Persistent halogenated pollutants in mothers´ milk

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Till Maria,

Det blev inte som det var tänkt – men det blev!

List of papers

The thesis is based upon the following articles and manuscripts that are referred to by their roman numerals (I–IV).

Paper I Jakobsson K., Fång J., Athanasiadou M., Rignell-Hydbom A. and Bergman Å., (2012) Polybrominated diphenyl ethers in maternal serum, umbilical cord serum, colostrum and mature breast milk. Insights from a pilot study and the literature. *Environment International*, **47**, 121–130

Paper II Fång J., Nyberg E., Bignert A. and Bergman Å. (2013) Temporal trends of polychlorinated dibenzo-p-dioxins and dibenzofurans and dioxin-like polychlorinated biphenyls in mothers' milk from Sweden, 1972–2011. *Environment International*, **60**, 224–231

Paper III Fång J., Nyberg E., Winnberg U., Bignert A. and Bergman Å. Spatial and temporal trends of POPs in mothers' milk – A global review 1995–2011 – including novel Swedish data.
(manuscript)

Paper IV Fång J. and Bergman Å. Non-destructive method for screening for novel persistent organic contaminants in mothers´ milk
(manuscript)

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Abbreviations

BDE-209	Brominated diphenyl ether #209
BMI	Body mass index
CB-118	Chlorinated biphenyl #118
CB-156	Chlorinated biphenyl #156
DCM	Dichloromethane
DDT	Dichlorodiphenyltrichloroethane
DL-PCB	Dioxin-like polychlorinated biphenyl
GC-ECD	Gas chromatograph – Electron capture detector
GC-MS	Gas chromatograph – Mass spectrometer
GPC	Gel permeation chromatography
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
LOD	Limit of detection
LOQ	Limit of quantification
MASE	Membrane assisted solvent extraction
MeO-PBDEs	Methoxylated polybrominated diphenyl ethers
NFA	National food administration
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
PCDD/F	Polychlorinated dibenzo-*p*-dioxins and/or dibenzofurans
POP	Persitent organic pollutant
POPUP	Persitent organic pollutant in Uppsala primiparae
PTFE	Polytetrafluoroethylene
QuEChERS	Quick, Easy, Cheap, Effective, Rugged, and Safe
SPE	Solid phase extraction
TEF	Toxic equivalent factor
TEQ	2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent
UNEP	United Nations Environment Programme
WHO	World Health Organization

1 Introduction

1.1 Defining POPs and persistency

The work comprised within this doctoral thesis concerns mothers´ milk and assessments of anthropogenic, persistent, halogenated, organic pollutants in mothers´ milk. In the scope of this thesis the term “persistent” is used in the same manner as the term is used by the Stockholm Convention on Persistent Organic Pollutants (POPs) (Stockholm Convention), i.e. substances that in general degrade slowly, are spread over the world by non-anthropogenic means (air deposition and subsequent re-mobilization from soil, sediments and water), accumulate in the fat rich tissues of biota, are enriched (bioaccumulated) along the food web (biomagnified) and are toxic both to wildlife and humans (Stockholm Convention). This does not mean that only the chemicals listed in the Stockholm Convention as POPs may be characterized and discussed as POPs. For clarification purposes the Stockholm Convention POPs, may thus be referred to as “legacy POPs”. Other, non-legacy POPs may not yet have been assessed in relation to all the criteria of POPs as defined by the Stockholm Convention. A few chemicals discussed herein are POPs but not yet legacy POPs.

It is notable that the use of “persistent” differs from “persistency” that is related to the abiotic inherent physicochemical and chemical reactivity properties of a chemical (Green & Bergman 2005). Their definition of persistency is:

“The persistence of a chemical is its longevity in the integrated background environment as estimated from its chemical and physicochemical properties with – in a defined model of the environment.”

1.2 Perspective on environmental contaminants

A second, more intensified, phase of the "chemical revolution" be considered to have started around the Second World War and really took off after the war ended. It is still important to remember that the initial phase leading up to this revolution started in the mid-19th century with intensive experimental undertakings in organic synthetic chemistry. As the chemistry industry grew,

from the 1940's, the standard of living improved for millions worldwide. New pharmaceuticals, pesticides, construction materials (polymers) as well as process chemicals for the production of, and additives to, materials and goods are examples of the development of chemicals. The chemicals promoted manufacturing of new items with sometimes spectacular properties which helped humans to undertake new and previously impossible tasks. As the chemistry industry grew, it became clear that in certain cases there were unforeseen downsides accompanying the advancements. Anthropogenic substances, such as mercury related compounds, organochlorine pesticides and technical products (PCBs) spread from their sources to the general environment and accumulated enough in wild life to cause adverse effects (Carson 1963, Cottam & Higgins 1946, Jensen 1966). The awareness of anthropogenic chemical risks, apart from occupational risks, starts during the 1960's when several chemical contamination problems developed in parallel, wildlife is affected and humans are intoxicated by environmental pollutants (Gee 2013).

In Sweden, the environmental effects of DDT and PCB exposure to top predators among mammals and birds, became evident at a rather early stage (Helander et al. 1982, Helle et al. 1976, Jensen et al. 1969). The effects were serious and posed threats to species survival, as was the case for seals species in the Baltic Sea (Olsson et al. 1992, and references given therein), and otters (Roos 2013, Roos et al. 2012) throughout the country, with the most pronounced problems among mammals in the Southern part of Sweden. Severe reproductive effects were reported from a number of experimental studies in which minks were subjected to food contaminated with PCBs (Kihlström et al. 1992, and references given therein).

Birds of prey, with Baltic Sea region habitats, were hit with effects leading to poor reproductive ability, almost leading extinction of several species, (Helander et al. 2002, Lundholm 1997, Nordlöf et al. 2012). As the pollution problem was realized, the scientific community started to investigate human exposure. Dr K. Norén was one of the very first researchers to address POPs in mothers´ milk (c.f. below).

These findings, together with other data, primarily from Europe and North America, led policy makers at UNEP to initiate, in 1995, work on a binding resolution that would subsequently restrict, decrease or ban the use of 12 POPs globally – sometimes referred to as "the dirty dozen". In 2001, the Stockholm Convention was amended and member states could sign the treaty (Stockholm Convention). The Stockholm Convention is dynamically growing and additions of POPs were made in 2009 and 2011 which means that the convention now encompasses 22 substances or classes of chemicals.

Stockholm mothers' milk sampling was initiated in 1967 for analysis of pollutants (Meironyté Guvenius 2002, Norén & Meironyté 2000, Norén & Westöö 1968, Norén 1987, Westöö & Norén 1978). WHO on their side, started working on POPs in mothers' milk and other food in 1976. WHO has also coordinated three international studies which focused on polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in 1987. This work resulted in a global monitoring plan for mothers´ milk (WHO 2009). The global monitoring plan has now been inherited by the Stockholm Convention and expanded to include chlorinated pesticides, e.g. the Stockholm Convention "dirty dozen" (UNEP 2009, 2011). The routes of exposure of POPs to the human population have been surveyed to better understand how to reduce the exposure of these chemicals.

The European population has been found to mainly be exposed to polybrominated diphenyl ethers (PBDEs) through the diet, and in particular from consumption of fatty fish (EFSA 2012). This is also true for the Swedish population which in numerous studies has been shown to be exposed to the traditional POPs, including brominated flame retardants (BFRs), through the diet (Darnerud et al. 2006, Sjödin et al. 2000, Törnkvist et al. 2011). There is cause for concern regarding infants´ exposure to POPs since many exhibit endocrine disrupting (EDC) properties, as presented by UNEP/WHO (UNEP & WHO 2013). Adverse effects include, but not limited to; endocrine related cancer, immune dysfunction and altered cognitive function (PCBs), changed thyroid hormone homeostasis (PBDEs) and increased cancer risk (PCDD/Fs) (Birnbaum 1994, IPCS 2002, UNEP & WHO 2013, Warner et al. 2011). For further information about the occurrence, exposure and adverse effects please see the introduction of **Paper III**.

1.3 Aims of thesis

The current thesis aim to problematize exposure analysis of mothers' milk, i.e. sampling, analytical methodology and reporting exposure levels to the nursing children. The goal was to also investigate changes over time regionally as well as to investigate the relationship of the mothers´ body burden and the transfer to the mothers´ milk.

