QSBMR
Quantitative Structure Biomagnification Relationships

Studies Regarding Persistent Environmental Pollutants in the Baltic Sea Biota

KATRIN LUNDSTEDT-ENKEL
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Abstract

I have studied persistent environmental pollutants in herring (Clupea harengus), in adult guillemot (Uria aalge) and in guillemot eggs from the Baltic Sea. The studied contaminants were organochlorines (OCs); dichlorodiphenyltrichloroethanes (DDTs), polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), hexachlorocyclohexanes (HCHs), and brominated flame retardants (BFRs); polybrominated diphenylethers (PBDEs) and hexabromocyclododecane (HBCD). The highest concentration in both species was shown by p,p’DDE with a concentration in guillemot egg (geometric mean (GM) with 95% confidence interval) of 18200 (17000 – 19600) ng/g lipid weight. The BFR with the highest concentration in guillemot egg was HBCD with a GM concentration of 140 (120 – 160) ng/g lw.

To extract additional and essential information from the data, not possible to obtain using only univariate or bivariate statistics, I used multivariate data analysis techniques; principal components analysis (PCA), partial least squares regression (PLS), soft independent modelling of class analogy (SIMCA), and PLS discriminant analysis (PLS-DA). I found e.g.; that there are significant negative correlations between egg weight and the concentrations of HCB and p,p’DDE; that concentrations of OCs and BFRs in the organisms co-varied so that concentrations of OCs can be used to calculate concentrations of BFRs; and, that several contaminants (e.g. HBCD) had higher concentration in guillemot egg than in guillemot muscle, that several (e.g. BDE47) showed no concentration difference between muscle and egg and that one contaminant (BDE154) showed higher concentration in the guillemot muscle than in egg.

In this thesis I developed a new method, “randomly sampled ratios” (RSR), to calculate biomagnification factors (BMFs) i.e. the ratio between the concentration of a contaminant in an organism and the concentration of the same contaminant in its food. With this new method BMFs are denoted with an estimate of variation. Contaminants that biomagnify are e.g., p,p’DDE, CB118, HCB, βHCH and all of the BFRs. Those that do not biomagnify are e.g., p,p’DDT, αHCH and CB101.

Lastly, to investigate which of the contaminants descriptors (physical-chemical/other properties and characteristics) that correlates to the biomagnification of the contaminants, I modeled the contaminants’ respective BMF_{RSR} versus ~100 descriptors and showed that ~20 descriptors in combination were important, either favoring or countercaturing biomagnification between herring and guillemot.

Keywords: Biomagnification, Multivariate modelling of environmental data, Transport of contaminants, Environmental toxicology

Katrin Lundstedt-Enkel, Department of Physiology and Developmental Biology, Environmental Toxicology, Norbyv. 18A, Uppsala University, SE-75236 Uppsala, Sweden

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To my Family with love!
List of Articles

This thesis includes the following articles. They will be referred to in the text by their Roman numerals I-IV.


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Introduction

Transport of contaminants

The Baltic Sea has unique qualities, and was designated a “particularly sensitive sea area” by the International maritime organization (IMO) the year 2004. It is the largest brackish water body in the world, with a salinity to low for most salt-water organisms, though to high for most fresh-water organisms, resulting in a species-poor ecosystem (1). The Baltic Sea is surrounded by a huge area that includes 9 countries with a total population of 85 million people meaning that large volumes of contaminated water enter the sea each year. As the Baltic Sea have limited exchange of water with the North Sea, giving it a turn-over time of ~30 years, the contaminants, if not degraded, will reside within the sea for a long time (2).

In a food-web of a specific ecosystem, nutrients are transported between the abiotic and biotic compartments as well as between individual organisms within the biotic compartment. Contaminants are transported in a similar way forming a contaminant-web, where fluctuations in concentrations are caused by variations in biotic factors (e.g., species composition, age, and health status of individuals) as well as in abiotic factors (e.g., temperature, wind, and precipitation). All together, this leads to variations in contaminant concentrations in any given individual at any given time (3-5). When modeling contaminant levels, transport, and biomagnification in an ecosystem, a thorough knowledge of variations in concentrations is important. Without this, modeling is difficult to perform at best, or glaringly faulty at worst (4,6-9).

Animals

Herring

Regarding transport of environmental contaminants in the Baltic Sea food-web, herring (Clupea harengus) is an important species (10). It is a planktivorous fish that feeds on selected pelagic zooplankton (11-13), and in turn, herring plays an important role as food for piscivore animals such as other fish (e.g., cod (Gadus morhua) and salmon (Salmo salar)), birds (e.g., guillemot (Uria aalge)), and mammals (e.g., grey seal (Halichoerus grypus)) and is an important human food source (14).
**Guillemot**

Common guillemot (*Uria aalge*) a member of the auk family (Alcidae) is a year-round resident in the Baltic Sea. It is a bird that weighs ~1000 g, is ~440 mm long (from the tip of the bill to the tip of the tail) and has a wing span (tip to tip measured on outstretched wings) of ~700 mm (I). Guillemots are some of the most long-lived birds in Sweden, the oldest reported living bird was 34 years in the summer of 2004 (15). Guillemots feeds almost exclusively on pelagic clupeids, sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) (16-18), two fatty fish species known to be contaminated with organochlorines (OCs) and brominated flame retardants (BFRs) (10,19-23).

The adult guillemots and eggs, used in this thesis, come from the colony at Stora Karlsö (see Materials and Methods, below) that hosts about 10 000 breeding pairs, two-thirds of the Baltic Sea population (24). From around 4-5 years of age, guillemot females breeds for the first time, laying one large (~110 g) egg in the spring each year (10,25). Guillemot eggs have been analyzed for their contaminant contents since 1969 within the Swedish monitoring program (26).

**Contaminants**

**Organochlorines**

**DDT**

Dichlorodiphenyl trichloroethane (DDT) was first synthesized by Zeidler in the year 1873. It acts as a neurotoxicant (27) and DDT’s insecticide properties were discovered 1939. The production of DDT increased rapidly and due to its widespread use, and to its physical-chemical properties, DDT together with its metabolites (see Figure 1 for *p,p’*DDTs) is now distributed world-wide (10,28-41).

