have also been observed between PBDE levels in breast milk and serum collected at the same time point (Lignell et al. 2013b). In addition, other studies have shown strong correlations between late pregnancy serum and breast milk levels of PBDEs (Needham et al. 2011). All these findings indicate that PBDE levels in breast milk can be used as an indicator of maternal body burden and probably of prenatal exposure.

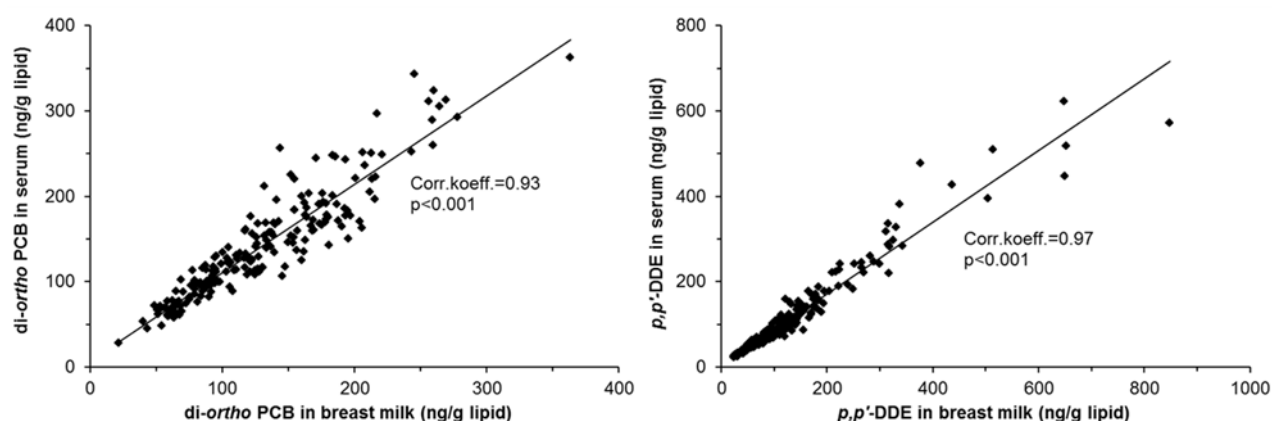


Figure 10. Correlations between serum (late pregnancy) and breast milk levels of di-ortho PCBs and *p,p'*-DDE in first-time mothers from the POPUP cohort (N=202). Pearson correlation coefficients are shown.

A major strength of this thesis concerns the relatively large number of participants. Precision is thus high in comparison with many other studies in this field. Another strength is that data on several lifestyle factors, including dietary habits, were available from the participants in the POPUP cohort. This availability of data enabled us to adjust the results in study III-V for a number of possible confounders. Variables that were chosen as covariates were either known to predict exposure (i.e., POP levels in breast milk), associated with the outcome (birth weight or thyroid hormone levels), or often included in similar studies. In paper V, only variables that were significantly associated with the outcome in bivariate models were selected as confounders.

3.6.1 Exposure assessment

Exposure assessment is often a critical determinant of the validity of epidemiological studies. An important strength of the POPUP study is the high quality of the exposure assessment, including chemical analysis of contaminants in individual breast milk or serum samples. Because chemical analyses of POPs are expensive, some studies in this field use fish consumption as a proxy for POP exposure. However, at least in the POPUP cohort, this is not a valid measurement of exposure because fish consumption only explains a small portion of the variation of body burden of POPs (Paper II).

Several chlorinated POPs (dioxins, DL-PCBs, NDL-PCBs, *p,p'*-DDE) as well as PBDEs were analysed in the samples from the POPUP cohort. Levels of the chlorinated

compounds are highly correlated because of similar sources of exposure and toxicokinetics in the human body. Hence, identifying the effect associated with, or caused by, a specific chlorinated compound is difficult. Moreover, other unmeasured compounds may contribute to the observed effects and interaction between chemicals may occur. The observed association between di-*ortho* PCB exposure and birth weight (Paper III) could seemingly be the result of exposure to the whole mixture of chlorinated compounds. PBDE levels in breast milk are to some extent correlated to the chlorinated compounds, but the correlations are much weaker. The analyses of both chlorinated compounds and PBDEs made it possible to adjust associations for concurrent exposure to these different groups of contaminants (Paper III). The results in Paper III show that it may be important to adjust for concurrent exposure to different contaminants.

3.6.2 Generalizability

Mothers in the POPUP cohort were randomly recruited among first-time mothers in Uppsala County. Both the mother and the child had to be healthy and the mother had to be born in a Nordic country to be included. During the study period, the participating rate varied between 42 and 82%. The age of the participants (mean 29.1 years in 2008-2010) was comparable with the mean age of all first-time mothers in Sweden (28.4 years in 2008-10) (Socialstyrelsen 2012). Since 2000, women who declined to participate were asked about their education. We found that participants generally had a higher level of education than non-participants. This may to some extent have influenced the results because of differences in lifestyle, i.e. dietary habits, between populations with different levels of education (Amcoff et al. 2012).