For clarification, the term "mothers´ milk" was chosen among others, such as "breast milk", "human milk" or "mother's milk", and used exclusively within this thesis. This is in line with the recent report from UNEP and WHO on endocrine disruptors (UNEP & WHO 2013) which have chosen to use the term "mothers' milk".

2 Mothers' milk as a sample matrix

2.1 Milk composition

Mothers´ milk is a biological fluid which is designed to provide all of a child's nutritional needs and provide immunological benefits (Kramer & Kakuma 2012). This is the basis why the WHO recommends exclusive breastfeeding for 6 months, in both developed as well as countries with economies in transition. Mothers´ milk can roughly be classified to belong in one out of three groups, depending on time from post-partum; colostrum (first week), transitional milk (second week) or mature milk (after the second week (Lawrence & Lawrence 2011). However, Lönnerdal reports that the protein composition and concentration has not stabilized until approximately 30 days post-partum (Lönnerdal 2003). The main components of mothers´ milk, other than water, are fat, proteins and carbohydrates (39, 7–8 and 72 g/L mature milk, respectively) (Lawrence & Lawrence 2011). The fat content of the early days after delivery seems to be quite homogenous (2.5–3.5% fat). As the time goes by, a higher degree of heterogeneity becomes apparent (2–7% fat). An association between maternal weight gain during pregnancy seems to influence the fat content in the later, mature milk but not colostral or transitional milk (Michaelsen et al. 1994). Changes during the actual course of feeding has been reported by Mizuno et al with a higher fat content in the milk in the later part of the feeding (Mizuno et al. 2009). The composition of different fatty acids is dominated by oleic (18:1) and palmitic acids (16:0), 36% and 21%, respectively, of the total fatty acids (Lawrence & Lawrence 2011). The protein content of the milk consists mainly of caseins and whey proteins. The ratio of whey proteins to caseins vary mainly with the varying concentration of whey proteins, which is higher in the early milk, approximately by a factor of two when comparing colostrum and mature milk. The ratio of the two proteins groups approaches 1:1 over time but reaches the ratio 6:4 at roughly 30 days, after delivery, and only slowly change back towards 1:1 after this (Lönnerdal 2003). This could in theory be a reason as to why BDE-209 has been reported colostrum but not in mature milk from the same group of mothers (Jakobsson et al. 2012), if one hypothesizes an association of BDE-209 to proteins. The carbohydrate portion of milk is almost exclusively made up out of lactose (Lawrence & Lawrence 2011).

2.2 Monitoring of mothers´ milk

There are a number of reasons as to why mothers´ milk is an important matrix for monitoring chemical pollutants; first and foremost it gives an answer to the dietary exposure of POPs of the nursing child. The mothers´ milk is the main pathway for POP exposure for humans in the early life. By monitoring mothers´ milk the POPs body burden of the mothers, and by extrapolation, the general public can be assessed. Other benefits of sampling mothers' milk for analyses include that the sampling is non-invasive and can be performed by the mothers' in their own home. It is also favorable that the milk has a rather high lipid content which promotes identification and quantification of a rather high number of POPs.

The correlation between blood concentrations of POPs and in mothers' milk is overall rather good. For PCBs, HCHs, DDTs, HCB, *trans*-nonachlor and Toxaphenes the concentrations are slightly lower in blood compared to mothers´ milk, the exceptions being CB-118 and CB-156 of which approximately half of mothers´ milk concentration was determined in the maternal blood, on a fat adjusted basis (Anda et al. 2007). Further support for this observation is given by Darnerud *et al.* (Darnerud et al. 2010) as well as Muckle *et al.*, which also reports umbilical cord concentrations of POPs. The ratio between umbilical cord and mothers´ milk concentrations are lower than for the corresponding maternal blood to mothers´ milk concentrations basis (Muckle et al. 2001). In general, two thirds of the POP concentrations found in mothers´ milk can be determined in umbilical cord blood, on a fat adjusted basis. For a more detail view, please see the review by Mannetje *et al.*, which have summarized data on serum to milk concentrations of PCDD, PCDFs, PCBs, PBDEs and a few selected traditional chlorinated pesticides. Wittsiepe and co-workers report that among the investigated analytes, PCDDs, PCDFs and DL-PCBs, substituted with a higher number of chlorine atoms were more abundant in blood compared to milk. In contrast, the opposite was found for the lower chlorinated compounds for which higher concentrations were reported in the milk. The authors propose that there is discrimination towards heavier compounds, either as a result of biological and/or physico-chemical factors. Note that the study was performed on paired serum and milk samples, i.e. from the same donor (Wittsiepe et al. 2007). In **Paper I** we report similar findings for PBDEs. The benefit of having paired samples as of serum to both colostrum and mature milk, as in **Paper I**, revealed that the transfer differed between the two types of mothers' milk/sampling times. The effect that lower halogen substituted analytes were found in higher concentrations, and vice versa, in the mothers´ milk were more pronounced in the mature compared to the early milk (**Table 3, Paper I**).

In a study which analyzed mothers´ milk from four different cities in Sweden; Lund, Gothenburg, Uppsala and Lycksele it is clear that there is no difference for PBDEs and PCBs between the cities, even though Lund and Lycksele is located more than 1000 kilometers apart, on a NNE axis (Glynn et al. 2011). One conclusion that can be made from the work in this study is that the human background exposure of classical POPs is likely to be quite similar in all of Sweden. In particular for PCBs and PBDEs, since that was the investigated analyst in the study. However, one should always be cautious drawing any firm conclusion since there might be point sources of different kinds at different places effecting the concentrations. Geographical distribution and exposure levels of the legacy POPs are discussed in detail in **Paper III**. However, it may be mentioned that the concentrations of several of the POPs are rather similar, but of course dependent on any point source close by and/or recent exposure to the chemicals. Other factors influences the POP concentrations found in breast milk. Pre-pregnancy body mass index (BMI) and weight gain during pregnancy were identified as factors affecting the concentrations negatively, i.e. associated with lower concentrations of POPs. The mothers´ age and post-partum weight loss were on the other hand affected the POP concentrations positively, i.e. associated with higher POP concentrations, to a smaller degree this association was also observed with mothers whom themselves had been breast fed and consumption of fish from the Baltic Sea (Lignell 2013, Lignell et al. 2011).

3 Methodological considerations

In **Papers I** and **II** established methods were used for the chemical analysis, whereas a new method was developed for the work presented in **Paper IV**. The work presented in **Paper III** is different that required an entirely different approach, except for the novel Swedish data included that is based on previously applied methodology. In this chapter general methodological considerations are discussed in brief for **Papers I–III** and in more detail for **Paper IV**, which also includes abandoned methods for possible benefit in other studies.

3.1 Paper I

The approach to meet the main objective of **Paper I** was to use an established method for the chemical analysis of the analytes, applicable for three matrices, umbilical cord serum, maternal serum and mothers milk. This was achieved by using the method described by Hovander and coworkers (Hovander et al. 2000) for the serum samples and the modified Hovander method for the milk extraction. The aim was to use as identical methods as possible to reduce any possible discrepancy due to difference in methods. The two versions of the method differs only in the first extraction step where the original Hovander method, the serum version, uses hydrochloric acid (HCl, 6 mol/dm^3) and methyl-*tert*-buthyl ether (MTBE) as a modifier of hexane acceptor phase. The latter method, for analysis of milk, concentrated formic acid is used to avoid protein precipitation during clean up, and diethyl ether instead of MTBE. The Hovander methods are both robust and well-known at the department, and among scientists globally, which also contributed to the choice of their use.

3.2 Paper II

Since one of the main objectives was to assess whether or not a time series could be elongated, the main concerns with the methodology for **Paper II** was to ensure that the samples for the overlapping years were made up of the very same samples as used by Norén and co-workers analyzed previously

(Norén & Meironyté 2000). Banked samples could also be selected to have more data points in the previously analyzed time series 1972–1997, three samples were added; 1978, 1988/99 and 1995, as well as an extension of the time series, up to 2011. Double sample pools were used in half of the extended series in an effort to give a better idea of the actual background concentrations. A certified commercial lab (Eurofins, Hamburg, Germany) was used to analyze the samples since the specialist equipment (high resolution mass spectrometer) for dioxin analysis is not available at the department.