As some of the other OCs in this thesis, DDT is listed as one of the twelve persistent organic pollutants (POP) in the Stockholm Convention, where it says;

“**POPs are chemicals that remain intact in the environment for long periods, become widely distributed geographically, accumulate in the fatty tissue of living organisms and are toxic to humans and wildlife. POPs circulate globally and can cause damage wherever they travel. In implementing the Convention, Governments will take measures to eliminate or reduce the release of POPs into the environment**” (42).
Sweden as well as many other countries banned DDT in the 1970s. However, DDT is still in use today, mostly in developing countries against malaria mosquitoes (43) (for a recent review see (44)).

Figure 1. Schematic chemical structures of \( p,p'\)DDT, \( p,p'\)DDE and \( p,p'\)DDD

**PCB**

Polychlorinated biphenyl (PCB) comprise of a biphenyl substituted with 1-10 chlorine atoms per molecule. This means that theoretically there are 209 different PCB congener. Commercial products (e.g. Aroclor and Clophen) that comprise of a mixture of various PCB congener (leading to variations in their properties) have been produced since 1929. As PCBs are resistant to high temperatures, and do not break down easily, PCB have been used in e.g., electrical transformers, as heat transfer agents, plasticizers, and hydraulic fluids (45). PCB was first detected in nature by Sören Jensen, in the year 1966 (46,47). Not all 209 congeners are found in nature and this have several causes e.g. \( a \), the commercial mixtures have varying content of different congeners (none include all 209) and \( b \), some of the congeners that reach nature may be selectively absorbed, degraded and excreted by organisms (48).

Due to the widespread use of PCB, and to its physical-chemical properties, PCB is now distributed world-wide (10,29,31,49-58). Most countries have banned PCB today though leakage from products containing PCB (e.g. from building material and transformers) is an ongoing problem (59).

**HCB**

Hexachlorobenzene (HCB) has been used since the year 1933 as a fungicide applied to seeds and to preserve wood. Also, in the industrial production of dyes and in polyvinyl chloride (PVC) (60). It is a persistent compound and was banned in many countries in the 1970s, though it is still in use in many developing countries (27).

Through emissions from industrial processes, and because HCB is a contaminant in other biocides, the persistent HCB is still released to the environment (for a recent reviews, see (61,62)) and present in many organisms (29,63-70).
HCH

Hexachlorocyclohexane (HCH) was first synthesized by Faraday in 1825. Its insecticidal properties were discovered in the early 1940s and HCH has now been in use for nearly 50 years (71). Like many other insecticides it acts as a neurotoxicant (27). The technical mixture of HCH contains several HCH isomers. It is made up by 10-15 % of the active isomer $\gamma$HCH (also called lindane) and the rest is $\alpha$HCH (60-70%), $\beta$HCH (5-12%), and $\delta$HCH (6-10%) (72-74).

Although HCH production is banned in most countries it is still in use. For instance, powders and creams containing $\gamma$HCH are sold in some countries (e.g., in USA though not in Sweden) for treatment of scabies and head lice in humans (72). HCH is wide spread in the environment and present in many organisms (29,35,66,75-77).

Chlordane

The insecticide Chlordane is a technical mixture that includes some 140 chlordane compounds e.g., a few percent of trans-nonachlor, that has shown increasing levels in the environment (72). Clordane is listed as a POP in the Stockholm Convention (42). Although its use has been banned in many countries, in China as late as 1999 (78), it is still present in the environment due to its persistency (79-83).

Brominated flame retardants

Flame retardants (FRs) reduce flammability and are used in products to prevent fires. The history of using FRs is long. The use of alum (potassium aluminum sulfate, KAl(SO$_4$)$_2$) on wood was practiced as early as 450 BC by the Egyptians (84). Today, modern FRs are used in many products, e.g., building materials, textiles, and plastics (in computers, TV sets, electric cables, and other electric products) and in expanded polystyrene (in furniture) (85-89).

In plastics the inclusions of FRs are performed in two ways: Reactive FRs are those that takes part of the polymerization (the reaction) and are chemically built into a polymer molecule. The majority of the reactive FR end up covalently bound to the plastic. An example of a reactive brominated FR is tetrabromobisphenol A (TBBPA). Additive FRs are those that are incorporated into the polymer either prior to, during, or (most frequently) following polymerization and are therefore more loosely incorporated in the plastic, making the additive FRs more prone to leaching in to the environment (87). An example of an additive brominated FR is hexabromocyclododecane (HBCD).
The use of FRs has grown substantially in the last decades and in 2001 the total world market demand for BFRs was 204 000 tonnes (90).

**Polybrominated diphenyl ether - PBDE**

PBDEs are additive FRs that are made up of diphenyl ethers that can be substituted with 1-10 bromine atoms per molecule (Figure 2). Like PCBs (see above) there are theoretically 209 different BDE congeners. The nomenclature suggested by Ballschmiter and Zell in 1980 for PCBs (91) is also adapted for the PBDEs.

Numerous evidences exists today that PBDEs have leached out into the environment and been transported, even to remote areas and are present in the living organisms, humans included (92-109). Most alarmingly, PBDE levels in mothers milk are increasing in USA and are now 10-1000 times higher than in Europe (99,110,111).

![Figure 2. Schematic chemical structure of polybrominated diphenyl ether (PBDE).](image)

**Hexabromocyclododecane - HBCD**

HBCD is substituted with six bromine atoms per molecule (Figure 3). These can be in various positions so that 16 individual stereoisomers of HBCD is possible (90,112). Numerous evidences exists today that HBCD have leached out into the environment and been transported, even to remote areas and are present in the living organisms, humans included (87,113-119). The use of HBCD globally is increasing and the estimated annual production was 16 700 tonnes world wide in the year 2001 (112).

![Figure 3. Chemical structure of 1,2,5,6,9,10-hexabromocyclododecane (HBCD).](image)
BFR restrictions

Since 2004 the two PBDEs, PentaBDE and OctaBDE have been banned in the European Union (120), and the major manufacturer in USA has agreed to phase out production of PentaBDE during 2005. Hexabromocyclododecane (HBCD), DecaBDE, and other BFRs are still in use though. This present change in BFR usage will lead to a change in the BFR composition in biota in the future. Although, the PentaBDE and OctaBDE already present in enormous quantities in products still in use will continue to contribute to the contaminant burden in biota for a long time.