To study regional differences in breast milk levels of POPs, first-time mothers have been recruited not only in Uppsala but also in other Swedish cities: Lund, Gothenburg and Lycksele (Glynn et al. 2011). Breast milk from these studies have been analysed for PCBs, *p,p'*-DDE and PBDEs. The results revealed small differences in contaminant levels between regions, indicating that POP exposure of the general population in Sweden is similar in different parts of the country (Glynn et al. 2011). The results presented in this thesis can thus be generalized to Swedish first-time mothers.

4 CONCLUSIONS

- Levels of PCBs and PCDD/Fs in breast milk from Swedish first-time mothers in the POPUP cohort have decreased by about 4-8% per year from 1996 to 2010. Temporal trends of PBDEs varied depending on congener studied, with decreasing levels of tetra- and penta-brominated congeners (BDE-47, -99, -100) (5-10% per year) and slightly increasing levels of the hexa-brominated BDE-153 (+1% per year). The decreasing trends for chlorinated POPs and lower brominated PBDEs show that efforts to reduce contamination of the environment and food have resulted in decreased human exposure levels.
- Factors that predicted higher levels of PCBs and PCDD/Fs in breast milk were high maternal age and fast weight loss after delivery. In contrast, high pre-pregnancy BMI and large weight gain during pregnancy predicted lower levels. Women who were breastfed during infancy, grew up on the east coast of Sweden (with high availability of contaminated fish from the Baltic Sea) and had a high consumption of fatty Baltic fish during the year before pregnancy had higher levels of some POPs in breast milk. These findings suggest that exposure to POPs during the whole lifetime of the individual is important for breast milk levels at the time of nursing.
- Prenatal exposure to di-*ortho* PCBs was associated with higher birth weight and PBDE exposure was associated with lower birth weight in the POPUP cohort. Both high and low birth weight has been linked to overweight, hypertension and insulin resistance later in life. If causal, the small PCB- and PBDE-induced shifts in birth weight distribution may influence future public health in populations with background exposure.
- The inverse association between PBDE exposure and birth weight proved stronger when maternal fish consumption was added to the statistical model, indicating that fish consumption may be a confounder in this relation because of its positive association with both exposure and outcome.
- The observed associations between PCB and PBDE exposure and birth weight were stronger in male infants, suggesting a higher susceptibility of male fetuses to POP exposure.
- Associations between maternal, fetal and postnatal exposure to PCBs, PCDD/Fs, *p,p'*-DDE and PBDE and thyroid hormone levels in mothers during pregnancy and in infants after delivery were mostly weak and non-significant. However, higher maternal body burdens of PCDD/Fs were associated with lower maternal levels of total T3. That the association was similar in early and late pregnancy strengthens its reliability. Thyroid hormones are essential for normal brain development. If causal, the observed association between PCDD/F exposure and thyroid hormone status may be of importance for neurological development in children.

- The margin between current body burdens of DL compounds in the POPUP cohort and the highest levels tolerable from a health perspective is small. In addition, exposure to DL compounds and PBDEs in breastfed infants is high.
- Based on the findings in this thesis, it is desirable that body burdens of PCBs, dioxins and PBDEs in young Swedish women continue to decrease to limit fetal and infant exposure. Efforts to reduce contamination of the environment and of the food chain should be continued and children and women in childbearing ages should restrict their consumption of contaminated fatty fish from the Baltic Sea to limit the risk of high exposure. In addition, women may be advised to have a normal weight increase during pregnancy and avoid fast weight loss shortly before pregnancy or after delivery. It is important to continue to follow the levels of POPs in breast milk to follow up further efforts to reduce human exposure.

5 POPULÄRVETENSKAPLIG SAMMANFATTNING

Persistenta (svårnedbrytbara) organiska miljöföroreningar (POP, persistent organic pollutants) är en grupp kemiska ämnen som på olika sätt har fått stor spridning i miljön. Exempel är polyklorerade bifenyler (PCB), polybromerade difenyletrar (PBDE) och dioxiner. PCB har framställts för användning i bland annat elektrisk utrustning. PBDE används för att förhindra brand i till exempel elektronik och textilier. Dioxiner kan bildas vid förbränningsprocesser och vid tillverkning av vissa kemikalier.

Människan får främst i sig POP via maten. För PBDE är inomhusmiljön (luft och damm) också en viktig källa. Eftersom POP är fettlösliga och bryts ner långsamt lagras de i kroppen under hela livet och förs över till fostret och det ammade spädbarnet via moderkakan och bröstmjölken. POP är giftiga och hög exponering kan bland annat påverka utvecklingen av nervsystemet, fortplantningsförmågan, immunförsvaret och hormonsystemen. Foster och spädbarn är extra känsliga för POP. På grund av ämnens oönskade egenskaper har användningen och utsläppen av PCB och dioxiner begränsats kraftigt sedan 1970-talet och användningen av PBDE sedan början av 2000-talet. Fet fisk från Östersjön (strömming, vildfångad lax) kan innehålla höga halter av dioxiner och PCB. Livsmedelsverket rekommenderar därför att barn och kvinnor i barnafödande ålder ska begränsa sin konsumtion av sådan fisk till 2-3 gånger per år. Syftet med rådet är att minska exponeringen av foster och spädbarn.