3.3 Paper III

The methodological approach for **Paper III,** which is a review paper, was to set up a set of demarcations on what to include and to exclude in the study. The method for the first crude collection of research papers was standardized so that more than one person could scan the literature. Furthermore, this was done to ensure that the search for all Stockholm Convention POPs was performed identically to avoid irregularities from differences in search engines and personal modus operandi. The collected articles were pooled in a database and duplicates removed. The aim was to give an insight to Stockholm convention POP concentrations worldwide, and not an accurate description of the historical development of the POPs since the beginning of environmental chemistry. This meant a temporal demarcation had to be defined, which was set to 1995. It can be argued that this year should be earlier or later, to include more studies or to better reflect the current situation, respectively. Furthermore some kind of quality control of the included data had to be defined. The minimum requirements were set down as follows; country of origin (samples), year (sampling), quantified concentrations from at least six individuals or from a pool consisting of at least six individuals. Time trend data was included only if the time series consisted of five or more data points and the concentration for each year was given. The latter requirement was imposed so that a statistical evaluation of all time series could be performed using the same parameters, thus ensuring greater comparability between the series.

3.4 Paper IV

In **Paper IV** a method is described and applied to large volume (200 mL) mothers' milk samples. The challenge for this particular study was to develop a method capable of handling large sample sizes without the use of destructive fat reducing agents such as sulfuric acid during the sample clean-up. The use of large sample size leads to a large quantity of fat in the sample,

200 mL mothers' milk at 4% fat gives 8 g of fat. Furthermore, the intent was to have as general method as possible, aimed towards lipophilic and neutral substances. The milk was spiked with HCHs, DDTs, PCBs, PBDEs, MeO-PBDEs and Dechlorane Plus's. The recovery for the substances is around 50–100%, with slightly better recoveries for the chlorinated analytes but with a lower standard deviation among the brominated analytes. The idea was that if the method is applicable for this range of substances, any unknown lipophilic, neutral halogenated compounds should be detectable if present.

3.4.1 Extraction and clean up method

The extraction method can be found in detail in **Paper IV**. However, in summary see **Figure 3.1** for a visual description of the method.

Clean up – pretreatment: The mothers´ milk sample (200 mL) is divided into eight subsamples each in a polypropylene centrifuge tube. The sub-samples are centrifuged at 3150 G for 5 min, at 8°C. The centrifugation allows the formation of a fat layer in the centrifuge tube. Subsequent refrigeration at 8°C for 2 hours hardens the formed fat layer. Once the fat layer is more or less solid a hole can be made to allow either decantation or removal by pipette of the water phase. The water phase has been investigated as to whether or not fat and analytes are retained (spiked with a mixture of chlorinated pesticides and PCBs). The amount of retained fat was equal 3% of the original sample and no analytes could be detected when the water phase was analyzed using the method (milk) described in **Paper I**.
The concept of pretreating the samples, i.e. to form the fat layer, comes from an abandoned extraction procedure *"Pre-treatment and top-layer extraction"* in which it was observed that the lipid con tents of a milk sample will form a fatty layer if exposed to mild heat (40°C) and cold (8°C). It was later found that cold centrifugation had been used in a study by Schlumpf *et al*. to isolate the fat from water phase in mothers´ milk samples. In the method, they first melt the lipid layer and subsequently ground with sodium sulfate before application on columns, from which the samples was extracted with organic solvents (Schlumpf et al. 2010).

Extraction: The fat layers were extracted by the addition of 20 mL acetonitrile, Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) extraction salt (4 g $MgSO_4$, 1g NaCl) and then vigorously agitated before centrifugation at 3150 G for 5 minutes. The extraction was repeated twice with 20 mL acetonitrile. The organic phases were pooled so that four subsamples were formed and solvent evaporated overnight. The extraction is developed from a method described by Restek (Misselwitz & Cochran 2012), discussed further in **3.4.2**, combined with an acetonitrile partition,

(Jensen et al. 1992, Jensen & Jansson 1976, Norström 2006). The acetonitrile partition works by agitation of lipids with dissolved analytes, in the presence of an abundance of acetonitrile, 1:9. The analytes will in varying degree be dissolved in the acetonitrile whereas the fat only to a smaller degree will be dissolved in the acetonitrile.

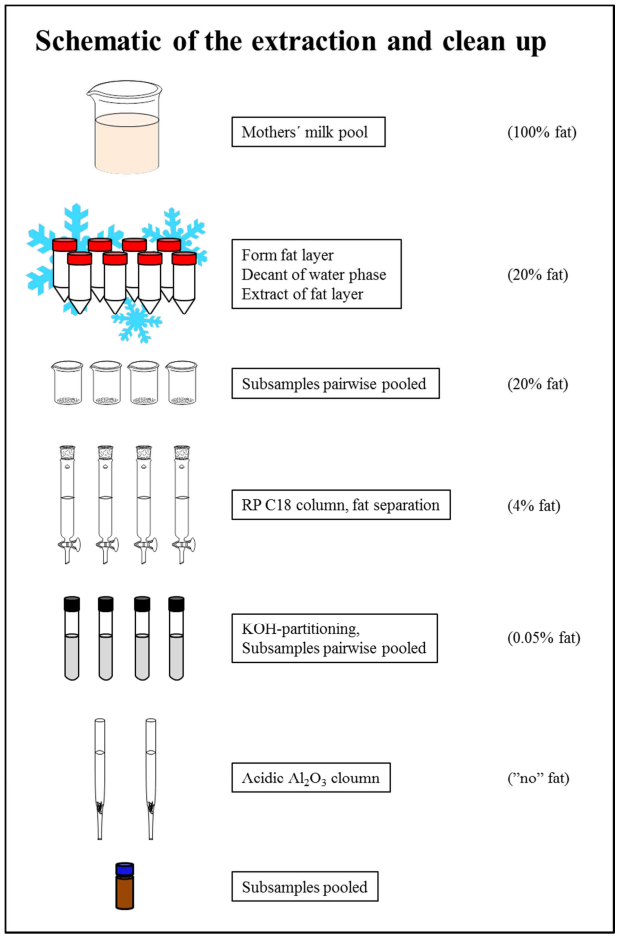

Figure 3.1 A schematic of the extraction and clean up described in **Paper IV.** The amount of fat still left in the samples after the corresponding treatment step is given within parenthesis, to the right.

C18 column: The sample was transferred using n-pentane to an open glass column containing C18-derivatized silica gel, with silica on top, 22 and 10 g, respectively. The pentane was evaporated from the column before eluting with 150 mL acetonitrile. The solvent was subsequently evaporated overnight.

Initially the samples were transferred using iso-hexane but it was noted that if the transfer solvent was present when elution of the column was initiated, the fats would break through and not retained on the column as intended. Thus the iso-hexane was replaced with the more volatile pentane, which was easier to evaporate before elution with acetonitrile was initiated.

The columns can be reused after a cleaning sequence consisting of 50 mL acetonitrile, 50 mL iso-propanol, 50 mL iso-hexane, 50 mL iso-propanol and 50 mL acetonitrile to recondition the columns. The columns are kept "wet" between runs to avoid formation of air pockets and to keep the columns conditioned.

Potassium hydroxide partitioning: Milk contains a substantial amount of fat, especially if compared to serum, so it is reasonable to expect at least some free fatty acids. Free fatty acids in the samples are readily ionizable using a base as a proton acceptor and can thus be separated from the sample. The potassium hydroxide partitioning was performed as described by Hovander *et al.*(Hovander et al. 2000), i.e. using 0.5 mol/dm^3 of potassium hydroxide dissolved in an ethanol/water mixture (1:1) as the acceptor of the deprotonated substances.

This proved to be a successful step, managing to take care of approximately 85% of the remaining fat in the samples. The amount of fatty acids, and other unknown deprotonated compounds, was enough to change the water/ethanol phase from colourless to yellow, indicating that sample undertook some profound change during the potassium hydroxide partitioning clean up step.

Acidic aluminum oxide column: The final clean up step proved to be particularly challenging. With almost all fat removed one might assume that it should be easy to get rid of the remaining fat residue. However, it must be appreciated that the final residue of fats comprises of compounds with properties highly similar to the analytes. If that was not the case, they would have been excluded from the samples during the previous clean up steps.

Three different types of aluminum oxide are commercially available; basic, neutral and acidic. Since the previous clean up step utilized a base, it seemed logical to use the acid aluminum oxide in the next step. Hence, the samples

the were pooled to form two subsamples before application on a 1.5 g aluminum oxide, topped with 0.05 g magnesium sulfate, columns packed in glass Pasteur pipettes. The columns were first conditioned and subsequently eluted, after sample application, with 3.5 mL of a mixture of acetonitrile and dichloromethane (DCM, 1:1000).