Effects

Effects caused by OCs and BFRs have been demonstrated in laboratory studies in a diverse range of organisms. As effects are outside the scope of this thesis only a few examples will be given for DDT, PCB and BFRs.

DDT

DDT and some of its metabolites (e.g. p,p'DDE) have been shown to cause numerous effects, e.g. increase in liver weight, increase in cytochrome P450 and aromatase activity, as well as decrease in corticosteroid secretion. DDT is a neurotoxicant and causes hyperactivity, decrease in learning capacity and tremors in laboratory animals. It is carcinogenic (liver tumors) in mice and rats and prolongs the oestrous cycle in female mice and induces oovestitis in the male bird as well as malformations in their Müllerian ducts (43,78,121-131). Further, p,p'DDE induces eggshell thinning in birds (for a review, see (132)).

In nature, increase in DDT levels are suspected to be detrimental to birds reproductive success e.g., to eagles (Haliaeetus albicilla) (133), peregrine falcons (Falco peregrinus) (134) and great black-backed gulls (Larus marinus) (135) as well as to mammals e.g. seals (136,137). For extensive reviews see e.g. (138-141).

PCB

PCB has been shown to cause numerous effects, e.g. increase in liver weight and induction of the hepatic drug metabolizing system. Also to reduce reproductive capacity, induce hyperactivity and a decrease in learning capacity in mice and rat, and cause alterations in rat maternal behavior. Further, lead to alterations in rat bone morphology, composition and strength, cause Leydig cell apoptosis in male mice, and induce alterations in fish swimming behavior (142-149).
In nature, PCBs are suspected to have caused negative effects on e.g. seal reproduction, skeleton and claws \((\text{136,137,150})\) suppression of seal immune system \((\text{151})\) and alter animal behaviors (for a review see \((\text{138})\)).

**BFR**

For the BFRs, laboratory experiments have shown reduced larval developmental rate after exposing the copepod *Nitocra spinipes* to PBDEs \((\text{152})\) and that PBDEs cause negative effects on the brain development in mice and in the rat \((\text{143})\) leading to hyperactivity and decrease in learning capacity. Exposure in ovo to BDE99 leads to a reduction in the level of Vitamin E in the liver of duck (*Anas platyrhynchos*) meaning that the BDE causes oxidative stress \((\text{153})\). Evidence of immunomodulation (e.g., reduced antibody-mediated response) in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs have been demonstrated \((\text{154})\). PBDEs have been shown to be an endocrine disrupting chemical (EDC) in that it causes a decrease in the levels of thyroid hormones \((\text{155,156})\).

Not many studies can be found regarding effects of HBCD. In one study HBCD lowers the uptake of glutamate in to the synaptosomes indicating a neurotoxic effect \((\text{157})\). HBCD inhibit EROD activity and increase the liver somatic index (LSI) in rainbow trout (*Oncorhynchus mykiss*) \((\text{158})\). HBCD modulates (enhances) the thyroid receptor-mediated gene expression meaning that HBCD have the potential to be an EDC \((\text{159})\).

**Biomagnification**

If the concentration of a contaminant in an organism exceeds that in the organism’s diet, biomagnification has occurred. The accumulation of the substance is supposed to be due to intestinal absorption. As our present knowledge regarding quantitative data on uptake routes (e.g., gastrointestinal tract, respiratory organs and integument) of contaminants in most animals in the wild is limited, this work use the term biomagnification in a wide sense, as defined by Gray \((\text{160})\). If the lipid normalized concentration of a substance is significantly higher in the predator than in the prey, regardless of how that higher concentration has been achieved, biomagnification of that substance has occurred.

A biomagnification factor (BMF) can be defined as the ratio between the concentration of chemical in the organism \((C_o)\) and that in the organism’s diet \((C_D)\).

\[
\text{BMF} = \frac{C_o}{C_D}
\]

The traditional method of calculating BMFs denotes the BMF as a single number without any estimate of the variation. In this thesis I propose a new
method, the randomly sampled ratios method, to calculate BMFs with an estimate of variation (see (III) and below).

Properties of the contaminant essential for biomagnification, mainly bioavailability and persistence, are determined by a number of chemical variables i.e., physical/chemical characteristics such as lipophilicity, molecular size and structure (161-163), as well as other chemical characteristics (IV). Biological variables important for biomagnification are related to the animals, e.g., feeding habits and habitats, lipid characteristics and fat content, sex, age, and the animals’ capacity to biotransform/excrete contaminants.

From an ecotoxicological point of view it is important to remember, as postulated in the early 1500 by Paracelsus (1493-1541), that it is the dose of a substance (the effect concentration) that determines if a substance is toxic or not (164). Thus, substances that biomagnify can have far-reaching biological effects (165) as the biomagnification of contaminants can lead to contaminant levels that ultimately gets high enough to cause adverse effects in organisms.

Modeling

There are many papers that deal with the issue of error/uncertainty propagation when modeling bioaccumulation/biomagnification of contaminants for instance, to evaluate how model output changes with variations in input variables (166,167) and/or how uncertainties in individual parameters can affect uncertainties in the results (168,169). In these papers Bayesian methodologies (e.g., Monte Carlo or Markov Chain Monte Carlo simulations) are used to generate new distributions of values e.g., for contaminant concentrations used as input values in the modeling. Often, in such papers new distributions are generated based on a low number of analyzed samples from short term experiments, not from field studies.

The US EPA recommends the use of techniques to clarify the impact/sources of uncertainty on the result of an ecological risk assessment but at the same time cautions that a poor execution of any method can obscure rather than facilitate understanding (170). In fact, Linkov and Burnistrov (8) found that “modeler uncertainty” and “choices made by modelers” contributed to as high as 7 orders of magnitude differences in model predictions.
In order to understand her environment, humans have always been trying to figure out relationships between a property or structure and an effect, e.g. Paracelsus ideas that there existed relationships between the shapes of plants and their beneficial effects on human disease (171). In the year 1863, in one of the first publications that investigated the relationships between a chemical property and the chemicals’ measured effect, the toxicity of some primary aliphatic alcohols were related to their water solubility (172). During the last 40 years an increasing number of studies dealing with relationships between a chemical’s structure and e.g., its potential for bioconcentration or toxicity have been published and the term QSAR - Quantitative Structure Activity Relationships is now in frequent use. A search in Scirus (www.scirus.com) gives close to 7000 articles that include the term QSAR. A wealth of papers relates chemical structure/structures to e.g. activity (173-175) to toxicity (176-185), to bioconcentration (186-188), to bioaccumulation (189,190), et cetera.

