# Improved Assessment in Environmental Monitoring of POPs - Using monitoring data from the aquatic ecosystem and human milk

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Till mamma och pappa 

# List of publications

This thesis is based on the following articles, which are referred to by roman numerals (I-V) in the text. Papers (I-V) are reprinted with kind permission of the publishers of Chemosphere, Ambio, Marine Environmental Research, and Environment International. My own contributions to each of the papers are presented in Appendix A.

I. Bignert A., Ericsson U., **Nyberg E.**, Miller A. and Danielsson S. 2014. Consequences of using pooled versus individual samples for designing environmental monitoring sampling strategies. Chemosphere 94: 177-182.

II. **Nyberg E.**, Danielsson S., Eriksson U., Faxneld S., Miller A. and Bignert A. 2014. Spatio-temporal trends of PCBs in the Swedish freshwater environment 1981-2012. Ambio 43: 45-57.

III. **Nyberg E.**, Faxneld S., Danielsson S., Eriksson U., Miller A. and Bignert A. 2015. Temporal and spatial trends of PCBs, DDTs, HCHs, and HCB in Swedish marine biota 1969-2012. Ambio 44: 484-497.

IV. Miller A., **Nyberg E.**, Danielsson S., Faxneld S., Haglund P. and Bignert A. 2014. Comparing temporal trends of organochlorines in guillemot eggs and Baltic herring: Advantages and disadvantages for selecting sentinel species for environmental monitoring. Marine Environmental Research 100: 38-47.

V. 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.

Additional relevant publications by the author:

Fång J., **Nyberg E.**, Winnberg U., Bignert A. and Bergman Å. 2015. Spatial and temporal trends of the Stockholm Convention POPs in mothers' milk- a global review. Environmental Science and Pollution Research 22: 8989-9041.

Jörundsdóttir H.O., Jensen S., Hylland K., Holth T.F., Gunnlaugsdóttir H., Svavarsson J., Ólafsdóttir Á., El-Taliwy H., Rigét F., Strand J., **Nyberg E.**, Bignert A., Hoydal K.S. and Halldórsson. 2014. Pristine Arctic: Background mapping of PAHs, PAH metabolites and inorganic trace elements in the North-Atlantic Arctic and sub-Arctic coastal environment.
Science of The Total Environment 493: 719-728.

Miller A., Hedman J., **Nyberg E.**, Haglund P., Cousins I.T and Wiberg K. 2013. Temporal trends in dioxins (polychlorinated dibenzo-p-dioxin and dibenzofurans) and dioxin-like polychlorinated biphenyls in Baltic herring (*Clupea harengus*). Marine Pollution Bulletin 73: 220-230.

Dittman T., Becker P.H., Bakker J., Bignert A., **Nyberg E.**, Pereira M.G., Pijanowska U., et al. 2012. Large-scale spatial pollution patterns around the North Sea indicated by costal bird eggs within an EcoQO programme. Environmental Science and Pollution Research 19: 4060-4072.

# Contents

# Abbreviations

| | |
|---|---|
| BMF | Biomagnification Factor |
| CL | Confidence Level |
| CV | Coefficient of Variation |
| DDD | Dichlorodiphenyldichloroethane |
| DDE | Dichlorodiphenyldichloroethylene |
| DDT | Dichlorodiphenyltrichloroethane |
| DL-PCBs | Dioxin-like polychlorinated biphenyls |
| EAC | Environmental Assessment Criteria |
| EcoQO | Ecological Quality Objectives |
| EPA | Environmental Protection Agency |
| EQS | Environmental Quality Standards |
| ESB | Environmental Specimen Bank |
| GES | Good Environmental Status |
| HCB | Hexachlorobenzene |
| HCHs | Hexachlorocyclohexanes |
| HELCOM | Helsinki Commission |
| LDT | Lowest detectable trend |
| LOD | Level or limit of detection |
| LOQ | Level or limit of quantification |
| MSFD | Marine Strategy Framework Directive |
| OSPARCOM | Oslo Paris Commission |
| PAH | Polycyclic aromatic hydrocarbons |
| PBDEs | Polybrominated diphenyl ethers |
| PCBs | Polychlorinated biphenyls |
| PCDD | Polychlorinated dibenzo-p-dioxins |
| PCDF | Polychlorinated dibenzofurans |
| POPs | Persistent Organic Pollutants |
| SC | Stockholm Convention |
| SIA | Stable Isotope Analysis |
| SMNH | Swedish Museum of Natural History |
| SNMPCFB | Swedish National Freshwater Monitoring Program for Contaminants in Biota |
| SNMPCMB | Swedish National Marine Monitoring Program for Contaminants in Biota |

| | |
|---|---|
| SNMPHH | Swedish National Monitoring Program for Human Health |
| TDI | Tolerable Daily Intake |
| TGD | Technical Guidance Document |
| TL | Trophic Level |
| TMF | Trophic Magnification Factor |
| WFD | Water Framework Directive |
| YQR | Number of years required to detect and annual change of 10% |

# 1 Introduction

American biologist Rachel Carson wrote the book *Silent Spring* in 1962. The book described the effects that the use of pesticides had on American wildlife, especially on birds (Carson 1962). The book put environmental issues on the table of the American public in particular, but was also globally noticed. Barely seven years later, Sören Jensen and co-workers found high levels of persistent organic pollutants (POPs) (e.g. PCBs and DDTs) in Swedish biota, and realised POPs were also a great threat to the Baltic Sea and Swedish freshwater ecosystems (Jensen et al. 1969). These findings were the starting point of continuous national monitoring programs for contaminants in biological matrices from the marine, freshwater and terrestrial environment in Sweden. At the same time, also as a result of the recognised pollution, Dr. Koidu Norén and co-workers started to collect and analyse organic contaminants in human milk from the Stockholm area to investigate human exposure.

The aim of the monitoring programs was primarily to monitor temporal trends to evaluate the rate of change in POPs when assessing measures taken to reduce the contaminant load. Since the start of the monitoring, overall significant decreases of about 70–90% have been observed for PCBs, DDTs, HCHs and HCB in fish and bird eggs from both the Baltic Sea and the Swedish freshwater environment, which implies that bans and restrictions implemented in the 1970s and 1980s have had the desired effect. Several of the classical POPs have also decreased considerably in human milk. However, concentrations of PCBs, DDTs and HCHs are still higher in the Baltic Sea compared to, for example, the North Sea.

When monitoring contaminants in biological matrices, there are several aspects that need to be considered, such as the choice of sample (species, organ etc.), sampling design, and how to evaluate the data statistically both for trend analysis and for compliance with a set target value. These aspects will be discussed in detail within this thesis.

## 1.1 Aim of thesis

The primary aim was to evaluate monitoring data collected for several decades within the national monitoring programs for contaminants in biological matrices, including the marine program, the freshwater program, and the human health program. The secondary aim was to assess the suitability of different matrices used for environmental monitoring of POPs for assessment of temporal and spatial trends, and to evaluate concentrations for compliance of set target values with the overall aim to improve the evaluation of results within the current monitoring programs. To evaluate and control efficiency and to guarantee quality in a quantitative way, statistical aspects have played a major role through e.g. power analysis.

Specific aims for each paper follow.

**Paper I:** To examine various scenarios using individual or pooled samples and the relationships between chemical analytical error and other sources of sample variance within the marine monitoring program for contaminants in biota; to outline advantages and disadvantages of pooled samples compared to individual samples. The article may be used as a technical guidance for countries setting up new monitoring programs.

**Paper II:** To examine temporal and spatial trends of PCB congeners in perch (*Perca fluviatilis*), pike (*Esox lucius*), and Arctic char (*Salvelinus alpinus*); to evaluate concentrations over time in relation to bans and restrictions, and in compliance with set environmental target levels; to investigate how monitoring design may affect the interpretation of these trends.

**Paper III:** To examine temporal, seasonal, and spatial relationships of PCBs, HCHs, HCB, and DDTs primarily in herring (*Clupea harengus*) and guillemot (*Uria aalge*) eggs, but also in cod (*Gadus morhua*), perch (*Perca fluviatilis*), eelpout (*Zoarces viviparous*), and blue mussel (*Mytilus edulis*); to evaluate concentrations over time in relation to imposed bans and restrictions; to investigate compound profiles that could indicate new releases or aging of residues, e.g. DDT/DDE, patterns of α, β, γ-HCH; and to examine concentrations in relation to environmental target values.

**Paper IV:** To evaluate the suitability of guillemot (*Uria aalge*) egg as a sentinel species for investigating Good Environmental Status (GES) within the Marine Strategy Framework Directive (MSFD) by comparing the tem-

poral trends of PCDD/Fs and DL-PCBs in guillemot egg with the temporal trends in herring (*Clupea harengus*).

**Paper V:** To assess temporal trends of PCDDs, PCDFs and DL-PCBs in human milk collected from nursing women in Stockholm since the 1960s; to compare the results with previous analysis of some of the older samples to evaluate if time series could be elongated if the sample population and analytical method remains the same.

# 2 Background

In the 1960s, the Baltic Sea was found to be severely polluted by PCBs and DDTs, two groups of persistent organic contaminants (Jensen et al. 1969, 1972). Jensen et al. (1969) analysed various marine species, including blue mussel *(Mytilus edulis)* at the bottom of the food chain to top predators such as grey, common, and ringed seals (*Halichoerus grypus*, *Phoca vitulina*, *Pusa hispida*). These contaminants were present in all species examined. At that time, little was known about the toxicological implications of the concentrations found in the environment (Jensen et al. 1969). Later studies revealed that the high concentrations of PCBs and DDT including its metabolites (DDE, DDD), caused severe reproduction problems among the Baltic seal species and white-tailed sea eagle (*Haliaeetus albicilla*) populations (Helle et al. 1976a, b; Helander et al. 2002; Bergman et al. 2003, Bredhult et al. 2008). These discoveries led to the start of an environmental contaminant research program at the Swedish Museum of Natural History (SMNH), which in turn initiated the launch of a comprehensive environmental monitoring program in the 1980s by the Swedish Environmental Protection Agency (SEPA). The Department of Environmental Research and Monitoring at the SMNH was given the responsibility of monitoring contaminants in marine and freshwater biota. The samples from these programs have been stored frozen in the Environmental Specimen Bank (ESB) at the SMNH.

The focus of these monitoring programs was primarily to follow temporal trends to estimate the rate of change in contaminant concentrations when following up bans and restrictions. The programs were also designed to indicate large-scale spatial differences, thereby detecting local contaminant incidents or widespread incidents.

Unlike the national strategy for monitoring levels of contaminants in the environment, a common national strategy was not set for monitoring human chemical exposure in Sweden. However, Dr. Koidu Norén, at Karolinska Institute, Sweden, initiated human health monitoring in Sweden when she began collecting and analysing organic contaminants in mothers' milk from

the Stockholm area in 1967 (Norén and Meironyté 2000). The milk was supplied by the Mothers´ Milk Centre in Stockholm (Meironyte et al. 1999). Dr Norén and her research group have analysed a wide range of persistent organic contaminants and their metabolites in human milk samples (Norén and Meironyté 2000, Meironyte et al. 1999, Norén et al. 1996, Norén and Lundén 1991). The samples, collected since 1972, were stored frozen for future re-analysis. In 1997, this milk collection was transferred to the ESB at SMNH.

In 1993, SEPA began a small scale program for human health monitoring after suggestions given in a report from the Institute for Environmental Medicine in 1992. SEPA's human health program examines heavy metals and organic contaminants in human material (blood, urine, milk) and also air pollutants, foodstuffs/drinking water, and road traffic noise. In 2010, the Department of Environmental Research and Monitoring at SMNH was assigned responsibility for the collection of human milk in the Stockholm area by the section for Health-related environmental monitoring (HÄMI) at the SEPA.

## 2.1 The Swedish National Monitoring Program for Contaminants in Marine Biota (SNMPCMB)

### 2.1.1 The Baltic Sea/ Monitoring Stations

The majority of the SNMPCMB monitoring sites (18 of 23) are located in the Baltic Sea (Figure 2.1). The Baltic Sea is one of the largest brackish inland seas by surface area, being almost 400 000 $km^2$ with an average depth of 52 m (HELCOM, 2009). It has a water turn-over time of approximately 25 years (Stigebrandt 2001) due to the narrow connection to the North Sea via Kattegat and the Danish Straits (Winsor et al. 2001). The Swedish part of the Baltic Sea is generally divided into three basins – the Bothnian Bay and the Bothnian Sea together referred to as the Gulf of Bothnia, and the Baltic Proper (Figure 2.1). The basins differ by size (both volume and surface area), sea surface temperature, pH, depth and salinity amongst other abiotic factors. Surface salinity is around 3‰ in the most northern parts of the Gulf

of Bothnia, and almost 10‰ close to the Danish Straits in the Baltic Proper (Winsor et al. 2001). The water masses are also vertically stratified, which prevents the water layers from mixing. The stratification is caused by differences in salinity, and seasonally, also by temperature differences (HELCOM 2009). The catchment area of the Baltic Sea is larger than 1 700 000 $km^2$ and is inhabited by approximately 85 million people (HELCOM 2009). The population density is much higher in the southern and south western parts of the catchment area, with more than 100 people per $km^2$ compared to the northern and north-eastern parts, with fewer than 1 person per $km^2$ on average. Land-use follows the same pattern, with a high proportion of cultivated land in the south, and mainly forests, wetlands, and mountains in the north (HELCOM 2009).

The sampling sites within the SNMPCMB are all located in areas regarded as locally uncontaminated and, as far as possible, uninfluenced by major river outlets, ferry routes, or urban and industrial areas, and are thus considered reference sites for regional and local monitoring and recipient control.

Data from 23 sampling sites are discussed in this thesis, and in **Paper III and IV** (Figure 2.1). The year of initial analysis varies between the selected sites, but altogether they span over more than 40 years, 1969-2012. However, sampling and analysis has been carried out annually throughout the duration of the program, with few exceptions. The stations are generally located in coastal areas, but a few of them are situated in off-shore areas.

The contaminant monitoring is integrated with fish population and physiology monitoring, carried out by SLU AQUA and the University of Gothenburg, at three of the monitoring sites – Holmöarna and Kvädöfjärden in the Baltic Sea and Fjällbacka in the Skagerrak, with perch and eelpout being the common species monitored (sites number 4, 14 and 12, Figure 2.1).