When this clean up step first was attempted non-modified DCM was used. However, poor recovery of MeO-PBDEs was observed. Some of the congeners eluted poorly (6-MeO-BDE47 and 6-MeO-BDE137), and some others appeared to be retained to the column indefinitely (6-MeO-BDE85, 6-MeO-BDE90, 6-MeO-BDE99 and 4-MeO-BDE121). The DCM was first modified with 10% methanol, which unfortunately leads to matrix effects in the GC-ECD analysis, and observed in the chromatograms. The methanol was replaced by acetonitrile, which seemed better suited as an aprotic solvent in the event of any acidic residues. In **Figure 3.2 a–d)** it is shown how the analytes (MeO-PBDEs) and the matrix were separated in different fractions by using lower amount of acetonitrile to modify the mobile phase (DCM), from 10% to 1‰.

To determine if the observed matrix effects, i.e. the negative peaks in the chromatogram shown in **Figure 3.2,** were due to fatty acids in the sample, a sample containing the negative peaks was subjected to triborofluoride (1 mL, 1.3 mol/dm^3) treatment dissolved in methanol, for 15 min at room temperature. The treatment should convert any fatty acids to the corresponding methyl esther and thereby removing or at least displacing the negative peaks in the chromatogram. The negative peaks remained unchanged however. Thereby the conclusion that the peaks are not related to fatty acids, but something else of hitherto unknown structure.

Analysis: After the acidic aluminum oxide column the samples were pooled prior to spiking with injection standards and analysis by GC-ECD and GC-MS.

3.4.2 Lessons learned from other methods tested

Hovander extraction: The Hovander method (Hovander et al. 2000) used in **Paper I** was up-scaled in a first attempt to get a good extraction method, for neutral and lipophilic organic compounds. However, simple direct up-scaling did not work. The extraction required more solvents on a relative basis and left fat un-extracted. The problems were mainly related to the formation of an emulsion layer in the separator funnel, which persisted over days, even after several re-extractions.

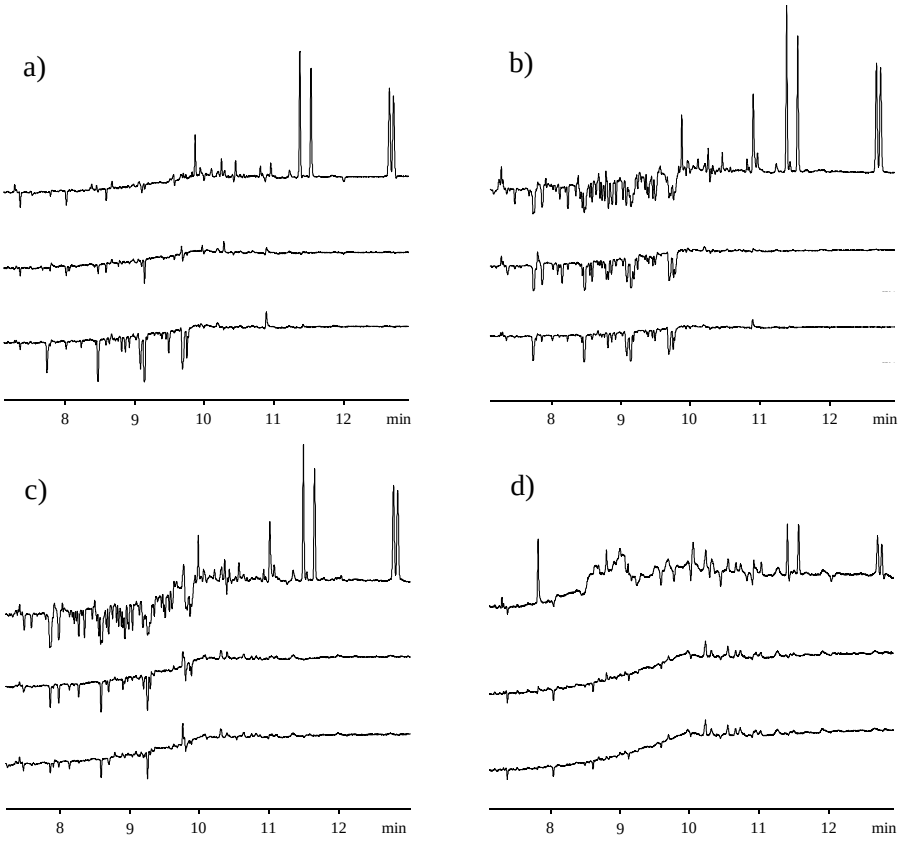

Figure 3.2 GC-ECD chromatograms of fractions from a 1.5 g acidic aluminum oxide column with DCM:acetonitrile as the mobile phase; **a)** (1000:1), **b)** (1000:6), **c)** (98:2) and **d)** (9:1). Each chromatogram represents a fraction of 3 mL, except for in **d)** where the fraction size was 5 mL. The first fraction is the top-most chromatogram in each figure, the second and third are the middle and bottom chromatogram respectively.

Membrane assisted solvent extraction (MASE): The principle of MASE can be explained by that analytes diffuse through a membrane, which the matrix cannot cross, to an acceptor phase in which analytes are more readily soluble than in the donor phase as illustrated in **Figure 3.3** (Hauser et al. 2004, Schellin & Popp 2003).

Attempts were made to create a large MASE cell using 250 mL glass bottles and a polypropylene membrane to be used in the clean-up of fat rich samples (Wojtalewicz et al. 2006). Though several ratios of solvents and sample were tried, the general approach was that one part of sample was mixed with one

part comprised of formic acid/water/2-propanol in a 250 mL glass bottle. A tube of polypropylene filled with an organic solvent (hexane, DCM or mixture) was inserted to the sample and the sample was stirred using a magnetic stirrer for various defined times (1, 2, 4 hours), before the solvent was collected and replaced. The collected solvent showed to contain low amounts of clear and colourless fat. The fat proved very difficult to get rid of, almost completely withstanding; regular silica column, sulfuric acid impregnated silica column, concentrated sulfuric acid treatments, potassium hydroxide partitioning, activated phosphoric acid column, Florisil column , potassium hydroxide activated silica column and 30% hydrogen peroxide at 70°C (1 hour).

A less destructive approach was tried to get rid of the peculiar fats and fractioning commenced. Straight alkanes; n-pentane, n-hexane and n-heptane was tried in combination with newly activated silica gel (1 g) was used to pack Pasteur pipette columns, spiked with a mixture of chlorinated pesticides, PCBs and PBDEs, as a fractionation test. The results are shown in **Table 3.1**, from which it is clear that n-heptane could be used to remove the fats from the analytes, discarding a pre-fraction of n-heptane (3 mL). After GC-ECD analysis it became apparent that the recovery was at around 20% and 40% for PCBs and PBDEs, respectively. The recovery was deemed too low and the method abandoned.

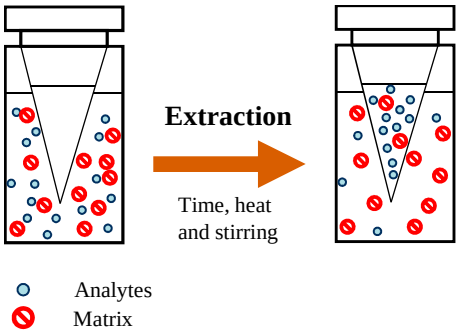

Figure 3.3 Schematic of MASE, in which the sample is extracted by immersion of an acceptor phase, organic solvent, contained within a polypropylene membrane. After time, heating and stirring most of the analytes have diffused though the membrane to the organic acceptor phase whereas unwanted matrix retained in the sample, donor phase.

Pre-treatment and top-layer extraction: When non-homogenized milk is left in room temperature a ”cream layer” is formed after some time. This layer comprises of most of the fats in the milk. As mentioned above, the rate of formation of this layer can be accelerated by raising the temperature and cooling of the milk, repeatedly. A triplicate of mothers´ milk samples (5 g), were mixed with formic acid (1 mL), 2-propanol (2 mL), iso-hexane (1.5 mL) and diethyl ether (1.5 mL). The samples were heated on a water bath at

40°C for 1 hour and subsequently cooled with water, before refrigeration for 1 hour. This was repeated twice after which the samples where left overnight in a refrigerator. A slightly yellow tinted organic phase was collected the following morning. The organic solvent was replaced with of iso-hexane (3 mL) and the heating and cooling process repeated as previously described. After another night or refrigeration the organic solvent fractions were pooled.