There are papers discussing how change in, for instance, the substitution pattern of PCBs influence the congeners potential to biomagnify (161,191-193) or their propensity to be biotransformed (162). However, modeling the structure of chemicals to their biomagnification in a food web is, to my knowledge, not attempted. In line with QSAR, in this thesis I explore QSBMR - Quantitative Structure Biomagnification Relationships.

Chemical structure and QSAR

In computational chemistry, for instance chemometrics, the chemical structures are represented as multiple numerical variables, many that are computer generated and not determined empirically, and it is today possible to generate thousands of variables. These variables are henceforth called descriptors and they are of varying types for instance, describing hydrophobic, steric, electronic or other properties of a given chemical. A QSAR is a model that relate e.g., the measured biological activity shown by organisms (after exposure to chemicals) to the chemicals’ descriptors. A QSAR generally takes the form of an equation;

\[
\text{Biological activity} = K + (C_1*S_1) + (C_2*S_2) + \ldots (C_n*S_n)
\]

The parameters S_1 through S_n are the generated descriptors for the chemicals included in the QSAR and the coefficients C_1 through C_n as well as the constant K, are calculated. This calculation can only be meaningful if the variation in biological activity correlates to variations in the generated descriptors S_1 to S_n.
Aims

- to present concentrations of organochlorines and brominated flame retardants in herring (*Clupea harengus*) and guillemot (*Uria aalge*) from the Baltic Sea (I, II, III and IV).

- to model the relationships between contaminant patterns and the animals’ biological variables (I, II, III)

- to model the relationships between the organochlorines and the brominated flame retardants in a sampled organism (I, II)

- to model the pattern of concentrations of organochlorines and brominated flame retardants in guillemot egg and compare it to the pattern in guillemot muscle (II).

- to show the usefulness of multivariate analysis techniques for analysis and interpretation of environmental data (I, II, III and IV).

- to propose a new method to calculate biomagnification factors (BMFs), the “randomly sampled ratios” (RSR) method, that denote BMFs with an estimate of variation (III).

- to model the contaminants respective BMF versus their physical-chemical properties and other descriptors to investigate what property or properties that favor or counteract biomagnification (IV).
Materials and Methods

The following is a brief summary of Materials and Methods used in this thesis. The more detailed descriptions can be found in articles I, II, III, and IV.

Animals

Herring (*Clupea harengus*) specimens (in III, IV) were sampled from fish catches at two sites, Landsort (co-ordinates: 58º 42’ N, 18º 04’ E) and Utlängan (co-ordinates: 55º 57’ N, 15º 47’ E). Landsort is situated to the north and Utlängan to the south of the guillemot colony at Stora Karlsö (see below) and both herring locations are within those birds’ large foraging area (25). All collecting and sample preparation was carried out and recorded in a standardized manner (10,194). The herring were from autumn (October to December) from the years 1996, 1997, and 1998 (III and IV) and from the years 2000, 2001 and 2002 (IV). A total of 144 herring samples (12 specimens/location/year) were individually analyzed regarding their dorsal muscle concentrations of a number of OCs and BFRs (see chemical analysis below).

The guillemot (*Uria aalge*) egg (II, III and IV) and adult birds pectoral muscles (see below) originated from the colony at Stora Karlsö (co-ordinates: 57º 17’ N, 17º 58’ E) that is situated ~6 km west of the island of Gotland. The eggs were sampled directly after egg laying, in April - May the years 1997, 1998, and 1999 (III and IV) and from the years 2000, 2001 and 2002 (II and IV). The adult guillemot pectoral muscles (in I and II) were sampled from animals that drowned in trawl nets in April year 2000, at time of egg formation. A total of 59 guillemot eggs (10 eggs/year, though one was lost during the chemical analysis) and 20 guillemot muscles were individually analyzed regarding their concentration of a number of OCs and BFRs.

Contaminant analysis

The OCs and BFRs that were determined are shown (Table 1). ΣDDT was calculated as the sum of: *p,p*’DDT, *p,p*’DDE, and *p,p*’DDD concentrations, and ΣPCB as the sum of ICES 7 marker PCBs’: CB28, CB52, CB101, CB118, CB138, CB153 and CB180 concentrations. Most chemical analyses were carried out at the department of Applied Environmental Science (ITM), at Stockholm University and some performed at the Special Analytical
Laboratory (RSL), Swedish Museum of Natural History, in cooperation with ITM.

Sample treatment, analytical method for quantization of individual OCs and BFRs isomers/congeneres as well as laboratory QA/QC procedures have been described in detail (I, II and III) and in (10,11,18,195,196). All samples were homogenized, the lipids were extracted and the lipid content (F%) determined gravimetrically at ITM. The concentrations are expressed as ng/g on a lipid weight (lw) basis unless otherwise specified.

Statistics

Basic statistics

For uni- and bivariate statistics regarding the biological variables and concentrations of OCs and BFRs the software GraphPad Prism 4.03 (197) was used. This included column statistics (e.g., mean, geometric mean, 95% confidence interval), correlation analysis (Pearson), un-paired two-tailed t-tests (on log10 transformed data for Gaussian distributions). The significance level was set to 0.05 for all tests (I, II, III and IV).

Multivariate data analysis

Multivariate data analysis (MVDA) is based on the assumption that the data include variables that to some part are dependant, that not all variables contain useful information and that the useful information that can be extracted from the data is extracted as latent variables, components. The MVDA used in (I, II, III and IV) were principal component analysis (PCA), partial least-squares projection to latent structures (PLS), soft independent modeling of class analogy (SIMCA classification), and PLS discriminant analysis (PLS-DA). All MVDA were performed using the software SIMCA-P 10.0.4 (198). The significance level used was 0.05 and data were centered and scaled (to variance 1) prior to modeling (199). Determinations of the significant number of components to extract were made by cross validations. Values of the explained variation, $R^2$, and predicted variation, $Q^2$, were calculated. $R^2$ values >0.7 and $Q^2$ values >0.4 are considered to denote an acceptable model when analyzing biological data (200).