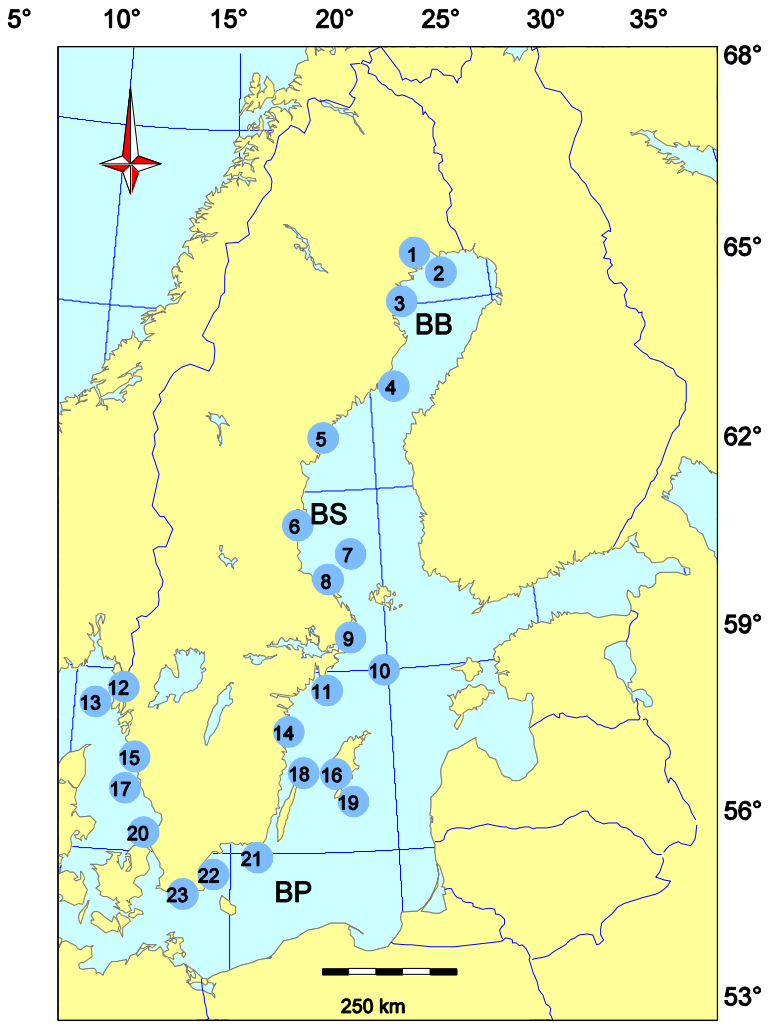


**Figure 2.1.** Sampling sites within the Swedish National Monitoring Program for Contaminants in Marine Biota. BB Bothnian Bay, BS Bothnian Sea, and BP Baltic Proper. ***Herring*****:** 1 Råneфjärden, 2 Harufjärden, 3 Kinnbäcksfjärden, 5 Gaviksfjärden, 6 Långvindsfjärden, 7 Bothnian Sea, offshore site, 8 Ängskärsklubb, 9 Lagnö, 10 Baltic Proper, offshore site, 11 Landsort, 13 Väderöarna, 17 Fladen, 18 Byxelkrok, 20 Kullen, 21 Utlängan, 22 Västra Hanöbukten, 23 Abbekås. ***Perch*****:** 4 Holmöarna, 14 Kvädöfjärden. ***Eelpout*****:** 4 Holmöarna, 14 Kvädöfjärden, 12 Fjällbacka. ***Cod:*** 19 SE Gotland, 17 Fladen. ***Blue mussel*****:** 12 Fjällbacka, 14 Kvädöfjärden, 15 Nidingen. ***Guillemot egg*****:** 16 Stora Karlsö

## 2.2 The Swedish National Monitoring Program for Contaminants in Freshwater Biota (SNMPCFB)

### 2.2.1 Swedish Lakes/Monitoring Stations

There are almost 100 000 lakes larger than 0.01 $km^2$ covering more than 9% of Sweden's total surface area. Most of the lakes are small (less than 0.1 $km^2$), however, 23 of the lakes have surface areas greater than 100 $km^2$, encompassing a third of the total lake surface area. Approximately 30% of all Swedish lakes are located in Norrbotten County bordering to the Bothnian Bay (www.smhi.se).

The 32 lakes monitored within the SNMPCFB (Figure 2.2) are distributed from the far north of Sweden (Lake Abiskojaure), located 200 km north of the Arctic Circle, to the southern-most parts (Lake Krageholmssjön), located 1800 km south of the Arctic Circle. The majority of the lakes are situated in the southern half of Sweden. The large distances between north and south (approximately 2000 km from the far northern border with Norway and Finland, to Malmö in the far south at the Danish border) leads to large temperature differences, thus some lakes are covered with ice for several months of the year, while a few in the far south remain ice-free. The length of time of ice coverage can have significant effects on oxygen conditions (Livingstone, 1993), which in turn influences phytoplankton diversity and production (Weyhenmeyer et al. 1999). The lakes also differ in size, nutrient status, general physical environment, and land use of surrounding areas. The smallest lake in the monitoring program is 0.06 $km^2$ (Lake Skärgölen), while the largest is 184 $km^2$ (Lake Bolmen) (Figure 2.2). These physical differences imply a great variability between the lakes concerning abiotic factors that might affect contaminant levels in fish.

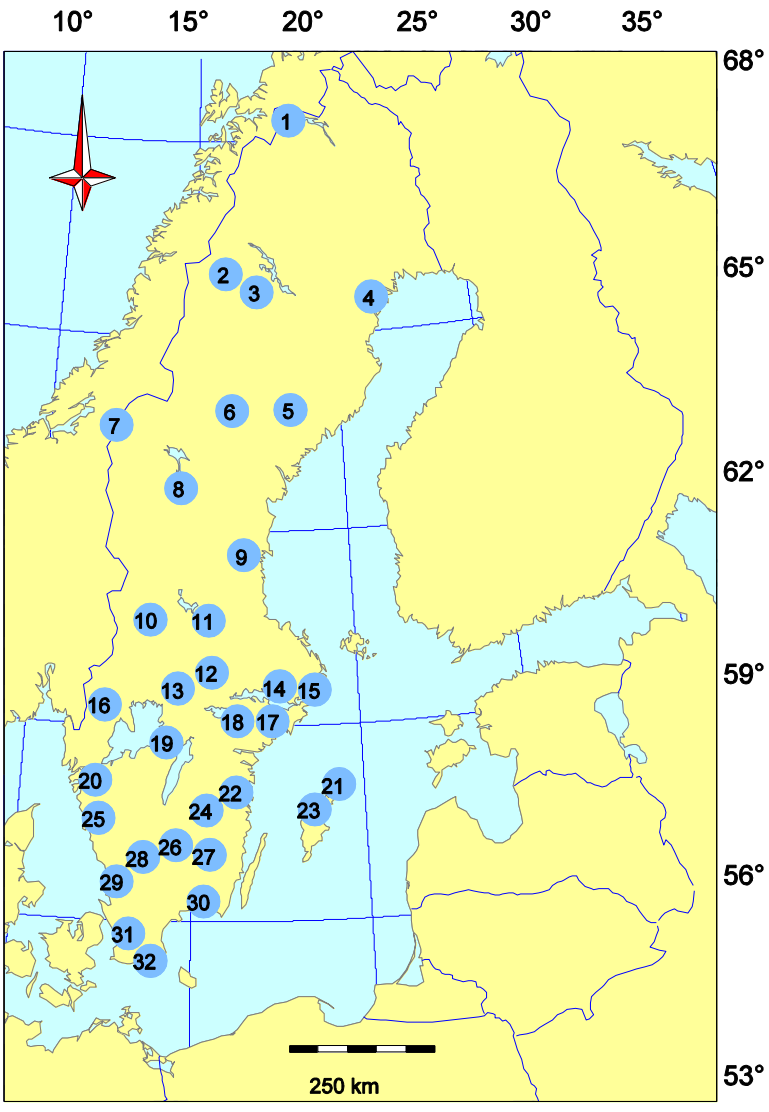


**Figure 2.2.** Sampling sites within the Swedish National Monitoring Program for Contaminants in Freshwater Biota. ***Arctic char:*** 1 Abiskojaure, 2 Tjulträsk, 7 Stor-Björsjön. ***Pike:*** 3 Storvindeln, 28 Bolmen. ***Perch:*** 4 Brännträsket, 5 Remmarsjön, 6 Degervattnet, 8 Stor-Backsjön, 9 Stensjön, 10 Gipsjön, 11 Spjutsjön, 12 Övre Skärsjön, 13 Limmingsjön, 14 Fysingen, 15 Tärnan, 16 Bysjön, 17 Stora Envättern, 18 Älgsjön, 19 Svartsjön, 20 Fräcksjön, 21 Bästeträsk, 22 Allgjuttern, 23 Horsan, 24 Skärgölen, 25 Lilla Öresjön, 26 Fiolen, 27 Hjärtsjön, 29 Stora Skärsjön, 30 Sännen, 31 Krankesjön and 32 Krageholmsjön.

The lakes within the monitoring program are selected using a number of criteria. The primary reason for the use of numerous criteria is that the lakes

should serve as reference sites, both nationally and regionally. The lakes must not be influenced by local contamination, thus land use in the surrounding areas is well investigated, and any areas with intensive agricultural activities are avoided. Preferably the lakes should be oligotrophic rather than eutrophic, because oligotrophic ecosystems display a faster response to changes in discharges of contaminants (Larsson et al. 2000). The lakes should also be placed as high up as possible in the drainage system to minimise the risk of local contamination. Liming activities in lakes (i.e. lime dumped into the lakes) have been common in Sweden since the late 1970s to counteract the negative effects of acidification (caused by atmospheric deposition of acidifying pollutants) on wildlife (Persson 2008). Areas where liming activities occur are avoided when selecting sampling sites. Preferably, the lakes should also have some protection against future exploitation. Another important factor is if other monitoring occurs (e.g. fish population, water chemistry), primarily for the evaluation of contaminant data, but sampling for the different programs could then also be coordinated for financial reasons.

## 2.3 The Swedish National Monitoring Program for Human Health (SNMPHH)

The SMNH is responsible for monitoring of human milk in the Stockholm and Gothenburg region; however, monitoring of human milk is also performed by the Swedish Food Administration in the Uppsala region. In 2000-2004 human milk was also collected from Lycksele in the north of Sweden, and Lund in the south of Sweden. A regional study of organohalogenated persistent compounds was performed, which included human milk from Uppsala and Gothenburg in the analysis. Statistically significant differences were found in most contaminant concentrations analysed between the four regions. However, the differences in concentrations between the regions were not large, hence indicating that there is a similar long-term exposure pattern of these compounds among nursing women from different regions in Sweden (Glynn et al. 2011). Due to similar exposure mechanisms, and also due to practical difficulties of collecting human milk samples, the current SNMPHH only consists of samples from Stockholm, Uppsala, and Gothenburg.

To reduce influence of confounding factors, the sample definition is narrow and restrictive. The selected mothers are healthy and non-smokers. They are

mainly primiparous, because studies have shown a correlation between contaminant level and the number of children a woman has given birth to (Dillon et al. 1981, Albers et al. 1996, Fitzgerald et al. 2001). Women of similar age are sampled because as age of the mother increases, levels of POPs in the fat generally also increase (Albers et al. 1996). However, the samples could be adjusted for age in the assessment. Samples are collected from 2 weeks up to three months after delivery to minimize variation in the milk composition. The mothers are born and have resided in Sweden for their entire lives to ensure that the contaminant level in the milk is representative of a Swedish contaminant load.

## 2.4 Monitoring matrices

### 2.4.1 Guillemot (*Uria aalge*) egg

The guillemot is a piscivorous bird species with a circumpolar distribution. It overwinters in the Baltic, mainly at Stora Karlsö, which is the largest breeding colony in the Baltic (Österblom et al. 2002) and preferably feed on sprat (*Sprattus sprattus)*, and herring (Österblom et al. 2001). Guillemot do not migrate far, thus their contamination concentrations are typically locally acquired. The breeding season begins in early May and normally only one egg is laid each year. However, if the first egg is lost, a second egg may be laid (Hedgren 1980). The egg has a high fat content, between 11-13%, which makes it suitable for analysis of substances that dissolve in the fat (Bignert et al. 1995). Guillemot eggs laid late in the season are presumably replacement eggs. These tend to contain significantly higher concentrations of organochlorines compared to eggs laid early (presumably first laid eggs) (Bignert et al. 1995).

### 2.4.2 Arctic char (*Salvelinus alpinus*)

Arctic char inhabit upland freshwaters of Swedish mountain areas. Their diet varies depending on prey availability, char size, and the presence of other competitive species. Small Arctic char generally feed on benthic invertebrates and plankton, while larger individuals feed on aquatic insects, and fish, including conspecifics (Hammar 2000). Large Arctic char are thus generally exposed to biomagnifying substances at the high end of the aquatic food chain. Arctic char muscle tissue is relatively fat, with 1-3% extractable

fat content (Nyberg et al. 2015). Spawning normally occurs during August-January (Kullander et al. 2012).

### 2.4.3 Cod (*Gadus morhua*)

Cod are found in both marine and brackish water but to be able to spawn successfully it demand a salinity and oxygen level above 11 PSU (Practical Salinity Unit) and 2 ml/l respectively (Nissling 1995). Baltic cod live below the halocline and feed mainly on clupeids (e.g. herring), but invertebrates and other fish species, as well as young cod, are also included in the diet (Pachur and Horbowy 2013). Spawning normally takes place between January (more common for Skagerrak cod) and July (more common for Baltic cod) (Nissling et al. 1998) on the Swedish west coast and in the Baltic Proper, which is the only area in the Baltic Sea where the salinity is sufficient for the eggs to remain afloat, which is necessary for successful spawning (Bagge and Thurow 1994). The cod liver is fat, which makes it appropriate for analysis of fat soluble contaminants, but the fat content is highly variable, between 12-52% extractable fat (95% confidence intervals) (Bignert et al. 2016a).

### 2.4.4 Eelpout (*Zoarces viviparous*)

The eelpout is also found in both brackish and marine waters and is regarded as a relatively stationary species. The eelpout is living quite close to the bottom (Jacobssen et al. 1993) and it feeds mainly on invertebrates and small fish (Ojaveer et al. 2004). The eelpout is viviparous (females give birth to fully developed fry) which enables measurements of number of eggs, fertilised eggs, larvae size, and embryonic development and in turn makes the species suitable for integrated studies of contaminants and reproduction (Jacobsson et al. 1993, Hedman et al. 2011). The mating takes place in August through September (Rasmussen et al. 2006). Eelpout muscle tissue is lean and contains approximately 0.5-0.7% of extractable fat (Bignert et al. 2016a).

### 2.4.5 Herring (*Clupea harengus*)

Herring is a pelagic fish species, living in both brackish and marine waters. Herring feed mainly on zooplankton, but fish and mysids are also included in the diet when herring size increases and availability of mysids is high (Casini et al. 2004). Herring is the most dominant commercial fish species in the

Baltic and important for a number of other fish eating species such as seals and guillemot (Lundin 2011). Herring is one of the core species for contaminant monitoring within the HELCOM convention area (HELCOM 2015). The herring muscle tissue has a fat content between 2 and 5%, which is considered as relatively fat, thus the species is suitable for analysis of fat soluble contaminants (Bignert et al. 2016a). The fat percentage is higher in herring on the Swedish west coast compared to herring in the Baltic Sea. Spawning typically occurs in spring or autumn, and in some cases it may even occur in summer and winter (Kullander et al. 2012).