Table 3.1 The results from a fractionation test on activated silica column (1 g) packed in a Pasteur pipette, using milk fat spiked with a mixture of chlorinated pesticides, PCBs and PBDEs. Note that in the third fraction for both n-pentane and n-hexane hexachlorobenzene (HCB) is the only analyte, circled in the table. The PBDEs eluted with the DCM fraction.

Fraction	n-pentane	n-hexane	n-heptane
1 mL			
2 mL			
3 mL			
4 mL			
5 mL			
8 mL DCM			

Analytes

Matrix

The extracted amount of fat was compared to samples, created from the same pooled mothers´ milk sample but extracted using the Hovander method (Hovander et al. 2000). A good agreement of isolated fat was observed independent of method, as shown by gravimetric fat determination, 2.6–2.9% and 2.5–2.8%, respectively, for the Hovander and “top-layer” methods. No recovery study was performed. The motif for this was that the method was considered too time consuming for the purpose, that is extracting a few, large mothers´ milk samples, and abandoned. That said, all fat was extracted. If one is interested in extracting a large number of mothers´ milk samples with respect to lipophilic organic compounds, this method might be a way to make the extraction less labor intense.

Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS): The method consists of liquid–liquid extraction of a sample diluted with water and an acceptor phase consisting of organic solvents. The extraction is carried out in polypropylene test tubes in the presence of “extraction salts” and phase

separation improved by centrifugation. The sample is purified on one or more SPE columns dependent of sample type. The mothers´ milk method described by Misselwitz and Cochran (Misselwitz & Cochran 2012) uses QuEChERS extraction salt, acetone and n-hexane (1:1) as the acceptor phase and 50 mL polytetrafluoroethylene (PTFE) centrifuge tubes. The original method dilutes the samples 1:1 with water and uses the same ratio between the donor and the acceptor phases. Three changes were made to the method during the present project; 1) The sample was not diluted with water to enable a larger sample volume per 50 mL tube, 2) the ratio of sample to organic phase was changed from 1:1 to 25:20, and lastly, 3) polypropylene centrifuge tubes were used instead of PTFE tubes. The two first changes were made to enable a larger sample size per tube and to still enable a mixing of the samples, which is difficult to pursue if the test tube is totally full, the last change made is due to cost reduction – polypropylene tubes are cheaper compared to PTFE tubes. The changes made gave rise to a specific problem. After agitation and centrifugation the sample consisted of a five layered system, excluding the magnesium sulfate precipitation, two in the water phase and three in the organic phase. Two lipid plugs had formed, one on the boundary between the water and the organic phase and one between the two different organic phases (each corresponding to the added amount of iso-hexane and acetone). Various solvent compositions were tested, including iso-propanol, acetonitrile, diethyl ether and formic acid. In the end, the problem was the sample volume per test tube was not big enough. However, if one tries to apply the whole sample, and not just an aliquot as stated in the method, the SPE cartridges (secondary-primary amine and silica columns) cannot cope with the amount of fat and there will be breakthrough and consequently poor recovery.

Gravity assisted, gel permeation chromatography (GPC): Some work using open, gravity assisted gel permeation chromatography columns were undertaken, to clean up milk fat. The separation is mainly due to size exclusion, which allows larger compounds to migrate through the column without entering the pores in the stationary phase. Smaller compounds enter the pores of the stationary phase beads and are thus retained to a higher degree. Thereby separation based on size can be achieved. The stationary phase consists of beads made up of a lattice of polymerized styrene and divinylbenzene as a cross linker. The formed beads are porous and the pores´ size is determined by the amount of cross linker and which solvent the beads are exposed to. The method has been used to separate lipids from halogenated organic pollutants, albeit assisted by a mechanical pump as reported by for instance Liem and co-workers (Liem et al. 1992).

Initially, open GPC was thought to be the first and primary clean up technique, to produce a crude sample which subsequently could be refined

by further clean up steps. The open GPCs were packed in glass funnels with a glass frit after swelling in a mixture of DCM and iso-hexane (1:1) overnight. Milk fat spiked with PCBs and PBDEs was applied on GPC columns packed with 30g of Biobeads S-X2, S-X3, S-X8 or S-X12, where a different amount of cross linker was used in the production of the gels. The columns were eluted with DCM/iso-hexane (1:1) and after fractionation tests it was found that S-X3 gave the best separation between the lipids and the analytes. A pre-fraction of 50 mL containing lipids could be discarded and the PCBs and PBDEs collected after an additional 80 mL mobile phase. As suspected, the GPC alone could not produce a clean enough sample. However, in tandem with the C18-column described in **3.1**., the GPC proved promising. Albeit, after changing the extraction technique and the addition of the potassium hydroxide partition step, the GPC was redundant. The GPC was also applied to try to remove the negative peaks in **Figure 3.2**, without success.

4 Dioxins time trend comparisons

This chapter contains comparisons between time series of dioxin levels in mothers´ milk as presented in **Paper II**, with other time trend series covering dioxins in mothers´ milk and in addition with dioxins in other matrices. “Dioxins” are, when used herein, referring to PCDDs, PCDFs and DL-PCBs when applicable, but specified as group of chemicals or as individual congeners when detailed data are discussed.

4.1 Mothers´ milk time series

In **Paper II** comparison of the time trend studies by Norén and co-workers (Norén & Meironyté 2000) and the new results from **Paper II** are performed. It is important to note that the majority of the samples in the two studies are from the same, banked, pools that makes the comparison extra valuable. The results from the comparisons of the overlapping samples show a good correlation of the sample concentrations, thereby leading to similar trends for the sums of PCDDs, PCDFs and DL-PCBs for the overlapping time period. However, the two whole time trends differ in rate of decline, see **Table 4.1**, with a faster decline for the 1972–1997 trend compared to the trend calculated for 1972–2011. A direct comparison is hard to make, since the time series compares different time periods and the annual change differs in the earlier part compared to the later part of the longer 1972–2011 series.

The National Food Agency has since 1996 sampled and analyzed mothers´ milk for POPs within the study Persistent Organic Pollutants in Uppsala Primiparas (POPUP). The most recent results show decreasing trends for all types of dioxins during the time period of 1996–2010 (Lignell et al. 2012) of a similar magnitude as reported in **Paper II**. A review was compiled by LaKind in which it is reported that the dioxin concentrations, on a TEQ basis, are decreasing on a global scale. In a clever way, data from all over the world was put together to create a global time trend (LaKind 2007) that is in the same range as presented in **Paper II**. In an older review from Alcock and Jones (Alcock & Jones 1996) trend data are summarized showing decreasing concentrations over time – but is perhaps more interesting from an historical point of view today.

From **Table 4.1** it seems that the Swedish dioxin ∑TEQ time series decrease faster than for the rest of the world, with the Global and Arkhangelsk trends decreasing at a similar rate. However, the two trends countries span over a short time period, 1999–2002 and 1996–2000, respectively. Hence a trend could be the result of a limited trend or flux. For instance a German study reported an increase of the annual dioxin concentration, 1998–1999, suspected to be due to dioxin contaminated citrus pulp used as cattle feed (Fig. 1, in Fürst 2006, (Fürst 2006). Most of the studies in **Table 4.1** have samples 10 to 15 years apart, but several only have two time points. Most studies seem to report an annual concentration decrease of around 4% or less. A Japanese study report a status quo for the sampling period, 2001–2004 (Kunisue et al. 2006) and from Russia there is a report of a small increase of ∑PCDF, ∑PCDF and ∑TEQ, with 2%, and a more substantial increase of mono-*ortho*-PCBs, 4%. No explanation or theory is given by the authors for this deviating trend (Polder et al. 2008). The two studies from Japan suggest a slower decrease of dioxins in mothers´ milk, possibly due to a high fish intake, lingering effects from Yusho accident.

Table 4.1. Relative annual change of dioxin concentrations over time in mothers´ milk worldwide on WHO TEQ_{2005} pg/g fat basis. Numbers in *italics* show a calculated annual change when a change in concentration was given for a whole time period rather than an annual average over the period. ∑non-ortho and ∑mono-ortho refers to non- and mono-*ortho*-PCBs, respectively.