Principal component analysis – PCA

Principal component analysis (PCA) is a method that should be applied when analyzing multivariate data (i.e., three or more variables determined) especially in biology where many of the variables are dependant on each other and/or correlate. PCA is useful to initially identify correlations between a set of variables, for instance analyzed contaminant concentrations
and/or biological measurements, generated from the individual observations (e.g., individual herring muscles or guillemot muscles/eggs) (I, II, III and IV). When using PCA on data originating from ecotoxicological studies, which often include of up to one hundred variables per observation, PCA summarize the data, helps to identify structures and/or correlations and/or trends in the data and display the results in graphs that are easy to interpret, more easily than e.g., presenting the data in tables.

Projection to Latent Structures – PLS

The full name of the PLS method is Projection to Latent Structures by means of Partial Least Squares analysis. PLS is a regression extension of PCA that is used to model the relationship between two matrixes (two blocks of variables, X and Y) that both can be multidimensional. A PLS model calculates the systematic change or changes in the X matrix and correlates this change to any systematic change or changes in the Y matrix. X and Y are hooked together in such a way that the explained X/Y covariance is maximized (201-203).

When analyzing data with many, collinear and even incomplete data matrices, the PLS-models’ stability and precision improves when the number of relevant X-variables increases. Therefore, PLS is especially useful when analyzing biological data, e.g., ecotoxicological and/or monitoring data, and I am convinced that the use of PLS will increase in biology as it has done for the last 30 years in chemistry (204).

In this thesis PLS was used, for instance to investigate the relationships between an individual observation’s (e.g., guillemot egg or pectoral muscle) concentrations of OCs and its concentrations of BFRs. If changes in the contaminant pattern correlates to changes in the biological variables. PLS was also used in article (IV) for the QSBMR modeling. Here the X-matrix was the generated chemical descriptors (see below) and the Y response was the calculated biomagnification factors (see below).

Soft independent modeling of class analogy – SIMCA classification

SIMCA classification (205) is a MVDA classification technique that makes it possible to investigate if there are significant differences between two or more groups (e.g. males and females) in their variables (e.g. contaminant concentrations). SIMCA classification is designed to be used on multivariate data (for univariate data, e.g., t-test can be used). The advantage, compared to other techniques, is that the class membership (e.g. “female” or “male”) is not denoted beforehand but tested unprejudiced. In SIMCA, the first is to select a training set which is modeled by PCA and then, subsequently, new samples (test samples) are fitted to this model. The test samples are classified as similar or dissimilar to the training set if they are classified within or without the confidence interval (0.95%) of the model, respectively. SIMCA classification in this work was for instance used to investigate if the con-
taminant pattern in guillemot egg differed significantly from that in guillemot muscle (II).

**PLS discriminant analysis – PLS-DA**

PLS discriminant analysis (PLS-DA) can be used in order to identify the discriminating variables that contribute to the separation of classes (e.g., male as one class and female as the other) (206). In the articles in this thesis where PLS-DA has been used (I, II, and III) so called dummy variables were introduced, e.g., 0 for the class Egg, and 1 for the class Muscle. The PLS-DA then quantifies which variable or combination of variables that discriminate between egg and muscle.

**Calculations of biomagnification factors (BMF)**

Biomagnification factors (BMF) are calculated as the quotient between the concentration of a substance in the predator (the eater) to the concentration of that substance in the prey (the food). Traditionally BMFs are calculated using the arithmetic mean (AM) or the geometric mean (GM) concentrations for a substance in the eater and relating it to the AM or GM in the food. To make comparisons between the different methods facilitated I denote these BMFs as BMF\textsubscript{AM} and BMF\textsubscript{GM}.

The traditional method gives the BMF\textsubscript{AM} and BMF\textsubscript{GM} as a single figure that do not contain any information regarding the distribution of the values in the original data. The aim of article (III) was to introduce a novel method to calculate BMF, the randomly sampled ratios (RSR) method that denotes BMF\textsubscript{RSR} with an estimate of variation.

In (III) BMF\textsubscript{AM} and BMF\textsubscript{GM} were calculated using the AM and GM concentration of a given OC in guillemot eggs for the 3 years 1997, 1998 and 1999 (n = 29) and the AM and GM concentration of the same OC in herring from Landsort and Utlängan for the 3 years 1996, 1997, and 1998 (n = 72). In addition, with the new RSR method proposed by me, BMF\textsubscript{RSR} were calculated by randomly selecting one guillemot egg out of the 29 and one herring out of the 72, using the BMF\textsubscript{RSR} computer program especially designed for the task (207). This program uses a *random sampling with replacement* technique where a single specimen is randomly sampled and can be sampled several times. The ratio between the concentrations of a given OC in that sampled guillemot egg and the same OC in that sampled herring muscle was determined. In this way, 50 000 iterations were performed, generating 50 000 observations of BMFs for each contaminant, and GM (denoted BMF\textsubscript{RSRGM}) with GM standard deviation (GMSD) were calculated for this new distribution of ratios.
Also, minimum and maximum BMFs for all organochlorines were determined. These BMFs were calculated as the ratio between the guillemot egg with the lowest concentration of a given contaminant and the herring muscle with the highest concentration of the same contaminant (BMF$_{\text{MIN}}$) and between the guillemot egg with the highest concentration and the herring muscle with the lowest concentration (BMF$_{\text{MAX}}$). The CB153 concentration in herring (the prey) and guillemot (the predator) is shown (Figure 4). Here it can be seen that the distribution in concentrations show considerable variations in both herring and guillemot as does BMF$_{\text{MIN}}$, BMF$_{\text{GM}}$, and BMF$_{\text{MAX}}$.

Figure 4. Concentration (ng/g of lipid weight) on a logarithmic scale of CB153 in herring (*Clupea harengus*) muscle ($n=72$) from Landsort and Uttlänken the years 1996, 1997 and 1998 and in guillemot (*Uria aalge*) egg ($n=29$) from Stora Karlsö, the Baltic Sea, the years 1997, 1998 and 1999. Biomagnification factors (BMFs) calculated as BMF$_{\text{MIN}}$ (3.0), BMF$_{\text{GM}}$ (24.7), and BMF$_{\text{MAX}}$ (158).
Generating descriptors

The physico-chemical and structural descriptors (henceforth denoted descriptors) were characterized using several methods.