### 2.4.6 Perch (*Perca fluviatilis*)

The European perch is found in both fresh and brackish waters (www.fishbase.org) and is sampled both within the SNMPCMB and the SNMPCFB. Perch is an opportunistic predatory fish that undergoes an ontogenetic shift in diet (Collette et al. 1977). Larger perch, at a higher level of the aquatic food chain, are thus exposed to higher concentrations of biomagnifying substances. The age at dietary shift is dependent on the growth rate of perch; growth rate can vary between lakes (Holmgren and Appelberg 2001) but also between sampling sites in the Baltic Sea (Bignert et al. 2016a). The perch is considered to be stationary (Thorpe 1977) and spawning takes place during April to June (Kottelat and Freyhof 2007). Perch muscle tissue is lean and contains approximately 0.6-0.8% of extractable fat (Bignert et al. 2016a).

### 2.4.7 Pike (*Esox lucius*)

Pike, sampled within the SNMPCFB, is a common fish in the Nordic countries and can be found in both fresh and brackish waters (Baltic Sea). Pike is a predatory, mainly piscivorous fish that will prey on conspecifics, but also feeds on frogs, small mammals and birds (www.fishbase.org). Spawning normally takes place during March to June (Kullander et al. 2012). Pike is a lean fish with an average muscle extractable fat content of 0.6% (Nyberg et al. 2015).

### 2.4.8 Blue mussel (*Mytilus edulis*)

Blue mussels are filter feeders, stationary, and function in a wide range of salinities and temperatures. These characteristics make them one of the most commonly used species for monitoring of contaminants. Size and biomass

decrease rapidly with lowered salinity, thus Baltic Sea mussels are much smaller than mussels from the Swedish west coast (Kautsky 1981). Blue mussel soft body contains approximately 1.0-1.5% of extractable fat (Bignert et al. 2016a).

### 2.4.9 Human milk

Women in Sweden or any other developed country have a highly variable diet. They consume products imported from all over the world and from different parts of the food chain. The composition of human milk, which apart from water mainly consists of carbohydrates, proteins, and fat, differs a lot depending on a number of factors. However, fat content is the most variable component (Mitoulas et al. 2002). The fat content has been shown to be affected by time post-partum (fat content increases with increasing time since birth), the portion of feeding (fat content increases during the course of a single feeding), number of children, and infections (La Kind 2002). A study by Jensen et al. (1996) reports an average fat content of 3-5% and similar numbers are also reported by Mitoulas et al. (2002). In the time trend study in **Paper V**, the fat content varied from 2.4-4.0 %, with a significantly higher fat content observed during more recent years.

## 2.5 Persistent Organic Pollutants (POPs)

The POPs discussed in this doctoral thesis (i.e. PCBs, DDTs, HCHs, HCBs and PCDD/PCDFs) have been of environmental and health concern for a number of decades. The POPs here in are presented in more detail under the Stockholm Convention (SC) (UNEP 2016).

Production of technical polychlorinated biphenyls (PCBs) started in 1929. Due to its chemical and physical properties (e.g. heat resistance) this group was mainly used in transformers, capacitors, hydraulic liquids and lubricants (UNEP 2016). The open use of PCBs have been nationally (SFS 1971:385) and internationally banned (OECD 1973) since the early 1970s, followed by a ban of all new uses in 1978, and finally, the total ban of PCBs in Sweden in 1995 (SFS, 1995: 1095).

Dichlorodiphenyltrichloroethane (DDT) is a potent insecticide that has mainly been used for mosquito control (UNEP 2016). DDT is transformed to dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloro-

ethane (DDD). The use of DDT has been banned since the 1970s in most countries. Currently it is only allowed to combat malaria (UNEP 2016), primarily in Africa and the Pacific Islands (Bogdal et al. 2013).

Hexachlorobenzene (HCB) has primarily been used as a fungicide and has been banned in the Baltic countries since mid-1970s (Gaul, 1992). HCB is also formed as a by-product in a number of industrial processes (e.g. electrolyte production, chlor-alkali processes, and waste incineration of materials containing chlorine), thus can still enter the environment (WHO 1997, Garı´ et al. 2014).

Hexachlorocyclohexane (HCH) has been produced since the late 1940s. Three of its isomers are of environmental interest, specifically α-, β- and γ-HCH. γ-HCH, known as lindane, is the most potent HCH isomer of this group; however, the technical mixtures of all three isomers have been widely used as commercial pesticides. The use of HCHs has been regulated or banned in a number of countries since the 1970-1990s (Willett et al. 1997). However, China and Romania did not cease lindane production until recently, while in India, lindane is still produced (Vijgen et al. 2011).

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) are unintentionally formed in a number of industrial processes and during most combustion processes, particularly if they are incomplete (e.g. waste incineration, forest fires, backyard burning, fossil fuel burning) (Baars et al. 2004). Pulp bleaching using chlorine gas has, in the past, been another source of PCDD/Fs to the Baltic Sea (Rappe et al. 1987, Wiberg et al. 1989) as were emissions related to the use of chlorinated pesticides (Baars et al. 2004). In the late 1970s, the use of dioxin-contaminated herbicides and chlorophenols (SFS 1977:246) were banned. During the 1990s, the efficiency of municipal waste incinerators in Sweden was greatly improved, and the pulp and paper industry gradually changed their bleaching technology towards a chlorine free process.

## 2.6 EU directives, conventions and the WHO's human milk program

### 2.6.1 The Water Framework Directive (WFD) and the Marine Strategy Framework Directive (MSFD)

The EU WFD was adopted in 2000, and primarily deals with freshwater, but the coastal-zone and estuary waters are also included in the directive, while the EU MSFD was adopted in 2008. The WFD aims to achieve good ecological and chemical status, with the common denomination of Good Environmental Status (GES) of all surface waters and ground water bodies in the EU. For the MSFD, the aim is GES in all European marine waters. GES, in accordance with the MSFD (MSFD, 2008/56/EC), is defined as "concentrations of contaminants at levels not giving rise to pollution effects".

The WFD requires that member states should report the chemical status of their water bodies to the EU Commission every six years (WFD, 2000/60/EC) concerning 45 priority substances. The evaluation of chemical status is done through monitoring of water, sediment, or biota, depending of the properties of the substance of concern. The EU has produced Technical Guidance Documents (TGDs) concerning different aspects of contaminant monitoring under the WFD umbrella, to facilitate the setup of new monitoring programs in countries with non-existent monitoring (European Commission 2010, 2011, 2013).

Within the MSFD, GES is evaluated through eleven qualitative descriptors that describe what the environment will look like when GES has been achieved. Two of the eleven descriptors deal with contaminants, namely "contaminants and pollution effects" and "contaminants in fish and other seafood" (MSFD, 2008/56/EC).

To evaluate if GES has been achieved, Environmental Quality Standards (EQSs) have been established for the priority substances. EQS values are set to protect the most sensitive organisms against harmful effects from hazardous substances. There are only 11 out of 45 priority substances that have EQS values set for biota. Biota standards are set for fish (except for polycyclic aromatic hydrocarbons (PAHs)), where the target species are crustaceans and molluscs, since PAHs are metabolised to a large extent in fish, in contrast to crustaceans and molluscs where the metabolic processes are less efficient (2013/39/EU, Elskus and Stegeman 1989). The EQS in fish is set

for the whole fish, if the most sensitive species consume the whole fish ($EQS_{biota}$), and for muscle tissue if the most sensitive species is human ($EQS_{human\_health}$), generally only consuming the fillet of most fish species. The $EQS_{biota}$ in fish is set for fish of trophic level (TL) 3.5 in freshwater food webs, and TL 4.5 in marine food webs, and the $EQS_{human\_health}$ in fish is set for fish of TL 4, all with a default fat content of 5% and a default dry weight content of 26%. To put TL in a perspective, filter feeders (feeding on primary producers), such as mussels, are at trophic level 2 in the food web. Marine top predators are at a trophic level of 5.5 or higher. This implies that the analytical data needs to be recalculated to represent the correct TL and also be normalized to the default fat or dry weight content depending on analyte (European Commission 2013).

### 2.6.2 The Oslo Paris (OSPAR) Convention and the Helsinki Convention

The convention for the protection of the marine environment of the North-East Atlantic (the OSPAR convention) was adopted in 1992 and entered into force in 1998, and is governed by the OSPAR Commission (OSPARCOM). The OSPAR Convention has been signed by all European countries with a border or connection to the Atlantic. At the same time (1992), a convention for the protection of the marine environment in the Baltic Sea, the Helsinki Convention, was signed by all states bordering the Baltic Sea. The Helsinki convention entered into force in 2000 and is governed by the Helsinki Commission (HELCOM). Both conventions have the focus to protect the marine environment from all sources of pollution through intergovernmental cooperation. OSPARCOM has the specific aim to achieve levels of naturally occurring substances close to background level and levels of man-made substances close to zero, while HELCOM's specific aim strives towards a healthy Baltic Sea with a restored ecological status and concentrations of hazardous substances close to natural levels.

Monitoring of contaminants in marine biota in the North-east Atlantic Ocean and in the Baltic Sea is performed within the framework of these two conventions. The objectives are primarily to assess temporal trends and spatial differences within the convention area through harmonized sampling strategies and monitoring species. In the OSPAR Joint Assessment Monitoring Programme (JAMP) guidelines (OSPAR 2009) the quantitative objectives, sampling strategy, species selection, storage, practical sampling, and sample treatment is specified to get results as comparable as possible between the

different countries. HELCOM has specified similar issues for the Baltic Sea in the Manual for Marine Monitoring in the Cooperative Monitoring in the Baltic Marine Environment (COMBINE) Programme of HELCOM, often referred to as the COMBINE manual (HELCOM 2015). The TGD for monitoring of sediment and biota (European Commission 2010) is to a large extent based on the guidelines within OSPAR JAMP and HELCOM COMBINE.

Within the OSPAR convention, Environmental Assessment Criteria (EAC) have been developed for interpretation of chemical monitoring data in sediments and biota (OSPAR 2014) in the marine environment for the chemicals listed in the Coordinated Environmental Monitoring Programme (CEMP). OSPAR EACs intend to represent "the contaminant concentration in the environment below which no chronic effects are expected to occur in marine species, including the most sensitive species" and may be considered as related to EQSs. HELCOM uses EQSs for the core indicator substances (HELCOM 2016) where they are available, and when not, other target values are used, in some cases OSPAR EACs.

### 2.6.3 The Stockholm Convention

All substances discussed in this doctoral thesis are included in the initial 12 of the 26 Persistent Organic Pollutants (POPs) included in The Stockholm Convention (SC) on POPs. The SC is an international agreement that requires measures to reduce and prevent the release of organic compounds that are persistent, have a potential for long-range transport, accumulate in fatty tissues, are found in higher concentrations higher up the food chain, and are toxic both to wildlife and humans. The SC was adopted in 2001 and entered into force in 2004 (UNEP 2016).

### 2.6.4 WHO's Human Milk Monitoring

Monitoring of human milk is a non-invasive way to monitor the exposure of both infant and mother. WHO started to collect and evaluate data on levels of POPs in human milk in 1976. Since then, they have organized four international studies to evaluate concentrations and trends of PCDD/Fs, and DL-PCBs (Colles et al. 2008). This work, and the ratification of the SC in 2004, has resulted in an international protocol for monitoring human milk published in 2005, which aims to produce comparable and reliable results between participating countries and to measure the response in human milk of

measures taken under the SC to reduce the release of certain chemicals into the environment. The guideline deals with questions such as type of samples (pooled vs individual), number of samples, selection of donors, how to practically collect and transport the samples, ethical aspects etc. (WHO 2005).

To evaluate if the level of contaminants in the milk impose a risk for the infant, the tolerable daily intake (TDI) of the specific contaminant is used. It represents the tolerable amount of a contaminant in food and drinkingwater that a person can consume on a daily basis without it being a health risk. The average daily intake of a contaminant could be calculated using figures on average daily human milk consumption.

# 3 Designing an efficient monitoring program

Environmental monitoring is a prerequisite for international organizations, national authorities, environmental agencies, and others, to assess the effects of measures taken to protect the environment and to discover new threats and emerging substances. Environmental authorities and politicians trust the monitoring to be sensible enough to discover new threats or inefficient reductions of known problems e.g. as a basement for legislation. Large amounts of resources are spent on these activities, yet many ongoing monitoring programs fail to detect relevant changes in environmental exposure (Bignert et al. 2004, Rigét et al. 2010). This implies an unacceptable waste of resources.

Several measures can be taken that may lead to more effective monitoring programs in terms of statistical power. Objectives need to be clearly defined and formulated in a quantified way. It is imperative to consider the magnitude of change that is necessary to detect and the risks of making the wrong conclusion one is prepared to accept based on statistical tests (i.e. Type-I and Type-II errors). Furthermore, unnecessary noise (variance) should be reduced as much as possible by means of efficient sampling strategies, censoring, and adjustments using statistical techniques. Finally, the selection of appropriate statistical tests should be guided by the objectives and properties of the current data. These aspects will be discussed in detail below.

## 3.1 Objectives

The aim of a monitoring programme could, for example, focus on investigating changes over time, estimating geographical differences, or assessing compliance by comparing levels in a required matrix with the set environmental target value. In most cases, the objectives involve all three questions, and the program has to be designed so it can answer them at a high statistical power.

### 3.1.1 Temporal, spatial, or compliance to Quality Standards

Temporal trend monitoring aims to evaluate how levels of contaminants are changing over time and to estimate the rate of changes found. Knowledge about how levels are changing over time could give a good indication on the effectiveness of measures taken to reduce the discharge of various contaminants, and also if the contaminants that are monitored are approaching background levels (naturally occurring substances), or are close to zero (man-made substances) (OSPAR 2009). Spatial monitoring may aim to assess the contaminant status of an area or detect large scale geographical differences, or to identify and possibly explain spatial patterns and suggest potential sources or to identify areas of special concern (hot spots).

Compliance monitoring aims to evaluate if levels of contaminants are below or above certain quality standards (target values such as EQS or EAC) in order to assess whether the levels pose a threat to the most sensitive organism. Compliance monitoring also assists in the more general assessment on whether a specified region has reached or failed to reach the goals set for the region, e.g. GES within the WFD and the MSFD (OSPAR 2009, European Commission 2010). Temporal trend monitoring in combination with compliance monitoring provides a more reliable status assessment because the between year variability is then also considered.

## 3.2 Quantitative objectives

Before any monitoring is started, it is essential that explicit monitoring objectives are determined (Philips and Segar 1986). Quantitative objectives within a monitoring program generally states the size of a change that the program should be able to detect at a certain power and significance level. The quantitative objectives will thus vary with the purpose of the investigation. For example, if the purpose of the investigation is trend monitoring, the quantified objective includes information on the annual change that the program should be able to detect, and the time period that needs to be monitored to detect the specified trend at a specified power (OSPAR 2009). Quantitative objectives are necessary for guidance on frequency, continuance of monitoring, sample size etc. (European Commission 2010). The main quantitative objectives within the SNMPCMB and the SNMPCFB are presented in Nyberg et al. 2015:

- "To monitor long term time trends and to estimate the rate of changes found. Q*uantified objective:* to detect an annual change of 10% within a 10 year time period, with a power of 80% at a 5% significance level. "

- "To detect incidents of regional impact or widespread incidents and to act as watchdog monitoring to detect renewed use of banned contaminants. Q*uantified objective:* to detect an increase of 200% in a single year, with a power of 80% at a 5% significance level."