			Annual change (%)							
Country	Locale	Period	∑PCDD	∑PCDF	∑PCDD/F	∑non-ortho	∑mono-ortho	∑DL-PCB	∑TEQ	Reference
Sweden	Stockholm	1972–2011	-6,1	-6,1				-6,9	-6,5	**Paper II**
Sweden	Stockholm	2002–2011	-10	-7,3				-12	-10	**Paper II**
Sweden	Stockholm	1972–1997	-15	-11					-15	(Norén & Meironyté 2000)
Sweden	Uppsala	1996–2010	-8.2	-5.4		-7.4	-6.8			(Lignell et al. 2012)
Globally		1999–2002							*-8,9*	(LaKind 2007)
Russia	Arkhangelsk	1996–2000	*-13*	*-12*		*-14*	*-11*		*-12*	(Polder et al. 2008)
Spain	Tarragona County	1998–2012			*-4,7*			*-4,9*	*-5*	Schumacher et al 2013
Russia	Murmansk	1993–2000	*-5,6*	*-5*		*-6,3*	*-3,5*		*-4,9*	(Polder et al. 2008)
Germany	North Rhine-Westphalia	1989–2003[b]			*-4,7*					(Fürst 2006)
Belgium		2006–2009						*-3,9*	*-4,6*	(Croes et al. 2012)
Australia		1993–2002/03[a]			*-4,3*			*-3,9*	*-4,2*	(Harden et al. 2007)
Russia	Irkutsk	1988/89–2002/03[a]							*-4,1*	(Mamontova et al. 2005)
France		1998–2007			*-3,9*					(Focant et al. 2013)
Russia	Kachug	1988/89–2001/2002[a]							*-2,6*	(Mamontova et al. 2005)
Japan	Osaka	1973–1996[a]	*-1,4*	*-1,7*	*-1,5*			*-3,1*	*-2,4*	(Hori et al. 1999)
Japan	Fukoka	2001–2004[a]	*-0,56*	*-0,2*		*-0,4*	*-0,32*		*-1,4*	(Kunisue et al. 2006)
Russia	Kargopol	1996–2002	*0,71*	*2,1*		*-0,71*	*4,6*		*2*	(Polder et al. 2008)

[a] $WHO\text{-}TEQ_{1998}$ (Van den Berg et al. 1998)

[b] I-TEQ (Kutz et al. 1990)

4.2 Dioxin time series in aquatic matrices from Sweden

The decline of dioxins concentrations in Swedish mothers´ milk are discussed in **4.1** and **Paper II**. When the declining trend for dioxins in mothers´ milk is compared with other biological matrices from Sweden, it is not clear that the concentrations of dioxins in human milk mirror the concentrations in the general environment. Comparisons between the time trends in **Paper II** and reported time trends from Swedish wildlife with similar time spans of sampling are summarized in **Table 4.2**. In general, the concentration of dioxins in Sweden show decreasing trends during the last 40 years of monitoring. However, whereas the mothers´ milk concentrations decline faster during the last decade of the time series this is at best partially true for some of the wildlife time series. In herring (*Clupea harengus)*, the concentrations decline faster the last ten years compared to the whole time trend for two out of four sampling sites (Harufjärden and Fladen). The two remaining sites (Ängskärsklubb and Utlängan) show a steeper decline for ∑PCDD the last ten years but no significant trend for any other group, even though there is a trend for the whole time series.

For the fresh water species, perch *(Perca fluviatilis)* and pike *(Esox lucius)*, the picture is less clear. In general there is a decreasing concentration trend for most groups of analytes for the whole time series. The exception is for ∑PCDD/F for which no statistical significant trend is reported (**Table 4.2**). The concentration trends for the last ten years in the time series are not significant for any group of analytes. Still, it is of interest that the reported, though not statistical significant, trend for ∑PCDD/F and ∑DL-PCBs in perch from Lake Skärgölen is an increasing trend at 8% and 6% per year, respectively. Increasing concentrations, though not statistical significant, are also reported for 2,3,7,8-TCDD in Pike from Lake Bolmen at 1% per year the last ten years. In Lake Skärgölen there are strong evidence for a changing trend of ∑PCDD/F and ∑DL-PCBs concentrations (see **Figure 4.2 a–b)**). Apart from the positive trends the last ten years, there are also a non-linear statistical significant trend which shows an increase of both ∑PCDD/F and ∑DL-PCBs concentrations during the latter part of the past decade. In the case of Lake Bolmen, when one considers the shape of the slope (see **Figure 4.2 c)**) it is possible that there is a trend change, even though it is unlikely.

Guillemot (*Uria aalge*) egg concentrations show the most similar dioxin concentration trend compared to the mothers´ milk, decreasing over the whole time series, and a faster decrease over the last ten years of the series, at least for the ∑PCDD/F.

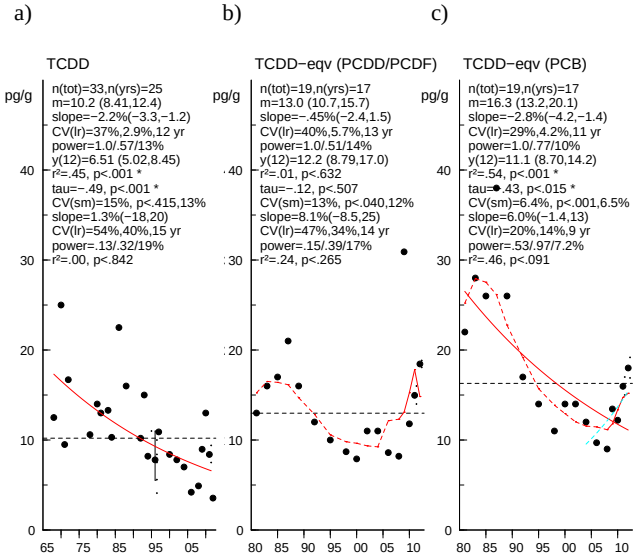

Figure 4.2 Figure **a)** shows the time trend of the concentration of 2,3,7,8-TCDD in pike from Lake Bolmen, **b)** ∑PCDD/Fs and **c)** ∑DL-PCBs in perch from Lake Skärgölen. Figure from "The National Swedish Contaminant Monitoring Programme for Freshwater Biota, 2013", Figure 29.4 and 29.7 (Nyberg et al. 2013).

As to why the concentrations of dioxins in mothers´ milk decrease in a more similar way to the marine biota, in general, rather than fresh water fish is not possible to explain. Possibly, smaller limnic systems represent a smaller local area, whereas the marine biota is part of a larger system, less sensitive to local conditions and events. This may be closer to the human situation. Support for this speculation may be taken from the fact that food stuffs are transported from around the world and that our background exposure is influence to a lower degree by local conditions. It is interesting to note that in a recent study on sediment cores from the Baltic Sea, the sediment concentration of PCDD/Fs peaks 1985–1993 in the off-shore sampling sites, one out of six peaks later, in 2003. In the coastal sediment cores from the same study it was found that the peak was earlier, average at 1975 (Assefa et al. 2014). The trends from the Baltic sediment cores doesn't fit with the trends observed in **Paper II**, but maybe the sediment trends could be indication of a general decrease in dioxin concentrations in the environment. This may be reflected in in the faster rate decrease for the last ten years of the time series as presented in **Paper II**.

Table 4.2. Relative annual change of dioxin concentrations in selected Swedish biota on WHO TEQ_{2005} pg/g fat basis. The upper half of the table is for the whole time series and the lower half is for the last ten years of the time series. Numbers in *italics* show annual change for 2,3,7,8-TCDD and -TCDF, respectively, in lieu of ∑PCDD and ∑PCDF. Blank fields are when nothing was reported and "---" when no trend was found.