Syftet med den här avhandlingen var att undersöka halterna av POP i kroppen hos svenska förstföderskor och hur halterna har förändrats över tiden. Vi ville också undersöka om det finns några samband mellan halterna av POP hos svenska förstföderskor och nivåerna av sköldkörtelhormoner i blodet hos kvinnorna och barnen eller barnens födelsevikt. För att kunna göra detta samlades prover av bröstmjolk och blod in från slumpvis utvalda förstföderskor och deras barn i Uppsala under perioden 1996 till 2010. Halterna av POP i modersmjolk ger ett bra mått på hur mycket POP barnet utsätts för under både foster- och amningsperioden.

Resultaten visade att de mammor som deltog i studien hade mätbara halter av PCB, dioxiner och PBDE i modersmjölken. Halterna av PCB och dioxiner minskade med 4-8 % per år under perioden 1996-2010. Det finns flera varianter av PBDE och halterna av vissa av dessa minskade, medan halterna av andra ökade något. De nedåtgående trenderna tyder på att de åtgärder som vidtagits för att minska spridningen av PCB, dioxiner och vissa av PBDE-varianterna till miljön har medfört att vi människor får i oss mindre mängder av dessa ämnen.

Äldre mammor hade högre POP-halter i bröstmjölken än yngre mammor, vilket beror på att ämnena lagras i kroppsfettet under hela livstiden och utsöndras i mjölken. Resultaten visade också att kvinnor som gick upp mycket i vikt under graviditeten hade lägre POP-halter i mjölken än de som gick upp mindre i vikt. De kvinnor som gick ner mycket i vikt efter förlossningen hade däremot högre POP-halter i mjölken. Detta beror sannolikt på att POP späds ut i en större mängd kroppsfett när man går upp i vikt och frigörs från kroppsf-

fettet vid viktnedgång. Högre halter av vissa POP uppmättes i mjölk från kvinnor som själva blivit ammade när de var spädbarn och från kvinnor som bodde på ostkusten under uppväxten (och sannolikt åt mer förorenad fet fisk från Östersjön än andra). Detta tyder på att de mängder POP man får i sig som barn via amning och fisk har betydelse för halterna i kroppen vid vuxen ålder. Konsumtionen av fisk senare i livet är också viktig och kvinnor som åt mycket förorenad fet fisk från Östersjön under året före graviditeten hade högre halter av vissa POP i sin mjölk än de som inte åt sådan fisk.

Födelsevikten är ett mått på barnets hälsa under fosterperioden. Dessutom kan både låg och hög födelsevikt öka risken för bland annat övervikt, högt blodtryck och hjärt- och kärlsjukdom i vuxen ålder. Den här studien visade ett samband mellan högre exponering för PCB under fosterperioden och högre födelsevikt hos barnen. För PBDE var sambandet det omvända, d.v.s. barn med högre exponering hade något lägre födelsevikt. Skillnaden i födelsevikt mellan hög exponering (75:e percentilen) och låg exponering (25:e percentilen) var ca 100 g högre födelsevikt för PCB och 80 g lägre födelsevikt för PBDE.

Sköldkörtelhormoner är viktiga för utveckling, tillväxt och ämnesomsättning. Under både fosterperioden och barndomen är de nödvändiga för att hjärnan ska utvecklas normalt. Sambanden mellan exponering för POP och nivåerna av sköldkörtelhormoner hos mammorna och barnen i den här studien var i de flesta fall svaga och inte statistiskt säkerställda. Det fanns dock ett samband mellan högre halter av dioxiner och lägre nivåer av sköldkörtelhormonet trijodtyronin (även kallat T3) i mammans blod. Även om sambandet var svagt skulle det kunna ha betydelse för hjärnans utveckling hos fostret.

Resultaten i den här avhandlingen har gett viktig kunskap om halterna av POP hos svenska förstföderskor som är användbar när riskerna med POP i mat ska värderas. Marginalen mellan de POP-halter som kvinnorna hade i kroppen och de nivåer som anses tolerabla var i vissa fall små. Vi observerade också samband mellan POP-exponering och födelsevikt respektive sköldkörtelhormonnivåer som kan vara viktiga för hälsan. Slutsatsen blir därför att det är önskvärt att halterna av dioxiner, PCB och PBDE hos svenska kvinnor fortsätter att minska. Viktigast är att fortsätta arbetet med att minska spridningen av dessa ämnen till miljön och till maten. Barn och kvinnor bör också begränsa sin konsumtion av fet fisk från Östersjön som kan innehålla höga POP halter. Det är viktigt att fortsätta följa halterna av dioxiner, PCB och PBDE i modersmjölk för att få veta om de åtgärder som sätts in för att minska befolkningens exponering har fått någon effekt.

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