- "To indicate large scale spatial differences. *Quantified objective:* to detect differences of a factor of 2 between sites, with a power of 80% at a 5% significance level."

### 3.2.1 Power

Power analysis is the statistical tool used to investigate sensitivity of a monitoring programme after the objectives are quantified. Generally, a statistical test compares the data with a null hypothesis ($H_0$), which almost always is of the type "no difference/impact". The test provides a probability that $H_0$ is true. A critical level ($\alpha$) is specified for the test. If the probability is less than $\alpha$, $H_0$ is rejected. Two types of errors can appear when interpreting the statistical test – Type I and Type II. The Type I error within environmental monitoring is when a trend is indicated even though no true trend exists. The acceptable risk of conducting this error is determined by $\alpha$. The related Type II error is when a true trend is not detected. The Type II error rate ($\beta$) is generally not fixed before the test is performed (Bignert 2002). However, in the quantified objectives regarding monitoring of contaminants in biota within OSPAR, the power (1-$\beta$) is set to 90% for some of the objectives (OSPAR 2009) and within AMAP (Arctic Monitoring and Assessment Programme) (AMAP 2014) and the Swedish national monitoring programs, power is set to 80%, while by convention, $\alpha$ is in all programmes set to 5%. The relevance of these fixed values of acceptable Type-I and Type-II-errors can be questioned. Mapstone (1995) suggests a different approach, where the critical changes that need to be detected are the primary focus, and the value of $\alpha$ is suggested to be determined by the balance of the cost of Type I/Type II errors.

Statistical power is the probability that a study will detect an effect, such as a temporal trend or a spatial difference, when there is a true trend or difference to be detected. High statistical power indicates that the probability of detecting a true trend, is high. Statistical power in temporal trend analysis is mainly affected by the magnitude of the trend, the length of the time-series, and the within- and between-year variation, which in turn is affected by sampling strategy, the number of samples, the type of statistical test applied, whether a two-or a one-tailed test is used, and the α-level. When interpreting results from statistical time series analyses, it is important to know with what power the temporal changes could be detected to be able to estimate the quality/sensitivity of the time series. However, even though a matrix is showing a high power, it is not always suitable for monitoring purposes if it does not respond to environmental changes in the contaminant being monitored fast enough (OSPAR 2009).

Reduction of noise (the unexplained variation between-samples and between-years) is an effective way to improve power. Noise reduction could be achieved, for example, by sample collection guidelines that aim at gathering as homogenous samples as possible regarding sex, age and other biological variables, guidelines for sample treatment, storage and preparation, and increased precision of the chemical analysis. Noise could also be reduced by using an appropriate base (i.e. fat, wet or dry weight) for expressing the contaminant concentrations, and by adjusting the data for relevant confounders. Reduction of noise will be discussed further in chapter 3.4, matrix selection.

## 3.3 Sampling strategy

The sampling strategy should be designed according to the quantitative objectives. As mentioned concerning the power, the recorded variability between samples should be reduced as much as possible. The accuracy and precision of the analytical performance should also be sufficient to meet the objectives, but normally the variation that originates from sample variation is much higher compared to variation due to uncertainty in the chemical analysis (**Paper I**).

Sample size is crucial for meeting the quantified objectives (**Paper I**). To estimate the required sample size when pooling of samples is considered, information on the precision of the chemical analyses (e.g. expressed as coefficient of variation (CVa)) and the total variance (e.g. expressed as coeffi-

cient of variation (CVt)) is needed. The specimen variance (expressed as coefficient of variation (CVs)) reflecting e.g. physiological difference, abiotic factors, and effects of long term storage and sample treatment, could be calculated from the analytical coefficient of variation and the total coefficient of variation using the relationship below (Råde and Westergren 1990):

$$CVt = (CVs^2+CVa^2)^{0.5}$$

The effect of various sample sizes on the random between-year variation (influenced by CVs and CVa) could then be estimated, and thus also a sample size that corresponds to the quantified objectives, represented here by the minimum trend that could be detected during a 10 year period with a power of 80% and $\alpha$=0.05 (Figure 3.1). The examples in **Paper I** are based on fish samples from the regular monitoring programs (SNMPCMB, SNMPCFB), where all fish specimens at one sampling site were caught at the same time in the same place, and therefore show both temporal and spatial autocorrelation (i.e. they cannot be considered independent observations of the current levels in fish from the region and season). To avoid problems with autocorrelation, the regression analysis (Figure 3.1) is based on geometric means of the yearly sample sizes, not the individual measurements. In a situation where the total CV is about 50%, analysing 12 individual specimens per year (but using the geometric means) are sufficient to detect an annual change of about 5-6% during a ten year period.

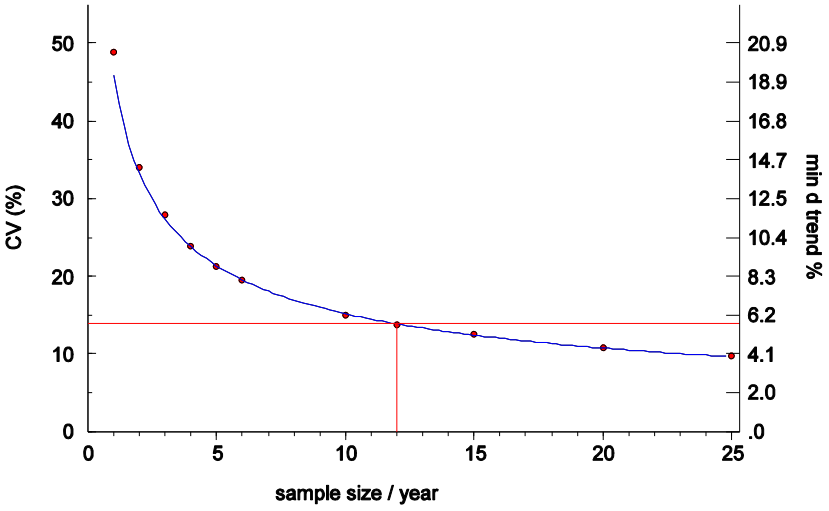


**Figure 3.1.** Individual samples from a log-normal distributed population, sampled over a 10 year period. In this scenario the total variance is set to 50% and analytical variance to 10%. The between-year variation is shown on the left y-axis, and minimum annual trend that could be detected at a statistical power of 80% is shown on the right y-axis. The red lines show the sample size needed to achieve a between-year variation of 14-15%, which is equivalent to a minimum detectable annual trend of 5-6%.

One important decision when selecting sampling strategy is the choice of individual or pooled samples (several individual samples homogenized into one pooled sample), both in regards to chemical analysis, and when storing samples for future analyses.

The effect of various numbers of pooled samples on the unexplained between-year variation was also investigated in **Paper I**. Analysing 2 pooled samples (i.e. increasing the number of collected specimens from 12 to 24 and reducing the number of chemical analyses from 12 to 2) per year, is sufficient to detect an annual change of about 5-6% during a ten year period at a maintained power of 80% (Figure 3.2).

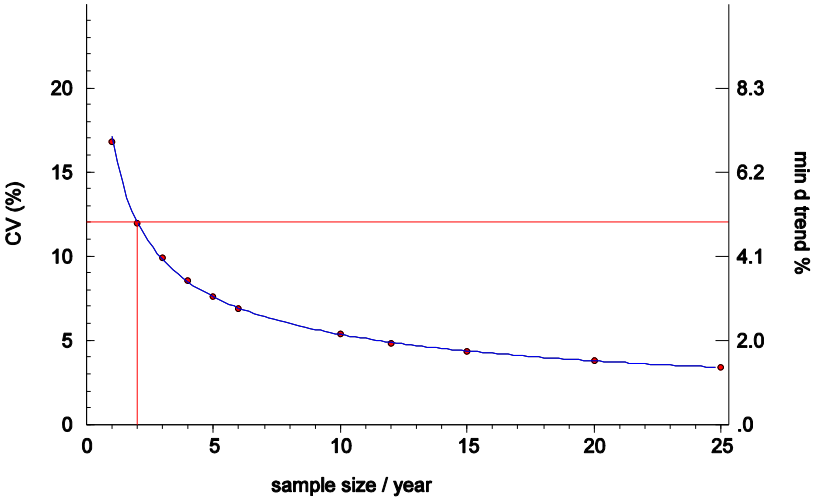


**Figure 3.2.** Pooled samples from a log-normal distributed population, using 12 individual specimens in each pool, over a 10 year period. The original total variance was set to 50%, and analytical precision to 10%. The between-year variation is shown on the left y-axis and minimum annual trend that could be detected with a statistical power of 80% is shown on the right y-axis. The red lines show the number of pools (consisting of 12 individual specimens each) needed to achieve a between-year variation of 14-15%, which is equivalent to a minimum detectable annual trend of 5-6%.

Advantages and disadvantages regarding the use of individual or pooled samples are discussed and evaluated in **Paper I** using monitoring data from the SNMPCMB. Situations when it is important to use individual samples are listed in **Paper I**, and are also as follows:

- When information about sample variance is important for its own sake e.g. identification of the sample distribution. Also because changes in variance are often the first sign of ongoing contamination (Bignert et al. 1993, Heffernan et al. 2014)

- When information on maximum value is crucial when the threshold level for a contaminant is set for the maximum concentration (**Paper I**).

- Individual samples allow the choice of an appropriate central measure, which is important because contaminants in biological matrices often show a right skewed distribution, in which case geometric or

median mean values are more appropriate. By contrast, pooled samples represent an arithmetic mean value (Caudill et al. 2007, Caudill 2012, Heffernan et al. 2014).

- Individual samples enable direct adjustments for confounding factors such as fat content and age, which could reduce variation within samples and in turn increase the sensitivity of the time-series (**Paper I**, Bjerkeng 2012).

- Individual samples facilitate the detection of extreme values (**Paper I,** Bjerkeng 2012).

- Individual samples allow an integrated comparison between contaminant concentrations and biological effects (Bignert et al. 1993).

The essential advantages of using pooled samples, as discussed in **Paper I**, are:

- Variation may be reduced and in turn the power increased within a smaller budget, if using pooled samples in cases where the cost of sampling and sample preparation are significantly lower than the cost of chemical analysis, and where the contribution of the biological variance is considerably larger than from the analytical error to the total variation (**Paper I**).

- Chemical analysis could be performed on very small samples, not providing enough material for individual analysis, if pooling them together (Gewurzt et al. 2011). A larger sample volume may also imply a reduced level or limit of detection (LOD) and thus a more precise estimation of the mean value than if individual samples, producing a number of LODs, were analysed (Schisterman et al. 2008).

One disadvantage of using pooled samples compared to individual samples is the so-called pooling error. The pooling error is discussed in Schisterman and Vexler (2008) and is a specific error connected to the physical process of pooling samples (e.g. effects of temperature, instrument, and technician variability).

Pooled samples are often preferable when the chemical analytical cost is considerably higher than the cost of sample preparation or if there is a wish

to reduce specimen variance. This is especially true when the individual measurements, at least to some degree, show autocorrelation, and it is questionable whether they can be considered independent observations. This is the case for most of the organic contaminants in the SNMPCMB and the SNMPCFB. Thus, during the last decade, a majority of the samples within the two programs are pooled. However, even though pooled analysis is performed, the samples are saved individually in the ESB to allow future individual analysis, if necessary.

## 3.4 Matrix selection

When choosing a suitable species for monitoring of contaminants there is a number of criteria that should be met for a species to qualify. These criteria have been highlighted in several publications (Moore 1966, Furness and Camphuysen 1997, Burger and Gochfeld 2004, Goodale et al. 2008, **Paper IV**) and monitoring guidelines (OSPAR 2009, European Commission 2010, HELCOM 2015).

The criteria include features such as the species should reflect the contaminant concentration in the surrounding environment and have the ability to accumulate the contaminant above the limit of detection, but without being toxicologically affected by the contaminant concentration (Moore 1966, OSPAR 2009). The species should be representative of and abundant throughout the whole study area and preferably also (fairly) stationary, thus representing the sampling location (Moore 1966, OSPAR 2009, European Commission 2010). It should be easy to identify, collect, transport, and handle, and large enough to yield sufficient amounts of material for individual chemical analysis (Moore 1966, Furness and Camphuysen 1997, OSPAR 2009, European Commission 2010, HELCOM 2015). The species should fit the demands of the marine conventions and the EU directives and therefore also be a potential food source for predators or humans, in order to be able to evaluate contaminant concentrations in the species against target values set for secondary poisoning or human consumption (European Commission 2010). In addition, it is also important that knowledge about the species biology and ecology is extensive to be able to decrease the variation within and between samples with the purpose of increasing statistical power (Moore 1966, Furness and Camphuysen 1997, Goodale 2008). It is also preferable if the species enable monitoring of biological effect parameters in addition to contaminant analysis. One must further consider if there are any ethical as-

pects involved in the sampling of the species, for example, if sampling affects the abundance of the species and thus future monitoring, or if permission is required to collect the samples.

For analysis of lipophilic organic contaminants, a high and stable fat content is an advantage. If the fish is too lean (fat content below 1%), the fat yield could be up to 25% too low, which in turn generates contaminant values presented on a fat weight basis that are correspondingly too high. The fat yield is very much dependent on the extraction method (Jensen et al 2003). The use of healthy looking specimens with undamaged skin is also preferable, because starved or unhealthy animals with very low fat content can show elevated concentrations of fat-soluble contaminants when expressed on a fat weight basis (Blomkvist et al. 1992, Bignert et al. 1993, HELCOM 2004).

When collecting biological specimens for the purpose of evaluating temporal changes in contaminant concentration, it is crucial to increase the comparability within and between years as much as possible by minimizing variability in the samples (OSPAR 2009) for increased power. A more recent contaminant load is generally represented by sub-adults, because many of these substances tend to bioaccumulate, and the contaminant levels often correlate with fish age (Dušek et al. 2005, Gewurtz et al. 2011). To increase the between-year comparability, younger specimens in the same age range are preferably collected. For herring, it has also been shown that a younger age class is less migratory compared to older age classes, thus are more representative of contamination at the sampling location (Parmanne 1990). Sex is another factor that can influence contaminant concentration in the sample, possibly explained by the elimination of lipophilic contaminants during roe release at spawning in female specimens (Scharma et al. 2009) or sex differences in energy allocation (Madenjian et al. 2011). Therefore, the same sex or the same proportion between sexes should be analysed each year. Contaminant concentrations could be affected by sampling season, especially close to spawning season (Bignert et al. 1993, Farkas et al. 2003), thus sampling close to or during spawning season should be avoided, and the sampling date should be kept consistent between years. Species within the SNMPCFB and SNMPCMB have been collected using the above criteria.

## 3.5 Statistical methods

One of the main aims of **Paper II-V** was to investigate the temporal trends of certain organic contaminants in biota and human milk. The trend analysis was carried out in three steps; first log-linear regression analysis was carried out, followed by the Mann-Kendall trend test, a non-parametric test, and finally non-linear trend components were identified.

Log-linear regression was performed for the entire investigated time period, and for the most recent 10 years, using the yearly geometric mean value in all papers, except **Paper V** where an arithmetic mean was applied due to having only one or two samples analysed per year in the study. Therefore, in this case, a geometric mean would have been equivalent to an arithmetic mean.