			Annual change (%)					
Matrix	Locale	Period	∑PCDD	∑PCDF	∑PCDD/F	∑DL-PCB	∑TEQ	Reference
Mothers' milk	Stockholm	1972–2011	-6.1	-6.1		-6.9	-6.5	**Paper II**
Guillemot egg	Stora Karlsö	1968–2012	*-4.9*	*-1.1*	-2.7			(Bignert et al. 2014)
Herring muscle	Harufjärden	1990–2009	---	---	---	-8.5	-9.5	(Miller et al. 2013)
Herring muscle	Ängskärsklubb	1979–2009	-8.5	-4.8	-6.5	-5.4	-6.1	(Miller et al. 2013)
Herring muscle	Utlängan	1988–2009	-2.6	---	-2.1	-2.1	---	(Miller et al. 2013)
Herring muscle	Fladen	1990–2009	---	---	-2.4	-14	-12	(Miller et al. 2013)
Perch muscle	Lake Skärgölen	1967–2012[a]	*-2.1*	*-1.4*	-0.45[c]	-2.8		(Nyberg et al. 2013)
Pike muscle	Lake Bolmen	1967–2012[b]	*-2.2*	*-1.5*	-2.6[c]	-4.4		(Nyberg et al. 2013)
Pike muscle	Lake Storvindeln	1967–2012[a]	*-4.2*[c]	*-0.27*[c]	-3.4[c,d]	-6.3		(Nyberg et al. 2013)
Mothers' milk	Stockholm	2002–2011	-10	-7.3		-12	-10	**Paper II**
Guillemot egg	Stora Karlsö	2003–2012	*-4.0*	*-0.24*[c]	-4.5			(Bignert et al. 2014)
Herring muscle	Harufjärden	2000–2009	-15	-10	---	-8.3	-9.5	(Miller et al. 2013)
Herring muscle	Ängskärsklubb	2000–2009	-13	---	---	---	---	(Miller et al. 2013)
Herring muscle	Utlängan	2000–2009	-6.3	---	---	---	---	(Miller et al. 2013)
Herring muscle	Fladen	2000–2009	-14	-7.8	-10	-14	-12	(Miller et al. 2013)
Perch muscle	Lake Skärgölen	2003–2012	*-4.4*[c]	*2.4*[c]	8.1[c]	6.0[c,d]		(Nyberg et al. 2013)
Pike muscle	Lake Bolmen	2003–2012	*1.3*	*-3.5*[c]	-0.31[c]	-3.9[c]		(Nyberg et al. 2013)
Pike muscle	Lake Storvindeln	2003–2012	*-26*[c]	*-7.4*[c]	-6.4[c]	-7.3[c]		(Nyberg et al. 2013)

[a] 1980-2012 for ∑PCDD/F and 1992-2012 for ∑DL-PCB
[b] 1994-2012 for ∑PCDD/F and ∑DL-PCB
[c] $p > 0,05$
[d] $p < 0,10$

5 Discussion and conclusions

To ensure data compatibility between background exposure assessment studies, the studies must follow coordinated criteria regarding sampling (c.f. **5.1** below) and for reporting results (c.f. **5.2**). The scientific community needs to harmonize studies to enable other scientist to use results more efficient, and hopefully to see the bigger picture. For instance, **Paper I** investigates the transfer of PBDEs from maternal blood to mothers´ milk to improve the understanding of the distribution of POPs between the two matrices. The study is somewhat limited in size, which can lead to statistical insignificance. A larger sample size is could have, at least in theory, allowed more statistically significant connections to be drawn, since larger samples sizes are required for detection of a smaller trend. Lacking a larger sample size, if all studies investigating PBDE transfer from maternal blood to mothers´ milk followed a harmonized sampling and reporting practice then secondary data could be used for statistical analysis, i.e. effectively creating a larger sample size by inclusion of results from previous studies. This could lead to a situation in which a small pilot study shows interesting results and other research groups can perform additional studies of the same type and pool the new data with pilot study. Thus generating enough support to draw more and accurate conclusions from a more comprehensive data set.

5.1 Sampling mothers´ milk

The sampling of mothers´ milk has some critical parameters which have to be considered when designing a study. UNEP have put together a list of criteria for the sampling of mothers´ milk to try harmonize the sampling to ensure a better compatibility when concentrations are compared within the global monitoring plan (UNEP 2012), given below:

- *Mother should be primiparae.*
- *Mother should be under 30 years of age (The National Coordinator might consult national health statistics for possible advice on setting the maximum age to assure a sufficient number of potential donors. In order to further reduce variability, an age range might be considered a useful criterion).*

- *Both mother and child should be apparently healthy, including normal pregnancy.*

- *Mother should be breastfeeding one child only (i.e., no twins).*

- *Mother should have resided in the represented area (country) for at least the previous ten years.*

- *Mother should not reside in local areas where emissions of POPs are known or suspected to result in elevated concentrations of POPs in the local population.*

- *Mothers should be available for sample collection within 3 to 8 weeks of delivery.*

It has been shown that a depuration of POPs from the mother occurs during lactation, the inclusion of only primiparae strives to reduce variability due to depuration from any previous pregnancy (Harris et al. 2001). The mothers´ age is also of great importance since POPs bioaccumulate and older mothers have in general a higher body burden of POPs compared to younger mothers. A lower age limit could possibly be useful to ensure a more homogenous sample group. Consideration should in such a case be taken to differences in number of years required to come of age in different countries. The exclusion of non-normal pregnancies is in part due to avoid confounding results, possibly caused by elevated POP exposure, but also from an ethical point of view. Multiple births are to be excluded to reduce variability due to a suspected higher rate depuration of the mother, which in turn affects the POP concentration in mothers´ milk. A mother should have resided in the area of interest to reflect the background contamination of the studied area. A newly immigrated person's body burden is likely to be more like the place of emigration rather than the current place of residence and a poor representative in a background exposure assessment study Likewise, a background exposure assessment cannot be performed if the donor is known or suspected to be exposed to elevated concentrations of POPs compared to other inhabitants from the same area. The sampling time post-partum of 3–8 weeks seems reasonable at first, possibly too close to the change from transitional milk to mature milk as discussed in chapter **2.1**. Even more so since Lönnerdal reports stabilization of milk protein ratios and concentrations at 30 days post-partum. I would argue that a more narrow sampling time, in weeks after delivery, 4–8 or even 5–8 would allow stabilization of milk composition and at least in theory, more uniform conditions.

The UNEP sampling instructions also includes a questionnaire which in information about for example the mothers eating and smoking habits can also be found in the same document. Some of this information might not be relevant for the study directly, but can be of importance for another research performing secondary analysis of the findings.

5.2 Reporting results

When reporting results from a study it is of outmost importance to report diligently, as much relevant data as possible. This is shown, and discussed in some detail in **Paper III**. Unfortunately the data commonly reported lack a minimum of information, apart from actual concentrations:

- Sampling year
- Sampling locale (country, city)
- Parity of the mother
- Number of samples in a mean/median value
- Number of samples in a pool
- Concentration basis (g/g fresh weight, fat)
- Individual sample values
- Conversion factors (e.g. WHO-TEF_{1998})
- Define sums
- Limit of quantification (LOQ) and detection (LOD)

Proper reporting helps other authors in process to undertake literature studies but in particular it is a requirement for risk assessments. With such information in place it gives the reader an insight as to how the study was designed and conducted. The importance of sampling year and place should obvious, and in fact these data are rarely missing. The parity of the mother should be stated, or number of primiparae if pooled samples are analyzed, due to the risk of depuration during pervious pregnancy/ies (Harris et al. 2001). Specification of the number of samples for analysis and included in

any calculated averages or the number of individual samples in the pool gives the reader information if this is a representative background sample, or if it is based on three individuals, and not as representative of a population.

The reported analyte concentrations should be given so that it is clear as to which concentration basis is reported, but it is also very important to report in such a manner that a reader can find, or recalculate, the results to another concentration basis. For instance, when reporting concentrations as g/g fat, it is easy to also report the lipid content in percent. This enables a reader to find correlations other than those reported in the study. When reporting mean or median values, please report individual values as well. This can enable statistical calculations not possible if only mean/median values are presented. When dealing with conversion factors such as the WHO-TEF (Van den Berg et al. 1998, Van den Berg et al. 2006) it is of importance to report at the very least mean/median values for each analyte. If the sum of analytes, i.e. ∑PCDD WHO-TEQ_{1998}, is reported and the individual sample concentrations, per analyte, or the mean/median value for each analyte is omitted, it is not possible to do correct comparisons with studies presenting such data. In other words, concentrations should be reported in such a manner that new conversion factors can be applied on reported results. A concentration that e.g. looks like "15 pg/g fat ∑WHO-TEQ_{1998}" has to be avoided. In this case an accurate translation to; the current WHO-TEQ_{2005}, back-compatibility to older conversion factors such as I-TEQ (Kutz et al. 1990), and likely future WHO-TEQ_{20XX} will be impossible. When a sum of analytes is reported, the analytes in the sum must be stated. A ∑PCBs for instance can differ significantly in the number of analytes. Because of a failure to this the need for the following instruction on data reporting from UNEP (UNEP 2012) has become reality:

"PCDD, PCDF and dioxin-like PCB are reported on the basis of toxic equivalents (TEQ) using the WHO toxic equivalency factors (TEF) as established by a WHO Expert Group in 1997 [21]. Only for comparison purposes, the TEQ levels can be reported which are based on use of the TEF factors as re-evaluated by a WHO Expert Group in 2005 [22]. As results of samples analyzed before 2005 were calculated on basis of the 1997 TEFs, use of these TEFs allows following time trends and therefore is the preferred reference in the data basis."