- With the software Tsar version 3.3 for windows (208) where the three-dimensional (3D) structures were generated using Corina included in the Tsar package. The descriptors describe charge, size, connectivity, number and type of atoms, group counting, et cetera (see the Appendix in IV for a list of all descriptors).

- To describe the position of the halogens in the molecules of PCBs and PBDEs binary fingerprint variables were generated (Figure 5).

- The log\(K_{ow}\) values were derived from references, as shown in (IV).

<table>
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<th>5</th>
<th>6</th>
<th>2'</th>
<th>3'</th>
<th>4'</th>
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<th>6'</th>
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</table>

Figure 5. Binary fingerprint variables for the PCB congeners describing the positions of the chlorine atoms in the biphenyl. Where there are halogen pairs (i.e., chlorine atoms in ortho-meta, meta-para positions) is shown by e.g., 23, 34, and 45.
Results and Discussions

The following are somewhat shortened results and discussions from the articles (I, II, III, and IV) included in this thesis. More extensive and detailed discussions can be found in the articles.

Article I

**Multivariate data analyses of chlorinated and brominated contaminants and biological characteristics in adult guillemot (*Uria aalge*) from the Baltic Sea.**

**Biological variables and Concentrations of OCs and BFRs**

Interestingly, there were no significant differences in either biological variables, or contaminant concentrations between the sexes, they were remarkable similar. Concentrations (GM ± 95% CI) in pectoral muscles are presented in Table 1. The total concentration of OCs was approximately 150 times higher than that of BFRs. The contaminant with the highest concentration in the bird muscles was *p,p'-DDE* with a concentration close to twice (1.95 times) as high as the concentration of ΣPCB. The same relationship exists also in guillemot eggs from Stora Karlsö with a *p,p'-DDE / ΣPCB* concentration ratio close to 2 (as shown in II and III). The opposite relationship has been found in tissues from other circumpolar seabirds with a *p,p'-DDE / ΣPCB* concentration ratio below 1 (64,209-211). The reasons for this relatively high *p,p'-DDE / ΣPCB* concentration ratio for guillemots from Stora Karlsö are not known but differences between the species regarding, for example, exposure and capacity to biotransform and excrete contaminants are likely to be involved.

The concentration in guillemot muscles of *βHCH* was ~20 times higher than that of both *αHCH* and *γHCH*. In (III) it was shown that *βHCH* biomagnifies ~20 times between herring muscle and guillemot egg while the *αHCH* and *γHCH* isomers do not biomagnify.

The concentration of ΣPBDE was not significantly different from that of HBCD. The concentrations of the various BFRs were in decreasing order: BDE47 = HBCD > BDE99 > BDE100 = BDE154 > BDE153. The use of the technical products PentaBDE, OctaBDE, and DecaBDE are the primary sources for PBDEs in the environment (85,88). The dominating congener in guillemot muscle was BDE47, a major constituent in the PentaBDE mixture. Apart from PentaBDE, some BDE47 might originate from
reductive debromination of other PBDE congeners. For instance, both OctaBDE and DecaBDE have been shown to undergo debromination to less brominated congeners (212-215).

Table 1. Concentrations (geometric mean (GM) with 95% confidence interval (-CI and +CI)) of organochlorines and brominated flame retardants in pectoral muscles of guillemots (*Uria aalge*) (*n* = 20) collected in 2000 south of Stora Karlsö in the Baltic Sea. For abbreviations, see the Materials and Methods section (I).

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<th>Female n=10</th>
<th>Male n=10</th>
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<td>HBCD</td>
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1 ND = not detected.
2 α-HCH, γ-HCH, and CB101 have individually determined LOQs
3 General level of quantification for trans-nonachlor (t-nonachlor) = 10 ng/g lw
4 ΣPCB= sum of ICES seven marker PCBs: IUPAC nos. CB28, CB52, CB101, CB118, CB138, CB153, and CB180. For CB52, ND = 0
5 ΣPBDE= sum of BDE congeners nos. BDE47, BDE99, BDE100, BDE153 and BDE154.

**Multivariate data analysis, MVDA**

In all MVDA no groupings regarding the sexes could be seen. There were no differences between females and males in any of the biological variables or in any of the contaminant concentrations. In mammals, *e.g.*, polar bears (*Ursus maritimus*), cetaceans, and pinnipeds, females exhibit lower contaminant concentrations than males (70,216,217), which can be explained mainly by excretion of lipophilic contaminants to the offspring via the milk. Articles dealing with differences in contaminant concentrations between male and female birds are rare in the literature. In white-tailed eagle (*Haliaeetus albicilla*), glaucous gulls (*Larus hyperboreus*), and Icelandic gulls (*Larus*...
 intoxicus) no sex-related differences in contaminant concentrations have been found (70,218). Probably, from a quantitative point of view, the excretion of contaminants from females to their offspring is of less importance in birds than in mammals. Female as well as male birds excrete lipid-containing secretions from their preen gland (uropygial gland) and spread the secretions over the plumage. Water birds, compared to other birds, possess a very large preen gland (219). The concentrations of DDT and PCB in the preen gland secretions from mergansers (Mergus merganser) were similar to those in bird muscle (220). The preen gland secretion, an excretion route for contaminants exclusive for birds, might be large and thus extinguish eventual sexual differences in contaminant concentration created by egg-laying.

PLS: Biological Variables versus Contaminants

The resulting PLS models with biological variables as Y and concentrations of OCs and BFRs as X showed that tail-feather length, liver weight, and lipid content (TL, LW, and F%) covaried with the concentrations of contaminants and generated significant models.

TL was positively correlated with the concentrations of contaminants. Other similar examples of this phenomenon were not found in literature and I refrain from speculation. More studies regarding e.g., POPs’ effects on feather growth are needed.