The non-parametric Mann-Kendall trend test was performed mainly to check that an indicated trend was not caused by points at the end of the line causing major leveraging. The test normally has a lower power than its parametric counterpart, but it is not affected by differences in magnitude of the concentrations, as it only measures the number of years where the concentration increases or decreases compared to the previous year (Gilbert 1987, Helsel and Hirsch 1995). Thus, if the outcome of the log-linear test is significant and the outcome of the Mann-Kendall test is not, it could either be because the Mann-Kendall test had a lower power or that extreme values at the end of the time series had an unjustifiably high influence on the slope.

In some cases, a non-linear trend is more suitable for description of contaminant development over time. In those cases, a running mean smoother was fitted to the annual geometric mean values. The significance of this line in comparison to the regression line was tested using ANOVA (Nicholson et al. 1998). In addition, non-linear trends could also be investigated using Change-Point detection. A method suggested by Sturludottir et al. (2015) has lately been applied to brominated and perfluorinated compounds that have been banned or phased out during the monitoring period. The method iteratively searches for a combination of two log-linear regression lines in different directions that explains significantly more of the total variance than that explained by a single regression line for the whole study period. In Figure 3.3, this method has been used for concentrations of the brominated flame retardant BDE-47 in guillemot eggs. A significant change point was detected in 1985. The advantage with the Change-point approach over the running

mean smoother, is that average slopes and halving/doubling times for two periods and a change-point year can be estimated quantitatively.

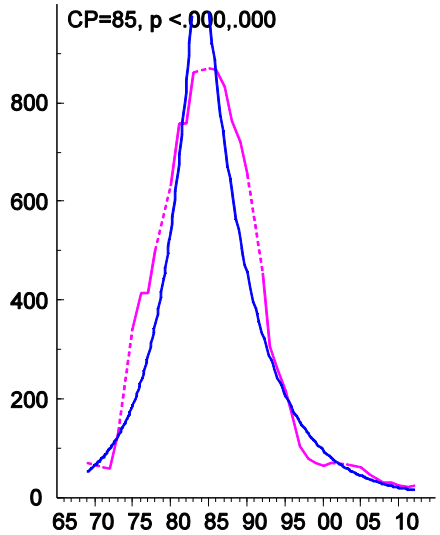


**Figure 3.3.** Temporal running mean smoother (5 years, purple line) and Change point (CP) detection (blue lines) of the brominated flame retardant, BDE-47, (ng/g fat weight) in guillemot egg 1969-2012.

In time series where contaminant concentrations are affected by confounders (confounding factors), adjustment for confounders is possible, at least if samples are analysed individually. Fat content can influence contaminant concentrations in cases where the fat content is very variable (Grimås et al 1985, Bignert et al 1993). Occasionally, this is of major importance for the evaluation of time series and sometimes it could even change the direction of a slope. The contaminant concentrations in cod liver and spring caught herring from Utlängan have hence been adjusted for varying fat content (**Paper II**). Age could also have a significant influence of contaminant concentration (e.g. Hg in herring muscle, Braune 1987). In these cases, an adjustment for age could improve the time series considerably. In Figure 3.4 the ∑PCDD/F (WHO-$TEQ_{2005}$) in human milk from Uppsala (individual analysis) is illustrated. The concentration is significantly affected not only by the change over time, but also by the age of the mothers, BMI (Body Mass Index), and the weight gained during pregnancy. In the time series adjusted for age, BMI, and weight gain, the CV has improved from 41% to 30%, and the lowest detectable trend (LDT) over a period of 10 years at a power of 80% im-

proved from 2.8% to 2.1%. The confounders could also be abiotic in character, such as temperature, pH, salinity, and productivity (Somero et al. 1977, Cusimano and Brakke 1986, Larsson et al. 1992).

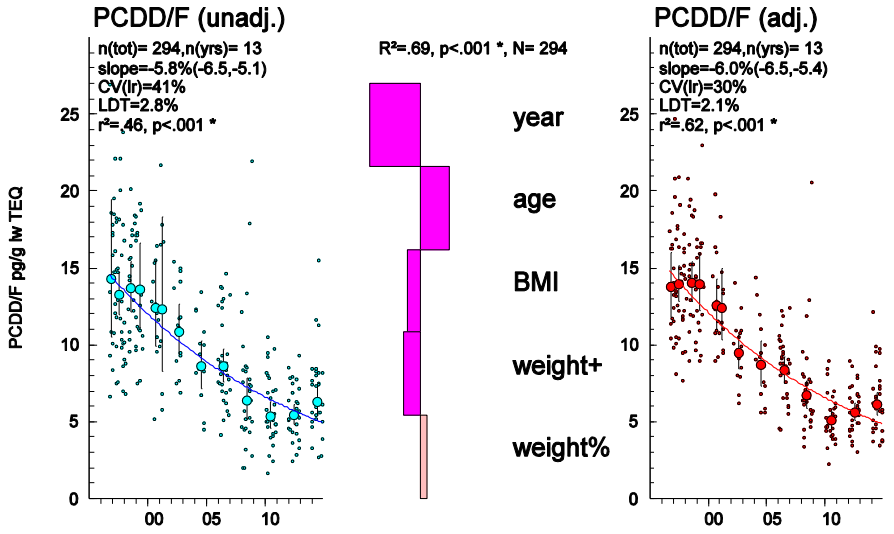


**Figure 3.4.** Temporal trend of ∑PCDD/F (WHO-TEQ$_{2005}$) (pg/g fat weight) in human milk from Uppsala. Unadjusted data on the left hand side, significant confounders in the middle and adjusted data on the right hand side. The colour of the blocks indicate p-values, purple:p<0.001 and pink: p<0.05 and confounders with blocks on the left of the central line affect the data negatively and on the right positively. **n(tot)** and **n(yrs)** are the total number of analysis and years, slope is the yearly change and its confidence intervals, **CV(lr)** is the coefficient of variation, **LDT** is the lowest detectable trend during 10 years at a power of 80%, and **r$^2$** is the coefficient of determination together with a p-value for a two sided test, (Figure slightly modified from Glynn et al 2016a).

Observations that are exceptionally far from the overall mean, the regression line, or the smoother, are of special concern. These observations are often referred to as outliers or extreme values. They could be caused by something unusual in the surrounding physical environment, a change in the pollution pattern, or errors in sampling procedure or chemical analysis. Outliers can have a major influence on the statistical power to detect temporal trends, either by changing the trend and/or masking its significance. A number of statistical methods have been proposed for the detection of outliers (Grubbs 1969, Hoaglin and Welsh 1978, Rosner 1983). However, it is very important that the removal of outliers is stated and motivated in order to avoid accusations of fraud (Wade 1976). In **Papers II-V**, the method described by

Hoaglin and Welsh (1978) was used; however, suspected outliers are indicated in the figures and still included in the statistical calculations.

Measurements below the quantification limit (LOQs) are calculated using LOD multiplied by 3. In the literature, there are several different ways describing how to treat LOQs, for example substituting them using the reported LOQ divided by 2, or divided by the square root of 2, but an even better technique is the regression based method described in Helsel (2005). However, the Helsel method requires a certain number of analysis above LOQ each year, and the method is thus not always suitable. In **Papers II-IV**, LOQs have been divided by the square root of 2; in **Paper V** LOQs have been divided by 2.

Assessing compliance towards a certain quality standard (i.e. comparing measured concentrations in biota with the standard) could play an important role in decision making, for example concerning permits, and when identifying risks from chemicals prior to implementing measures. Compliance checking could be performed in different ways, either by comparing the mean of a number of samples with the target value, or by using more statistical approaches that take into account the uncertainty in the measured values. The latter approach is required if the compliance assessment should be supported by some sort of estimate of the confidence of the outcome of the assessment (European Commission 2013).

Two scenarios assessing compliance using confidence intervals are illustrated (Figure 3.5, 3.6) and are referred to here as the "Brown" test (favours the polluter, Figure 3.5) and the "Green" test (favours the environment, Figure 3.6). The probable outcome, if a fail or pass decision is made on the basis of the lower confidence interval (Figure 3.5), is that a poor investigation with larger confidence intervals will give a pass even though the mean fails to reach levels below the quality standard. On the contrary, an investigation of high quality is awarded within the Green test, where smaller confidence intervals will lead to a pass where both the mean and the upper confidence level are below the quality standard. A poor investigation will provide a fail with the upper confidence level above the quality standard (Figure 3.6) (European Commission 2013).

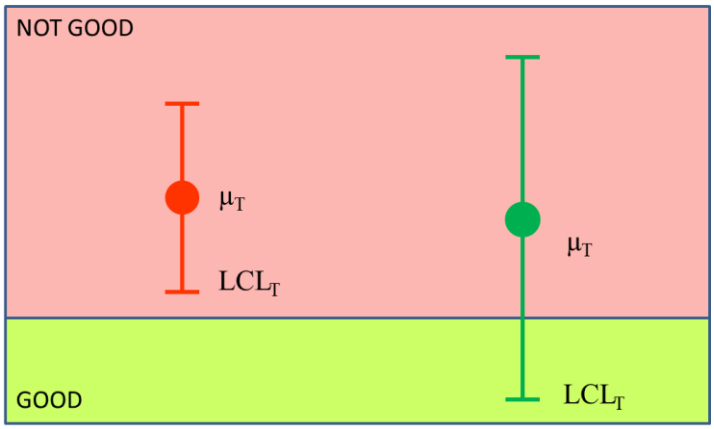


**Figure 3.5.** The "Brown" test within compliance assessment. To be significantly above the Quality Standard (indicated by the black line between good and not good status), the Lower Confidence Level ($LCL_T$) must not go below the Quality Standard.

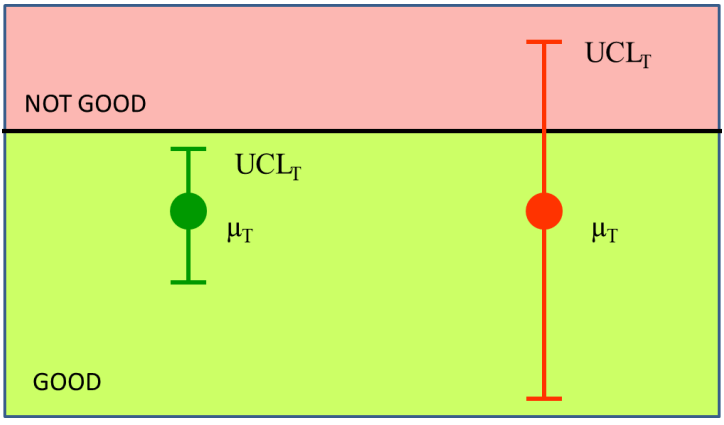


**Figure 3.6.** The "Green" test within compliance assessment. To be significantly below the Quality Standard (indicated by the line between good and not good status), the Upper Confidence Level ($UCL_T$) must not go above the Quality Standard.

When assessing compliance, the choice of value (e.g. mean, upper mean CL, or upper population CL) compared to the target level is central. Figure 3.7 is illustrating the difference between these three choices when assessing what year a contaminant (here ∑PCDD/F+DL-PCB) will reach compliance with a target level in human milk. The upper CL of the population will reach a body burden of ∑PCDD/F+DL-PCB regarded as safe, from a conservative fetal health point-of-view, in 2028.

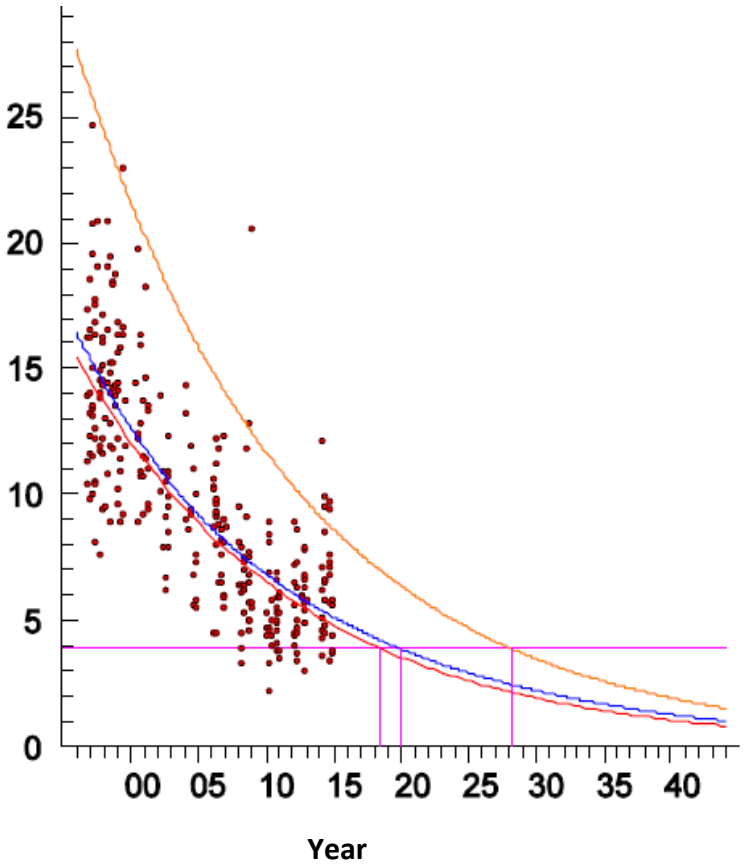


**Figure 3.7.** Extrapolated trends of temporal total ∑PCDD/F+DL-PCB (WHO-$_{TEQ2005}$ pg/g l.w.) concentrations in human milk, 1996-2014. The orange line represents the 95% confidence interval for the whole population, the blue line represents the 95% confidence interval for the mean, and the red line is representing the mean. The purple lines are showing when the trend reaches a body burden of 3.9 WHO-$_{TEQ2005}$ pg/g l.w.. The upper orange line is crossing a body burden of 3.9 pg WHO-$_{TEQ2005}$ pg/g l.w. in 2028. This maternal body burden, which is half of the US EPA non-cancer reference dose (Rfd) body burden of 7.9 pg WHO-$_{TEQ2005}$ pg/g l.w., could be regarded as safe from a fetal health point-of-view. The figure has been published in Glynn et al. 2016b.

# 4 Species/matrix comparison

One area where assessment could be improved is within the selection of monitoring species/matrices. In order to elucidate which species/matrices are more suitable than others, a number of issues related to key features for a monitoring species are discussed. Many of these features regarding guillemot egg and herring are discussed in more detail in **Paper III** and **IV**. The contaminants used for comparison of the species are PCBs (e.g. CB-153, the congener which is normally found in the highest concentrations in biological samples) and the sum of the toxic equivalents for PCDD/Fs, as data on these groups of substances are presented for most species/matrices in the publications in this thesis (**Paper II-V**).

Species within the SNMPCFB and SNMPCMB have all been chosen because of their suitability as monitoring species, hence many of the criteria presented in chapter 3.4, such as: their potential as a food source for top predators or humans; representativeness of recent contaminant load; abundance throughout the study area; size; ease to identify, transport and handle; good knowledge about species biology and ecology; suitability regarding the demands of the marine conventions and the EU directives; and their appropriateness regarding ethical considerations, are already in general fulfilled for all species. Therefore these features will not be discussed further within this thesis.