In other words this has lead us to a situation where newer data, based on refined toxicological methods and studies which have led to the new WHO-TEF_{2005} are disregarded in favor for older TEF values. The reasoning behind the quote is understandable but not sound, it makes a better comparison if you employ the same conversion factors, i.e. the same TEFs but if weight based data were reported initially, and in the future, recalculation can be

done. It is a shame that problem has occurred, a situation which easily could have been avoided by better reporting by the scientific community in the first place. If space to include the data in the actual journal aticle is difficult, due to imposed page restrictions, please use function of Supplemental Information provided by most, if not all, journals.

The question of reporting LOQ/LOD is of importance as well, since this is the measurement on how low concentrations could be detected in a given study, which is important when comparing concentrations from different studies. It is important to state how a value below LOQ or LOD in regards to calculating averages and statistical analyses is handled. There are different approaches to this problem, substitution to a given value is common; LOQ/2, LOQ/$\sqrt{2}$, 0 are some examples. Other studies choose to exclude values below LOQ/LOD from an average or a statistical analysis.

A good initiative to help researchers is the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE, http://www.strobe-statement.org/) and the addendum; STROBE – Molecular Epidemiology (STROBE-ME) which is an extension aimed towards the molecular epidemiological sciences (Gallo et al. 2011). The article gives a checklist which when applied will give the reader a better insight in the concept of the study as well as the strengths and limitations thereof. The intention to facilitate and to make studies´ results accessible to other researchers is of importance to further advance environmental science.

6 Svensk sammanfattning

Arbetet i denna avhandling handlar om antropogena, svårnedbrytbara halogenerade organiska föroreningar i modersmjölk, även kända som persistenta organiska mijöföroreningar (POPar). Det vill säga sådana ämnen som i allmänhet bryts ner långsamt, är spridda över hela världen, ansamlas i fettrikavävnader i biota (bioackumuleras), anrikas längs näringsväven (biomagnifieras) och är skadliga för både djur och människor.

Den ”kemiska revolutionen” kan anses ha börjat runt andra världskriget för att verkligen ta fart efter andra världskrigets slut. Det är fortfarande viktigt att komma ihåg att den inledande fasen som sedemera leder fram till denna revolution startade i mitten av 1800-talet med intensiva experimentella verksamhet i syntetisk organisk kemi. Allteftersom kom detta arbete att leda till en lång rad förbättringar för miljoner över hela världen. Nya läkemedel, t.ex. antibiotika , bekämpningsmedel (DDT för malariabekämpning), nya byggmaterial m.m. möjliggjorde tillverkningen av nya varor med ibland spektakulära egenskaper som bl.a. hjälpt människan att göra nya och tidigare omöjliga uppgifter möjliga. Med den snabbt växande kemiindustrin kom det såsmåningom att stå klart att fanns nackdelar. Antropogena ämnen, såsom kvicksilverföreningar, klororganiska pesticider (DDT, Lindan) och tekniska produkter (PCB) spreds till den allmänna miljön och hade eftersom ackumulerats tillräckligt i miljön för att orsaka negativa effekter.. Medvetenheten om antropogena kemiska faror, utöver arbetslivsrelatade risker väcks under 1960-talet när djurlivet påverkas negativt och människor är förorenade av miljöföroreningar. I Sverige ledde miljögifterna DDTs och PCBs exponering till toppredatorer uppenbart på ett ganska tidigt stadium till synliga effekter. Effekterna var allvarliga och utgjorde ett hot mot flera arters överlevnad i Östersjöområdet, så som säl, utter och för de stora rovfåglarna, bl.a. havsörn. Rovfåglarna drabbades framförallt av äggskalsförtunning vilket ledde till dålig reproduktion och återväxt.

De vetenskapliga upptäckter av POPar i både människa och miljö som gjordes, framförallt i Europa och Nordamerika, ledde beslutsfattare vid UNEP till att inleda arbetet på ett internationell fördrag för begränsingen av användning och spridning av POPar. Detta fastställdes 2001 i Stockholm och kallades därefter för Stockholmskonventionen och träde i kraft 2004. Initialt

så var inkludrades 12 POP'ar i konventionen men i.o.m. ny beslut så har 10 nya POP'ar tagits upp i konventionen, 2009 samt 2011, så att totalt 22 POPar omfattas.

Dr Koidu Norén var en av de första forskarna som undersökte POPar i modersmjölk och påbörjade sitt arbete i Stockholm 1967. Den europeiska och sålunda även den svenska, befolkningen utsätts främst för POPar via kosten, i synnerhet fet fisk. Det är av vikt att övervaka modersmjölk då flertalet POPar uppvisar endokrint störande egenskaper – vilket kan leda till oönskade effekter.

Min avhandling syftar till att probematisera exponeringsanalys av modersmjölk, d.v.s. provtagning, analysmetodik och rapportering. Målet var att även undersöka förändringar över tid av "dioxiner" regionalt i Stockholm samt att undersöka överföring av POPar från modern till modersmjölken. Haltbestämning av POPar i modersmjölk beskriver inte bara spädbarnets direkta exponering, utan ger även en bild av moderns exponering för POPar, och i förlängningen – den svenska befolkningens.

I avhandlingen så undersökts (**Paper I**) överföringen av polybromerade difenyletrar, en klass av ämnen bland de bromerade flamskyddsmedlen, från moderns blod till modersmjölken. Det konstateras att överföringen till mjölken skiljer sig beroende på hur lång tid efter förlossningen som proven tas. Det konstateras även att det finns ett samband mellan storleken på molekylerna och graden av överföring till mjölken.

Dioxinhalter i arkiverade prov av modersmjölk från Stockholm med början 1972 och slut 2011 undersöks i **Paper II**. Det går att konstatera att koncentrationerna av dioxiner, furaner och dioxinlika polyklorerade difenyletrar (DL-PCBer) minskar under hela tidsperioden. Särskilt positivt är att halterna årligen minskar snabbare under den senare delen av tidserien, 2002–2011. Jämförelse sker även med tidigare analyser av samma arkiverade modersmjölkprov. Analysresultaten överensstämmer väl, vilket ger stöd för att historiska tidserier i allmänhet kan förlängas.

En omfattande litteraturstudie som rör de POPar som rapporterats i modersmjölk världen över från 1995 och framåt har sammanställts och bearbetas för att presenteras i **Paper III**. Utöver redovisning av POP-halter så konstateras det att vissa ämnen är avsevärt mer väl undersökta än andra; DDT, PCB och hexaklorcyklohexan (HCH) jämfört med Aldrin och Toxaphene. Samt att vissa geografiska områden är mer väl undersökta än andra, Europa kontra Afrika. Vidare kritiseras studier då rapporting av resultat alltför ofta är bristfällig. Väsentlig information så som provtagningsland, -år och antal prover utelämnas t.ex. i visa studier.

I **Paper IV** så beskrivs en metod som utvecklats för att snabbt kunna upparbeta en större mängd modersmjölk, 200 mL per prov. Metoden är utarbetad på så vis att den vanligt förekommande och effektiva uppreningen med svavelsyra undviks. Detta för att kunna analysera föreningar som är känsliga för svavelsyrabehandling. Metoden är anpassad för att kunna analysera ett så brett spektrum av neutrala, lipofila föreningar som möjligt. Metoden tillämpas även på ett svenskt och ett kinesiskt samlingsprov, från 10 respektive 36 mödrar, för att påvisa skillnader i exponeringmönstret av POPar i Sverige och Kina.

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8 Appendix A

Contribution to Paper I–IV

A short description of the contribution to the Papers included in the thesis is given below.

Paper I	Performed the analytical work, participated in discussions and writing of the paper.
Paper II	Planned the study and sample selection in collaboration with co-author Elisabeth Nyberg and pooled archived mothers´ milk samples. Did the interpretation of the results and wrote the paper.
Paper III	Planned the study and collected and summarized the data in collaboration with particularly E. Nyberg. Participated in writing the manuscript in collaboration with my co-authors.
Paper IV	Planned and carried out the study, i.e. performed method development beyond the work presented in this manuscript (c.f. Chapter 3, above), interpreted all results and wrote the manuscript.

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