LW was negatively correlated with the OCs and BFRs (Figure 6). These relationships appeared not to be caused by dilution of the contaminants due to higher lipid content because there was no correlation between LW and F% (P = 0.41, r² = 0.038) and were also seen when concentrations were expressed on a wet weight basis. I assume that the size of the liver to some extent reflects the capacity of an individual to biotransform and excrete foreign compounds, and from that point of view it is not surprising to find a negative correlation between liver weight and concentrations of OCs and BFRs.

Figure 6. Coefficient plot with 95% CI for the respective variables for PLS model (R²X = 0.50, R²Y = 0.34, Q² = 0.09) between liver weight (LW) (g) and concentrations of organochlorines and brominated flame retardants in individually analyzed muscles (n = 20) from guillemot (Uria aalge) from Stora Karlsö, Baltic Sea, 2000. For abbreviations, see the Materials and Methods section (I).
Due to lipid dilution, the concentration of contaminants in lipid weight is often negatively correlated with the animals F% (48). Interestingly, a PLS regression with F% as Y and contaminant concentrations on lw basis as X gave a model that showed that the concentrations of most OCs but not of BFRs decreased with increasing F%. As far as I know, this anomalous behavior of the BFRs is not described in the literature.

**PLS: Predicting BFR Concentrations from OC Concentrations**

A PLS regression with BFR concentrations in guillemot muscles as Y and OC concentrations as X gave a model (R²X= 0.94, R²Y= 0.79, Q²= 0.48, three components) that showed groupings of the variables. Within the groups the contaminants covaried positively with each other. One group was formed by BDE47, BDE99, BDE100, BDE153, CB138, CB153, and CB180 and another by HBCD, BDE154, p,p’DDE, and β-HCH. These correlation patterns found in guillemot muscles are very similar to the patterns revealed by me in 49 individually analyzed guillemot eggs collected during 1996-2000 at Stora Karlsö (221).

The correlation patterns found in the eggs were believed to be related to differences between the studied contaminants in metabolism and pathways from food via the female to the egg. Apparently, as the present work describes the same correlation patterns in muscles, the transport to the egg is of no importance in this respect. The groupings are most likely caused by similarities of the contaminants within a group regarding physiochemical properties, exposure routes, bioavailability, distribution, biotransformation, and/or excretion.

I believe that it is possible to select a number of *indicator contaminants* from each group and based on their residue levels calculate and predict the levels of a number of other contaminants. *Most important*, a model used for such purposes will have to be tested and validated at close intervals to ensure its continuous validity.
Article II

Organochlorines and brominated flame retardants in Baltic Sea guillemot (Uria aalge) egg and muscle.

**Biological variables and Concentrations of OCs and BFRs**

There were no differences between eggs from the three years, either in biological variables, or in the concentrations of OCs and BFRs. The contaminant concentrations are shown (Table 2). In both egg and muscle the concentration of ΣDDT was ~2 times higher than the concentration of ΣPCB.

Table 2. Concentrations (ng/g lw) as geometric mean (GM) with lower and upper 95% confidence interval (-CI and +CI) of organochlorines and brominated flame retardants in egg and pectoral muscle of guillemot (Uria aalge) from Stora Karlsö, Baltic Sea. Ratios between the concentrations of contaminants in egg and in muscle (C<sub>Egg</sub>/C<sub>Muscle</sub>). Eggs collected years 2000, 2001 and 2002 (n= 30). Muscles sampled year 2000 (n= 20). For abbreviations, see Materials and Methods (II).

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<th>Muscle ng/g lw (n= 20)</th>
<th>C&lt;sub&gt;Egg&lt;/sub&gt;/C&lt;sub&gt;Muscle&lt;/sub&gt;</th>
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<td>0.98 (P=0.86)</td>
</tr>
<tr>
<td>BDE153</td>
<td>1.8 1.4 2.3</td>
<td>1.6 1.2 2.1</td>
<td>1.13 (P=0.51)</td>
</tr>
<tr>
<td>BDE154 †</td>
<td>3.3 2.9 3.7</td>
<td>4.3 3.9 4.9</td>
<td>0.76 ***</td>
</tr>
<tr>
<td>ΣPBDE †</td>
<td>77.4 62.2 96.2</td>
<td>80.2 63.0 102</td>
<td>0.96 (P=0.82)</td>
</tr>
<tr>
<td>HBCD</td>
<td>138 120 160</td>
<td>64.7 53.2 78.7</td>
<td>2.14 **</td>
</tr>
</tbody>
</table>

1 Not included in the PLS-DA modeling (egg vs muscle) due to concentrations ~LOQ.
2 ND = not detected.
3 ΣPCB = sum of ICES 7 marker PCBs: IUPAC no. CB28, CB52, CB101, CB118, CB138, CB153 and CB180 (contaminants with ND= 0 ng/g lw).
4 BDE100 not analyzed year 2000, (n= 20).
5 BDE154 not analyzed year 2002, (n= 20).
6 ΣPBDE = sum of BDE congeners no BDE47, BDE99, BDE100, BDE153 and BDE154.
* P<0.05; ** P<0.01; *** P<0.001 (t-test, egg vs muscle, log<sub>10</sub> transformed contaminant concentrations).
**PLS, egg biological variables versus contaminants**

One of the aims article II was to analyze relationships between the biological variables of guillemot eggs, and the residue levels of OCs and BFRs in the egg. A PLS with biological variables as \( Y \) and the concentrations of contaminants as \( X \) revealed that egg weight and egg shell thickness (Weight and Sh Thickn) covaried with the concentrations of contaminants, generating significant models.

Eggs with lower weight had higher concentration of \( p,p' \) DDE (\( P=0.03, r^2=0.27 \)) and HCB (\( P=0.001, r^2=0.42 \)). The relatively lower concentrations of \( p,p' \) DDE and HCB in the heavier eggs can not be explained by lipid dilution (48) as there was no significant correlation between Weight and F\% (\( P=0.37, r^2=0.03 \)). Whether or not there are causalities behind these correlations are not known. Further studies including e.g. effects of \( p,p' \) DDE and HCB on yolk formation and albumen formation/deposition are necessary.

Eggs with thicker shells had higher concentrations of HBCD (\( P=0.013, r^2=0.20 \)) and BDE154 (\( P=0.014, r^2=0.29 \)). Whether or not there is causality behind these correlations are not known. Further studies including e.g., effects of the BFRs on the shell gland are necessary.