## 4.1 Representativeness of the sampling location

The species representativeness of the sampling location is central to temporal and spatial studies. Within the Baltic Sea, herring is a migratory fish species (Parmanne 1990) and the specific location where the contaminants are accumulated is therefore not known. However, a study by Bignert et al. (2007) on concentrations of PCDD/Fs in herring muscle sampled along the Baltic coast, showed spatial autocorrelation between samples in the range of about 100 km. Measured concentrations were relatively representative of the sampled region during the sampling season. Studies on Atlantic cod have

shown that the species could show both stationary and migratory behaviour depending on if it is a coastal population and the age of the cod (Godø 1995, Storr-Paulsen et al. 2004). Baltic cod also show variation in their migration pattern, with both stationary and long distance migration observed (Neuenfeldt et al. 2007). Perch and eelpout are considered fairly stationary fish species (Thorpe 1977, Jacobssen et al. 1993), hence they acquire their contaminant burden in the surrounding area. Guillemot remain in ice-free areas of the southern Baltic Proper year round (Stolt et al. 1991), thus is also relatively stationary. Contaminant concentration could be considered as locally acquired. Adult blue mussels are sessile, thus providing a time-integrated picture of local contamination (Cantillo 1998).

Human milk is not very representative of the sampling location. The major source of POPs in human milk is via food intake (Darnerud et al. 2006), with the exception of polybrominated flame retardants (PBDEs) where indoor dust may be considered as equally important for exposure (Fredriksen et al. 2009). Food consumption is becoming more and more globalized, at least in industrialized countries. In a study on regional differences of contaminants in human milk from four different cities in Sweden, concentrations of PCBs and PBDEs were quite similar even though there was spatial variability in the examined regions (Glynn et al. 2011). However, it should still be stressed that studies of human milk give representative and important measures of the range and average of the exposure to the fetus in the surveyed regions.

## 4.2 Fat content

All POPs discussed in this thesis dissolve in fat, hence the fat content of a monitoring matrix is therefore important. The advantages of high and stable fat content in a matrix/organ for monitoring of fat soluble contaminants are discussed in more detail in section 3.4.

Guillemot egg has a relatively high and stable fat content (extractable fat content 11-13%). Cod liver also has a high extractable fat content, however it is very variable (12-52%). Herring muscle tissue from the Bothnian Bay has an extractable fat content of around 2%. The fat content increases towards the Baltic Proper and the Swedish west coast where it can reach levels above 5%. The fat content in herring is not as stable as in guillemot egg, and stability varies between sampling sites, with the highest variability observed in spring caught herring from Utlängan (Baltic Proper) and autumn caught

herring from Väderöarna (Swedish west coast), 2-10% and 1-6% respectively (Bignert et al. 2016a). Arctic char muscle has extractable fat content in the same range as herring from the Bothnian Bay/Sea (1-3%). Blue mussel have slightly lower extractable fat content of 1-1.5%. Perch (0.6-0.8%), eelpout (0.5-0.7%), and pike (0.6%) muscle all have fat content below 1%.

## 4.3 Integrated monitoring

Integrated monitoring, including fish community and population variables, biological effect parameters and contaminant analysis, allows an assessment of any actual harm caused by contaminants from a subcellular to community level. Potential synergistic, antagonistic, and cocktail effects, and the influence of natural stressors such as salinity or temperature, are also included in the integrated assessment. Analysing and assessing the three classes of parameters at the same time has several advantages. If no correlations between monitored contaminant and biological effects are found, a search for new potential chemical candidates needs to be initiated. Changes in the biological effect parameters may also have an effect on growth, reproduction, and survival. Alterations at ecosystem level may be found, but possible explanations are often difficult to find from population studies alone (Sandström et al 2005).

The choice of effect parameters aims to reflect population characteristics and important physiological functions such as growth, energy storage and metabolism, reproduction, liver function, and immune defence. Effect parameters are in some cases related to specific groups of contaminants, for example certain organic chemicals and EROD (Ethoxyresorufin-O-deethylase) activity, and estrogenic substances, and vitellogenin in male plasma (Sandström et al 2005).

Species used for integrated monitoring should be fairly stationary, preferably during all life stages, since they are more suitable for monitoring effects of local pollution with one of the aims of linking observed effects in the field with the source/sources of the pollution. It is also important that the species provides the ability to separate contaminant-related effects from influences that could be caused by other factors such as natural variability, temperature, salinity, food availability, and so on. It should also be fairly sensitive to contaminants (ICES 2012). In Sweden, integrated fish monitoring is focused on perch and eelpout, two species which have a rather stationary behaviour

(Thorpe 1977, Jacobssen et al. 1993, Sandström et al 2005). The eelpout is viviparous, which enables a number of studies related to eggs (e.g. larval deformities and dead or malformed larvae) (Hedman et al 2011). Other parameters are monitored within the fish effect monitoring program such as EROD, GSI, vitellogenin, DNA-adducts, PAH-metabolites in bile, liver histopathology, intersex, and externally visible fish disease. Perch is not viviparous, thus only sampled for the latter analysis; however, perch is one of the most dominant species in the Baltic archipelagos and freshwater ecosystem.

Bird eggs are also suitable for integrated monitoring because the eggshell can be affected by chemicals (Bignert et al. 1995, Helander et al 2002, Bignert and Helander 2015) and measurements of physical parameters such as shell thickness, length and shell index, are sufficient for effect monitoring. Shell parameters have been measured in guillemot eggs since the start of monitoring in 1969. Furthermore, guillemot is relatively stationary since it remains in ice-free areas in the southern Baltic Proper all year.

Mussel is a species commonly used for measurements of biological effects of contaminants. Mussels are common, wide spread, sessile, and display a wide range of biological responses to contaminants (Salazar and Salazar, 1995, ICES 2012). However, blue mussel are not yet included in the Swedish integrated contaminant monitoring. Eelpout and blue mussel have been identified as key species for integrated monitoring within the OSPAR area (OSPAR 2012).

## 4.4 Temporal trends

CB-153 has been analysed temporally in the matrices discussed in this doctoral thesis. Results of the temporal analysis are shown (Table 4.1, Figure 4.1). Here, CB-153 was decreasing significantly over time, in the range of -2.2 to -8.8% per year, in all marine matrices except for cod liver (both sampling sites), eelpout muscle (Holmöarna and Fjällbacka) and herring muscle (Väderöarna). In the freshwater environment, CB-153 was decreasing significantly in Arctic char muscle (both sampling sites) and pike muscle from Storvindeln, between -3.6 to -7.2% annually. The concentrations of CB-153 in human milk samples from the Stockholm area were also decreasing significantly over time, at -4.8% per year. The most rapid decrease (-8.8%) was seen in eelpout muscle from Kvädöfjärden, most probably due to exceptionally high concentrations observed at the beginning of the monitoring period

(Table 4.1). Decreases in CB-153 concentration seen in a majority of matrices since the 1970s is a result of the ban of all new use of PCBs in 1978, and a number of measures taken to reduce concentrations in the environment.

In order to combine temporal trends for CB-153 at various contaminant levels in the same graph (Figure 4.1), data were transformed to percent of maximum concentration (within a specific time series and because the length of the time series varies, data were transformed to percent of the concentration recorded in 1995) for representative species (long time series or geographically close). Even though levels of CB-153 have decreased in most species and sites since the beginning of monitoring, the non-linear trends differ (Figure 4.1). Guillemot egg (St Karlsö), human milk (Stockholm), Arctic char (Abiskojaure), pike (Storvindeln) and eelpout (Kvädöfjärden) display quite similar trends, while herring (Utlängan), cod (south of Gotland), perch (Kvädöfjärden) and blue mussel (Kvädöfjärden) show quite large between-species variations.

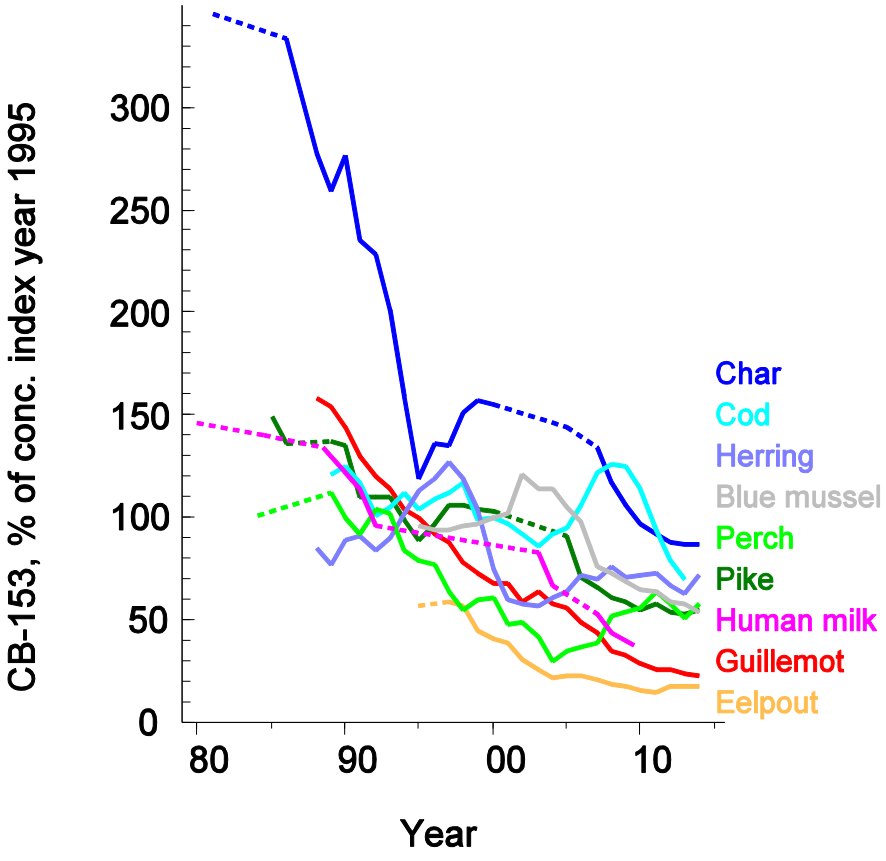


**Figure 4.1.** Temporal running mean smoother (5 years) of CB-153 (expressed as % of the value recorded in 1995) in autumn caught herring muscle (light blue line) from Utlängan (Baltic Proper); guillemot egg (red line) from St Karlsö (Baltic Proper); eelpout muscle (yellow line) from Kvädöfjärden (Baltic Proper); perch muscle (light green line) from Kvädöfjärden (Baltic Proper); blue mussel (grey line) from Kvädöfjärden (Baltic Proper); cod liver (turqoise line) from south of Gotland (Baltic Proper); pike muscle (dark green line) from Lake Storvindeln (northern Sweden); Arctic char muscle (dark blue line) from Lake Abiskojaure (northern Sweden) and human milk (purple line) from Stockholm. Dotted line segments indicate that one or more years are missing.

Temporal analysis of PCDD/Fs (represented by ∑PCDD/F (WHO-$_{TEQ1998}$)) has only been carried out for a few of the matrices and sampling sites discussed here (Table 4.2, Figure 4.2). Concentrations were decreasing significantly in guillemot egg, herring from Ängskärsklubb and Fladen, and in human milk from the Stockholm area (-2.8, -5.8, -0.77 and -5.9%, respec-

tively). By contrast, no trends were observed in the perch and pike time series from the freshwater environment. However, the levels were very high both in milk and herring from Ängskärsklubb in the beginning of the monitoring period, which was not the case for the freshwater fish.

Figure 4.2 illustrate the combined temporal trends of ∑PCDD/F (WHO-$_{TEQ1998)}$) for the species listed in Table 4.2 (because the length of the time series varies, data was transformed to percent of the concentration recorded in 1995). The figure shows that the temporal trend for guillemot egg and human milk are very similar until the late 1990s, after which it appears the ∑PCDD/F (WHO-$_{TEQ1998)}$) continue to decrease in milk while the trend in guillemot egg is levelling out. This may be explained by the fact that the advice concerning food for girls, reproductive aged, and pregnant women, developed by the Swedish Food Agency, has become more detailed and brought to the public's attention during the last decades. The dietary advice should, if reaching its goal, result in girls, reproductive aged, and pregnant women eating less food containing high levels of PCDD/Fs. Thus the levels in milk could continue to decrease at the same rate although the temporal trend in the environment has levelled out. In addition, food consumption patterns have changed considerably during recent years, representing a more global contaminant burden. The temporal trends in herring, perch and pike start much later and indicate a trend resembling the one seen in guillemot during the last decades.

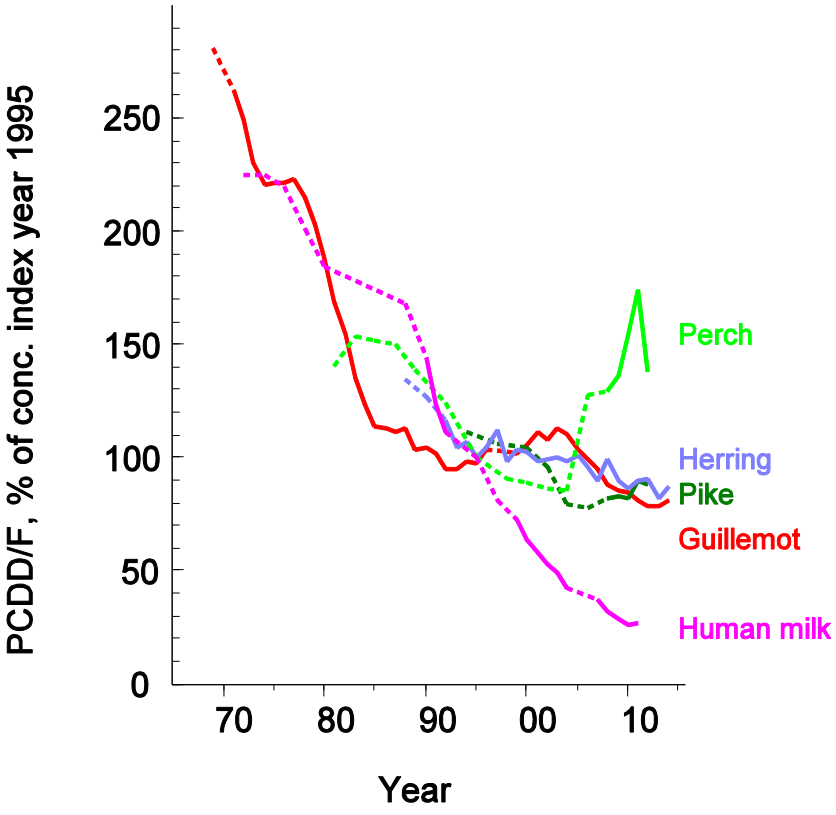


**Figure 4.2.** Temporal running mean smoother (5 years) of ∑PCDD/F (WHO-TEQ1998)) (expressed as % of the value recorded in 1995) in herring muscle (light blue line) from Utlängan (Baltic Proper); guillemot egg (red line) from St Karlsö (Baltic Proper); perch muscle (light green line) from Lake Skärgölen (southern Sweden); pike muscle (dark green line) from Lake Bolmen (northern Sweden); and human milk (purple line) from Stockholm. Dotted line segments indicate that one year or more is missing.

A small coefficient of variation (CV) is essential for detecting small changes in contaminant concentration (Gilbertson et al. 1987). The CV is, as mentioned before, affected by a number of things, e.g. physiological difference of the samples, abiotic factors, sampling strategy, as well as effects of long term storage, sample treatment, and analytical precision.

The time series presented in Table 4.1 and 4.2 are not always based on the yearly geometric means of individual analysis. In some cases, pooled samples are used and in others there is a variety of pooled and individual sam-

ples over the years. Time series with individual values are calculated using geometric mean values, which are less sensitive to extreme values compared to arithmetic mean values, which tend to decrease the CV slightly. Pooled samples are equal to an arithmetic mean value and in the case of a mixture of pooled and individual samples, geometric mean values are used for the years where individual analysis is performed and arithmetic for the years using pooled samples. The CV when using pooled samples is affected by the number of samples in the pool and also their sensitiveness to extreme values (**Paper I**).

Here, for CB-153, the median CV is 35%, ranging from 15 to 64%. A CV of 35% corresponds to a lowest detectable trend (LDT) in 10 years of 12-13% (ranging from 5.4-23%) and an estimated number of years required (YQR) to detect an annual change of 10% of 12 years (ranging from 8-16 years), both at a power of 80% (Table 4.1.).

The largest CV was seen in eelpout muscle from Holmöarna (64%) (Table 4.1). However, the monitoring of eelpout stopped in 2007 at Holmöarna, so that site may not be comparable to the other sites. The second largest CV was observed in perch from Lake Stensjön and Kvädöfjärden (55% and 54% respectively) followed by eelpout from Kvädöfjärden (49%) and herring from Harufjärden (44%). Kvädöfjärden has been subjected to intensified monitoring lately because some biological effect parameters have indicated that the area might be affected by contaminants. By contrast, blue mussel from Kvädöfjärden have in general a lower CV (26%) similar to the CV in blue mussel from the Swedish west coast (26-37%).

The lowest CV was seen in perch from Skärgölen (15%), but the temporal trend was based only on pooled samples (except one year) and it was a short temporal series (1999-2012, 7 years analysed), and no trends were indicated. In contrast to Skärgölen, Lake Stensjön had one of the highest CV, however here quite a low number of years were also analysed (1997-2012, 12 years analysed), but 50% of the samples analysed were individual and a trend was indicated during the last ten years. The second lowest CV was observed in guillemot egg from Stora Karlsö (17%) closely followed by pike from Storvindeln (19%). The CV seen in herring from the same area as the guillemot egg (Utlängan) was higher (36%).

The median CV for ∑PCDD/F (WHO-TEQ1998) is 30% (range 19 to 50%). A CV of 30% corresponds to a LDT in 10 years of 11% (range 8.1-18%),

and an estimated YQR to detect an annual change of 10% for 11 years (ranging from 9-14 years), both at a power of 80% (Table 4.2).

The highest CV seen for ∑PCDD/F (WHO-TEQ1998) was in herring from Ängskärsklubb (50%) (Table 4.2), most probably explained by the high and varying concentrations observed at the beginning of the monitoring period. The second largest CV was observed for pike from Storvindeln (42%) followed by perch from Skärgölen (39%). Human milk had the lowest CV (19%) closely followed by guillemot egg (22%). The temporal trend in milk was only based on pooled samples and the temporal trend in guillemot egg was mainly based on pooled samples.

## 4.5 Concentrations and compliance

In general, POPs bioaccumulate and biomagnify, which means that concentrations increase with increasing trophic level (HELCOM 2004, OSPAR 2007). All species within the SNMPCMB, SNMPCFB, as well as humans, bioaccumulate PCBs and PCDD/Fs to a certain extent. Regarding guillemots, the contaminant concentration seen in the adult females is mirrored in their eggs (Furness and Camphuysen 1997; Goodale et al. 2008)). This is also the case for human milk where the levels correlate well with levels measured in maternal serum (Darnerud et al. 2010, Darnerud et al. 2015). Hence, within the marine ecosystem the assumption could be made that the eggs are of similar trophic level as the adult birds. Guillemot from Stora Karlsö in the Baltic Sea mainly feed on sprat and herring (Österblom et al. 2001) and the levels (on a lipid weight l.w. basis) of ∑PCDD/F (WHO-$TEQ_{1998}$) (783 pg/g l.w.) and CB-153 (1.41 ug/g l.w) are about 30 and 18 times higher respectively in guillemot egg compared to herring muscle from sampling sites located in the south Baltic Proper (Utlängan and St Karlsö, Table 4.1, 4.2). This implies that PCDD/Fs and PCBs bioaccumulate in the egg to a high extent. Perch, eelpout (muscle), and blue mussel from Kvädöfjärden have concentrations of CB-153 in the same order of magnitude as in herring. The levels in cod liver from the south of Gotland are slightly higher than in herring (**Paper II and IV**; Table 4.1, 4.2). For the freshwater species it is difficult to make a general assumption because they are from different lakes where the contaminant input could vary a lot. However, the levels in pike (on a lipid weight basis) are slightly higher than in Arctic char from remote lakes in the north of Sweden and perch from the same area has levels comparable to the levels seen in Arctic char (**Paper III**,

Table 4.1). The levels seen in Swedish human milk after the year 2000 for PCDD/Fs (**Paper V**, Table 4.2) are only a tenth of the concentration seen in herring, whereas concentration of CB-153 (Fång et al 2015, Table 4.1) is only slightly lower than in herring.

The biota EQSs for evaluation of compliance are primarily set for fish within the WFD and MSFD, as mentioned in chapter 2.5.1 (European Commission 2013). OSPAR has target levels (EACs) for fish, mussel, and oyster for most CEMP substances (OSPAR 2014). Regarding the EQSs set for fish, it is not specified which species or TL that should be sampled. It is merely recommended in the guidelines (European Commission 2013) that the data should be transformed to a TL according to a protection goal of the established EQS, which in the freshwater environment is set to 3-4 and to 5 in the marine environment. To be able to adjust the data for a specific species to an accurate TL, knowledge about the species TL is needed and also information concerning the TMF, which are factors describing the average biomagnification (BMF) of a substance per trophic level. Stable isotope analysis (SIA) determining the ratio of the isotopes $^{15}N/^{14}N$ ($\delta^{15}N$) can be used to estimate the trophic level of a species (Peterson and Fry 1987). To establish the correct TL, a baseline also has to be determined using SIA (European Commission 2013).

The trophic position for the different fish species could be calculated using an equation from Post (2002);

Trophic position = $2 + (\delta^{15}N$ fish$– \delta^{15}N$ baseline organism$)/3.4$

In the Baltic and at the Swedish west coast, blue mussel can be used as the baseline. However, both the $\delta^{15}N$ for the baseline (blue mussel) and the TL for herring, cod, and eelpout differed between the Baltic Sea and the Swedish west coast (Bignert et al. 2016a). This implies that the difference in TL between the same species from different lakes should not be underestimated.

TMFs are calculated using the relationship between the contaminant concentrations and the TL of organisms in the food web (Fisk et al 2001). Several studies on BMFs and TMFs for organochlorines in different food webs are available (e.g. Kiriluk et al. 1995, Kidd et al. 1995, Kidd et al. 1995b, Kidd et al.1998, Kucklick and Baker 1998, Kid et al. 2001). However, the TMF approach assumes that diet is the dominant route of exposure to contaminants and that TL is the major driver for accumulation of contaminants (Broman et al. 1992). This implies that other factors that are important for

the contaminant concentration in a sample, such as age and size, can have a confounding effect on the regression line between TL and contaminant level (Borgå et al 2012). Borgå et al. (2012) discuss a number of factors that may affect TMFs, for example properties of the organism (affecting the metabolic rate), variables affecting the characterization of the food webs using stable isotopes, ecosystem characteristics (e.g. productivity, species composition, salinity) etc.

The EQSs in fish are set for either whole body, if the protection goal is secondary poisoning of a top predator, or the muscle fillet, if the protection goal is human. The most sensible matrix to monitor (e.g. regarding fat content or dry content) might not be whole body or muscle. Metals, with the exception of mercury and perfluorinated substances, are generally monitored in the liver because the concentrations are higher and generally above LOQ. Recalculations between organs are in these cases needed for compliance assessment and the recalculation factors might differ between species and also within species from different areas with varying contaminant concentrations (Boalt et al. 2014). The various adjustments and assumptions (TL, TMF, organ conversions) that facilitate comparisons between various media and matrices and are required to evaluate compliance with quality standards may add a lot of uncertainty. It is a prerequisite that robust and reliable methods are developed for this important issue to make these assessments meaningful.

EQSs for the PAHs represented by Benzo[a]pyrene and Fluoranthene are set for crustaceans and molluscs. No conversions between species or areas are suggested in the guidance document regarding the compliance against the set target levels (European Commission 2013). EQSs for bird eggs have not yet been established within the EU, and neither has OSPAR developed EACs for bird eggs. The guidance on biota monitoring states that solely pelagic food chain species, which excludes birds and mammals, could be adjusted by TMFs. However, OSPAR has done some work on Ecological Quality Objectives (EcoQOs) for eggs of Common and Arctic tern (*Sterna hirundo* and *Sterna paradisaea*) and oystercatchers (*Haematopus ostralegus*) in the North Sea to evaluate environmental health. The EcoQOs are target levels that estimate the expected status after a complete stop of any further input of pollutants.

Levels of contaminants in milk are straight forward when it comes to compliance. Concentrations of organic contaminants could be evaluated directly

against the TDI set for infants if the daily milk consumption is known. It is often easier to evaluate human milk because infants generally feed exclusively on human milk, at least during the time period when the milk is collected.

**Table 4.1.** Trend for the entire period (in %) for **CB-153** assessed from the annual geometric mean if the number of analysis per year is > 2, otherwise it is based on the mean (μg $g^{-1}$ lipid weight) in guillemot egg, Arctic char, cod, eelpout, herring, perch, pike, blue mussel, and human milk. P shows the p-value for a log-linear regression. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. CV% is the coefficient of variation around the regression line as a measure of between-year variation. Last year's CB-153 concentration values are estimated from the trend if $p<0.05$ and from the mean if no trend is present. The total number of years for the various time-series are shown in column three.

| Matrix, Sampling site | Period | N years | Trend % | P | YRQ | LDT % | Power | CV % | Last year μg/g l.w. | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **Guillemot egg** | | | | | | | | | | |
| Stora Karlsö | 88-12 | 25 | -7.5 | 0.00 | 8 | 6.2 | 1.0 | 17 | 1.4 | **Paper III**, Bignert et al. 2014 |
| **Arctic char muscle** | | | | | | | | | | |
| Abiskojaure | 81-12 | 23 | -4.4 | 0.00 | 11 | 11 | 1.0 | 31 | 0.009 | **Paper II,** Nyberg et al. 2013 |
| Tjulträsk | 86-12 | 9 | -7.2 | 0.00 | 12 | 13 | 0.45 | 34 | 0.014 | **Paper II,** Nyberg et al. 2013 |
| **Cod liver** | | | | | | | | | | |
| SE Gotland | 89-12 | 24 | -1.2 | 0.11 | 10 | 9.1 | 1.0 | 25 | 0.16 | **Paper III**, Bignert et al. 2014 |
| Fladen | 89-12 | 23 | .39 | 0.72 | 12 | 13 | 1.0 | 37 | 0.47 | **Paper III**, Bignert et al. 2014 |
| **Eelpout muscle** | | | | | | | | | | |
| Holmöarna | 95-07 | 11 | -3.0 | 0.54 | 16 | 23 | 0.32 | 64 | 0.12 | **Paper III**, Bignert et al. 2014 |
| Kvädöfjärden | 95-12 | 17 | -8.8 | 0.00 | 12 | 12 | 1.0 | 34 | 0.069 | **Paper III**, Bignert et al. 2014 |
| Fjällbacka | 95-12 | 18 | -.33 | 0.85 | 14 | 18 | 0.99 | 49 | 0.26 | **Paper III**, Bignert et al. 2014 |
| **Herring muscle** | | | | | | | | | | |
| Harufjärden | 87-12 | 24 | -2.6 | 0.04 | 13 | 16 | 1.0 | 44 | 0.037 | **Paper III**, Bignert et al. 2014 |
| Ängskärsklubb | 89-12 | 24 | -5.2 | 0.00 | 13 | 14 | 1.0 | 39 | 0.064 | **Paper III**, Bignert et al. 2014 |
| Landsort | 87-12 | 26 | -5.9 | 0.00 | 13 | 15 | 1.0 | 43 | 0.042 | **Paper III**, Bignert et al. 2014 |
| Utlängan | 88-12 | 25 | -2.2 | 0.03 | 12 | 13 | 1.0 | 36 | 0.069 | **Paper III**, Bignert et al. 2014 |
| Fladen | 88-12 | 25 | -5.9 | 0.00 | 10 | 9.4 | 1.0 | 26 | 0.019 | **Paper III**, Bignert et al. 2014 |
| Väderöarna | 95-12 | 17 | -3.0 | 0.11 | 13 | 15 | 1.0 | 42 | 0.016 | **Paper III**, Bignert et al. 2014 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Perch muscle** | | | | | | | | | | |
| Holmöarna | 95-12 | 18 | -6.4 | 0.00 | 12 | 13 | 1.0 | 36 | 0.039 | **Paper III**, Bignert et al. 2014 |
| Kvädöfjärden | 89-12 | 25 | -3.3 | 0.03 | 15 | 19 | 1.0 | 54 | 0.033 | **Paper III**, Bignert et al. 2014 |
| Skärgölen | 99-12 | 7 | -1.6 | 0.30 | 8 | 5.4 | 0.77 | 15 | 0.017 | **Paper II,** Nyberg et al. 2013 |
| Stensjön | 97-12 | 12 | 0.57 | 0.83 | 15 | 19 | 0.52 | 55 | 0.028 | **Paper II,** Nyberg et al. 2013 |
| **Pike muscle** | | | | | | | | | | |
| Bolmen | 88-12 | 21 | -1.2 | 0.21 | 12 | 12 | 1.0 | 34 | 0.213 | **Paper II,** Nyberg et al. 2013 |
| Storvindeln | 85-12 | 22 | -3.6 | 0.00 | 9 | 6.9 | 1.0 | 19 | 0.061 | **Paper II,** Nyberg et al. 2013 |
| **Blue mussel** | | | | | | | | | | |
| Nidingen | 88-12 | 25 | -8.1 | 0.00 | 10 | 9.4 | 1.0 | 26 | 0.015 | **Paper III**, Bignert et al. 2014 |
| Fjällbacka | 88-12 | 24 | -4.4 | 0.00 | 12 | 13 | 1.0 | 37 | 0.029 | **Paper III**, Bignert et al. 2014 |
| Kvädöfjärden | 95-12 | 18 | -2.9 | 0.00 | 10 | 9.5 | 1.0 | 26 | 0.043 | **Paper III**, Bignert et al. 2014 |
| **Human milk** | | | | | | | | | | |
| Stockholm | 72-09 | 13 | -4.8 | 0.00 | 11 | 11 | 0.99 | 25 | 0.04 | Fång et al 2015, unpub.data* |

*The time series is presented in Fång et al. 2015, but not the data in the table.

**Table 4.2.** Trend for the entire period (in %) for **∑PCDD/F (WHO-$_{TEQ1998)}$** assessed from the annual geometric mean if the number of analysis per year is >2 otherwise it is based on the mean (pg $g^{-1}$ lipid weight) in guillemot egg, herring, perch, pike and human milk. P shows the p-value for a log-linear regression. YRQ: years required to detect an annual change of 10% with a power of 80%. LDT: lowest detectable trend within a 10 year period with a power of 80%. CV% is the coefficient of variation around the regression line as a measure of between-year variation. Last year's ∑PCDD/F (WHO-$_{TEQ1998)}$ concentration values are estimated from the trend if p<0.05 and from the mean if no trend is present. The total number of years for the various time-series are shown in column three.

| Matrix, Sampling site | Period | N years | Trend % | P | YRQ | LDT % | Power | CV % | Last year pg/g l.w. | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| **Guillemot egg** | | | | | | | | | | |
| Stora Karlsö | 70-11 | 41 | -2.8 | 0.00 | 9 | 7.9 | 1.0 | 22 | 783 | **Paper IV**, Bignert et al. 2013 |
| **Herring muscle** | | | | | | | | | | |
| Harufjärden | 90-11 | 21 | 2.0 | 0.11 | 12 | 13 | 1.0 | 36 | 36.2 | **Paper IV**, Bignert et al. 2013 |
| Ängskärsklubb | 79-11 | 29 | -5.8 | 0.00 | 14 | 18 | 1.0 | 50 | 21.9 | **Paper IV**, Bignert et al. 2013 |
| Utlängan | 88-11 | 22 | -0.77 | 0.39 | 10 | 9.7 | 1.0 | 27 | 24.4 | **Paper IV**, Bignert et al. 2013 |
| Fladen | 90-11 | 22 | -1.9 | 0.03 | 10 | 8.7 | 1.0 | 24 | 6.52 | **Paper IV**, Bignert et al. 2013 |
| **Perch muscle** | | | | | | | | | | |
| Skärgölen | 81-11 | 16 | -0.83 | 0.40 | 13 | 14 | 1.0 | 39 | 11.4 | Nyberg et al. 2012 |
| **Pike muscle** | | | | | | | | | | |
| Bolmen | 94-11 | 10 | -1.7 | 0.34 | 11 | 11 | 0.75 | 30 | 62.2 | Nyberg et al. 2012 |
| Storvindeln | 91-11 | 12 | -3.6 | 0.09 | 13 | 15 | 0.59 | 42 | 13.7 | Nyberg et al. 2012 |
| **Human milk** | | | | | | | | | | |
| Stockholm | 72-11 | 22 | -5.9 | 0.00 | 9 | 8.1 | 1.0 | 19 | 4.00 | **Paper V**, unpub. data* |

***The annual concentrations are presented in **Paper V**, but not the trend assessment.

# 5 Conclusion and future perspectives

A number of central aspects regarding the choice of samples and sampling design within environmental monitoring of contaminants in biological matrices are highlighted in this thesis. For example, it is essential to quantify the objectives before initiating a monitoring program; the quantitative objectives could differ depending of the aim of the monitoring e.g. if the monitoring program is focused on investigation of temporal or spatial differences or assessing compliance, or as in most cases all three types of questions; power analysis is an important tool for evaluation of the quality of the time series and the uncertainty of the trends that are observed; the estimation of sample size needed to meet the objectives and the choice of using pooled or individual samples are very much dependant on the precision of the analysis and the specimen variance within and between years; essential features regarding the choice of monitoring species; and that it is crucial to minimize the natural variability within and between samples by choosing samples of the same sex, age, sampling period etc. to increase the power of the time series.

Statistical methods for evaluation of temporal trends are discussed and the conclusion is that the choice of method for evaluating the trend is very dependent of the trend itself. Is it a linear trend, a non-linear trend, or is the trend changing direction over time? It is easy to miss something of importance by only using one method for trend evaluation. The importance of adjustment for possible confounding factors and the treatment of extreme values and levels below quantification within temporal trend assessment are also pointed out.

How to assess compliance against a target value is a hot potato for policy-makers. Should a test be used that favours the environment (a Green test) using high quality investigations, or a test that favours the polluter (a Brown test) where investigations of poor quality are rewarded? For me, as an environmental scientist, the choice is quite easy, but the chemical industry with its lobbyists seems to have a major influence on central decisions regarding environmental monitoring today.

The marine species most representative for their respective sampling site are the stationary eelpout and perch, the sessile blue mussel, and the relatively stationary guillemot. Herring and cod are, to a varying extent, integrating contaminant accumulation over a larger area. Eelpout, perch, and blue mussel are also key species within the integrated monitoring of contaminants, biological effects and fish populations. If fat soluble contaminants are analysed, a high and stable fat content is desirable, and therefore species such as guillemot (egg), herring (muscle) and Arctic char are preferred. Likewise, human milk has a relatively high fat content.

Variation within and between years (CV) for a time series is crucial for the power and the sensitivity of temporal trend analysis (e.g. the number of years it takes to detect a trend and the size of the trend that could be detected). The time series showing the lowest CV for PCBs were perch from Skärgölen (pooled samples, short and no trend), guillemot eggs from Stora Karlsö (individual analyses) and pike from Storvindeln (mainly individual analyses). In contrast, pike from Storvindeln had one of the highest CVs regarding PCDD/F analysis, but only pooled samples were analysed and the number of analyses performed were relatively few. Human milk and guillemot egg showed the lowest CVs for the PCDD/F (pooled analyses). Pooled samples will automatically generate a lower CV (**Paper I**) and Lake Skärgölen is in addition located high up in the drainage area and most likely only affected by diffuse contamination. Guillemot eggs have a high and stable fat content and have higher concentrations generating lower analytical errors, thus a lower CV. Changes in contaminant concentrations can be detected faster; alternatively, a smaller trend may be detected (Table 4.1, 4.2) when using a matrix with a lower CV (lower variability).

Target values established for assessments under the WFD/MSFD and OSPAR are primarily set for fish, but a few of the target values are also set for mussels. Regarding birds eggs, OSPAR has determined Ecological Quality Objectives for a few bird species. The TGD for biota monitoring under the WFD addresses several difficulties regarding compliance assessment with biota EQSs and gives advice on how to handle e.g. normalisation of data to the correct basis, recalculation of concentrations between different organs, and adjustment for trophic level. However, information on TMFs for different food webs and recalculation factors between organs for different fish species are lacking for many substances and species.

Regarding the choice of species and matrices within Swedish contaminant monitoring, it is essential that the species and organ fit the purpose of the monitoring. In summary, two of the clearest advantages for using guillemot eggs are the high lipid content and the relatively low CV in both time trends presented herein. The largest drawback of using guillemot egg lies in the limitations imposed on collection, that it is only present at a few sites in the Baltic Sea, and the lack of relevant target values. Regarding fish species, each has its own advantages and disadvantages. Perch is stationary, found all over Europe, and frequently used for biological effect monitoring, similar to eelpout, but both species have very low muscular fat content. Pike also has a low muscular fat content. Thus, they are not very suitable for analysis of fat soluble contaminants. Cod is a common species within the OSPAR area and the liver is high in fat but the fat content is very variable. However, adjustment for fat content is possible within the assessment. Herring is widely distributed, has a relatively high and stable fat content, and is commonly used for human consumption, but it is not very stationary. Blue mussel are stationary, but target levels are lacking for many substances.

The temporal trends presented for PCBs, representative for many classical organochlorines, for which concentrations have decreased since the 1970s, are good examples that measures taken to reduce concentrations have been effective. Regarding PCDD/Fs, the picture is not as clear. These contaminants have decreased in matrices in which the concentrations were very high in the beginning of the monitoring period, but show no significant changes in any direction in other matrices with lower levels in the beginning of the monitoring. This might imply that some of the measures taken to reduce the levels, such as the ban of dioxin contamination in herbicides, the cease of chlorine bleach within the pulp and paper industry and the improvement within waste incineration have had an effect, but there are still sources that are keeping these contaminants at a relatively stable level in marine and freshwater organisms compared to decreases seen in human milk.

In the future, for the assessment of compliance towards priority substances within OSPAR, HELCOM, and the WFD/MFSD, it would be of particular interest to develop relevant TMFs for the Baltic Sea food web. In addition, research regarding the distribution of contaminants in different organs is also needed to establish recalculation factors suitable for the most common monitoring species.

# Sammanfattning (summary in Swedish)

Östersjön anses vara ett av världens smutsigaste havsområden. Det var under slutet av 1960-talet som det upptäcktes att Östersjön var svårt förorenat av långlivade organiska miljögifter såsom PCB och DDT. Det visade sig senare att dessa ämnen orsakat reproduktionsproblem hos både säl och havsörn, vilka är affischarter i den svenska marina miljön. Upptäckterna ledde till att ett omfattande miljöövervakningsprogram startades, finansierat av Naturvårdsverket. Enheten för Miljöforskning och Övervakning vid Naturhistoriska Riksmuseet fick ansvaret för övervakning av miljögifter i marin och limnisk biota (främst fisk, musslor, fågelägg). Idag ansvarar enheten också för den hälsorelaterade miljöövervakningen av miljögifter i bröstmjölk insamlade från Stockholmsområdet. Syftet med övervakningsprogrammen var i första hand att uppskatta förändringar över tid för att kunna utvärdera om åtgärder och förbud haft någon effekt.

De åtgärder som vidtogs har varit effektiva. Sedan övervakningen påbörjades noteras att halterna av PCB och DDT har minskat i både fisk, fågelägg och bröstmjölk, dock är koncentrationerna av majoriteterna av de klassiska miljögifterna (DDT, PCB, HCH) fortfarande avsevärt högre i Östersjön jämfört med andra havsområden.

Miljöövervakning är en förutsättning för att myndigheter och andra intressenter ska kunna bedöma hur effektiva åtgärdsprogrammen är och för att upptäcka nya hot i miljön. Stora summor läggs årligen på miljöövervakning, men dessvärre är många av programmen inte tillräckligt känsliga (har tillräckligt hög statistisk styrka) för att upptäcka förändringar i miljön i den storleksordning man skulle önska. För att förbättra bristfälliga program är det första steget att formulera syftet med miljöövervakningsprogrammet på ett kvantitativt sätt. Hur stor förändring måste kunna upptäckas och vilka risker kan accepteras när det gäller att dra fel slutsatser, att felaktigt påstå att det skett en förändring (Typ I fel) eller att felaktigt förmoda att ingen förändring sker fastän halterna ökar/minskar i miljön (Typ II fel). Dessutom bör onödigt brus (varians) minimeras genom en ändamålsenlig provtagningsstra-

tegi. I provtagningsstrategin ska provstorlek och val av individuella eller samlingsprov definieras. Matrisvalet, till exempel art, kön, storlek och organ, är också viktigt för att minska bruset och kan skilja sig åt för olika typer av miljögifter. Det är också mycket viktigt att den statistiska utvärderingen anpassas efter befintlig data.

Min avhandling syftar främst till att utvärdera övervakningsdata som samlats in under mer än 4 decennier inom de nationella övervakningsprogrammen för miljögifter i biologiska prov (marin, limnisk och human hälsa) i både tid och rum samt i förhållande till uppsatta miljögränsvärden som ska skydda den känsligaste arten mot toxiska effekter. Målet var även att utvärdera olika matrisers lämplighet för övervakning av långlivade organiska föroreningar i miljön med det övergripande målet att förbättra utvärderingen av resultaten i övervakningsprogrammen. Olika statistiska frågeställningar har en central roll i avhandlingen.

I **Paper I** undersöks hur valet av analys av individuella eller samlingsprov påverkar den statistiska styrkan. Den statistiska styrkan i temporala och geografiska studier bestäms av provvariationen, vilket i sin tur kan delas upp i analysfel och biologisk variation. Information om olika typer av variation är därmed avgörande för att kunna designa en effektiv provtagningsstrategi. Resultaten visar att variationen kan minskas med samlingsprov, vilket genererar en ökad statistisk styrka till en lägre kostnad i de fall där kostnaden för insamling och provtagning är betydligt lägre än kostnaden för kemisk analys. Emellertid finns det också ett flertal fördelar med individuella prov som bör beaktas.

Temporala (1981-2012) och geografiska trender av PCBer i abborre, gädda och röding från 32 svenska sjöar undersöks i **Paper II**. Generellt sett så minskar PCB koncentrationerna över tid, dock gäller detta inte alla PCB kongener och djurarter som studerats. Även kvoten mellan låg- och högklorerade kongener minskar över tid vilket indikerar att det inte sker någon större nyemission av PCB. Halterna av kongenerna CB-118 och CB-153 visar en geografisk gradient, med högre koncentrationer i stadsnära områden. Koncentrationen av CB-153 i abborre ligger under miljögränsvärdet, medan gränsvärdet för CB-118, som är dioxinlik och därmed mer toxisk, överskrids i vissa sjöar.

**Paper III** sammanfattar geografiska och temporala trender (1969-2012) av PCB, DDT, HCH och HCB i svensk marin biota (strömming, abborre, torsk,

tånglake, blåmussla och sillgrissleägg). Halterna av de undersökta ämnena har minskat över tid med 70-90% sedan övervakningen började. Miljögränsvärdet för CB-118 och DDE (metabolit till DDT) överskrids i vissa arter på några lokaler.

I **Paper IV** diskuteras fördelar och nackdelar med att använda strömming och sillgrissleägg för övervakning av klorerade fettlösliga föreningar genom att bland annat jämföra trender av dioxiner i de två arterna. Fetthalten i sillgrissleäggen var hög och variationen i proven låg, men äggen är relativt svåra att samla in. Strömming visade en högre variation i proven, men är lätt att samla in och halterna i strömming kan åldersjusteras.

Halten av dioxiner i bröstmjölk från Stockholmsområdet analyserades retrospektivt, 1972-2011, i **Paper V**. Halten av dioxiner minskar över tid och den årliga minskningen är större under de 10 sista åren. Resultaten från nyanalysen jämfördes också med gamla analysresultat av samma prov och överenstämmelsen var god, vilket indikerar att gamla tidsserier kan förlängas om samma analysmetod används.

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# Appendix A

## Contribution to **Paper I-V**

**Paper I** Planned the study together with the co-authors and participated in the data evaluation, discussions and the writing of the paper.

**Paper II** Planned the study together with the co-authors and was responsible for the data evaluation and the writing of the paper.

**Paper III** Planned the study together with the co-authors and was responsible for the data evaluation and the writing of the paper.

**Paper IV** Participated in the data evaluation, discussions and the writing of the paper.

**Paper V** Planned the study and sample selection together with first author Johan Fång. Participated in the data evaluation, discussions and writing of the paper.

All the papers rely on high quality chemical analyses for which environmental chemist have been responsible also for the choice of analytical methods. I have not been involved in these activities.

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