**PLS, calculate BFR concentrations from OC concentrations**

Another aim was to investigate if concentrations of OCs and BFRs in guillemot egg co-varied with each other. The PLS with guillemot egg concentrations of BFRs as \( Y \), and OCs as \( X \) (\( R^2X= 0.91, R^2Y= 0.79 \) and \( Q^2= 0.64, three components \), showed with the high \( R^2 \) and \( Q^2 \) values, that the OCs and BFRs co-varied to a high degree (similar as in article I).

The coefficient plots (Figure 7) for BDE47, BDE99, BDE100, and BDE153 were almost identical. These BFRs were significantly positively correlated with CB138, CB153 and CB180 and these patterns were very similar to the ones shown in guillemot muscles (I). The coefficient plots for BDE154 and HBCD differed from those of the other BFRs. Both BDE154 and HBCD co-varied positively with \( \beta \) HCH, and BDE154 co-varied positively also with \( p,p' \) DDE and the PCBs.

In guillemot muscles the patterns for BDE154 and HBCD were even more similar to each other (I). These groupings are most likely caused by similarities between the contaminants within a group, regarding physiochemical properties, exposure routes, bioavailability, distribution, biotransformation, and/or excretion.
Figure 7. Coefficient plots with 95% CI for the respective Y variables for a PLS model ($R^2_X = 0.91$, $R^2_Y = 0.79$ and $Q^2 = 0.64$, three components) between concentrations of organochlorines (as X) and brominated flame retardants (as Y) in individually analyzed guillemot (*Uria aalge*) eggs from the Baltic Sea, the years 2000, 2001, and 2002 ($n = 30$). For abbreviations, see Materials and Methods (II).

**SIMCA classification and PLS-discriminant analysis, contaminants in muscle versus in egg**

When performing univariate statistical tests (e.g., t-tests) the risk for spurious results increase with the number of tests performed. Multivariate data analysis eliminates that risk as well as facilitates the interpretability of the complex patterns (relationships) that exist within the data. One important question was whether egg and muscle differ significantly from each other in their contaminant patterns. To answer this, the separation between the two classes, egg and muscle, was first tested using SIMCA classification and the result showed that the two classes were completely separated. In order to identify the important contaminants, and quantify their contribution to the class separation, PLS-DA was applied to the data. The analysis showed that the concentrations of 7 ($\beta$HCH, CB153, CB180, BDE47, BDE99, BDE100,
and BDE153) out of the 14 contaminants did not differ between egg and muscle, that 6 contaminants (p,p’DDE, HCB, CB28, CB118, CB138, and HBCD) had higher concentration in egg than in muscle, and that one contaminant (BDE154) had lower concentration in egg than in muscle.

The concentrations of lower chlorinated biphenyls (CB28 (*tri*), CB118 (*penta*), and CB138 (*penta*)) but not of higher chlorinated biphenyls (CB153 (*hexa*) and CB180 (*hepta*)) were higher in egg than in muscle. Similar results have been obtained from a study on nesting tree swallows (*Tachycineta bicolor*) (222) and also from a study with experimentally dosed hens (*Gallus domesticus*) where the calculated egg to hen concentration ratio for CB105, CB156 and CB189 showed that there were higher relative transport to the egg of the lower chlorinated CBs (223).

The finding here that several of the contaminants showed higher concentration in egg than in muscle is contrary to results from studies showing that concentrations, on a lipid weight basis, of contaminants in egg did not exceed those in tissues from adult birds (224-228) or alligators (*Alligator mississippiensis*) (229).

These diverging results might be due to differences between the studied species, and/or that many studies are of short duration and/or based upon a low number of observations and/or based upon a low number of analyzed contaminants.

When the numbers of studied contaminants are low (e.g., only include CB153) the risk increases of excluding the few contaminants that have properties favouring accumulation in the egg.

A higher contaminant concentration in egg than in muscle might be due absence/low activity of metabolizing enzymes in the egg and/or differences in lipid composition between the two matrices. In the future, to be able to calculate the maternal transfer of contaminants it is essential to sample mother-egg pairs. This has been done, to my knowledge, in surprisingly few studies.
Article III

A statistical resampling method to calculate Biomagnification factors exemplified with organochlorine data from herring (Clupea harengus) muscle and guillemot (Uria aalge) egg from the Baltic Sea.

Concentrations

The OC with the highest level in herring muscle was $p,p'$DDE with a concentration (GM with 95% CI) of 370 (320 – 430) ng/g lw and this OC also showed the highest concentration in guillemot egg of 21 200 (19 400 – 23 200) ng/g lw.

Multivariate Data Analysis

A PLS-DA with contaminant concentrations in herring versus those in guillemot ($R^2_X = 0.78$, $R^2_Y = 0.95$, $Q^2 = 0.94$, two components) showed complete separation between the two species. The coefficient plot (Figure 8) showed that $p,p'$DDE, $\beta$HCH, HCB, CB28, CB118, CB138, CB153, and CB180 all had higher concentrations in guillemot egg, while $p,p'$DDD and CB101 had higher concentrations in herring muscle.

![Figure 8. Coefficient plot with 95% CI for the respective variables from PLS-discriminant analysis (PLS-DA) based on concentrations of contaminants in guillemot (Uria aalge) egg (n= 29) from Stora Karlsö and herring (Clupea harengus) muscle (n= 72) from Landsort and Utlangan, from the Baltic Sea, 1996-1999. PLS-DA model for guillemot versus herring ($R^2_X= 0.78$, $R^2_Y= 0.95$, $Q^2= 0.94$, two components). For abbreviations, see Materials and Methods (III).](image-url)
Biomagnification Factors

The BMFs calculated using different methods are presented (Table 3, page 38 and in III). The OCs that biomagnify, (BMF significantly higher than 1) were e.g., \( p,p' \)DDE that biomagnified with a BMF_{RSRGM} of 51 (29-113) and \( p,p' \)DDD that did not biomagnify (BMF_{RSRGM} of 0.42).

Worth noticing is the large variation in the BMFs as shown by the large SD.

Number of Iterations

When I performed the series with increasing number of iterations (3, 5, … 100 000) 10 times each using the CB153 concentration in herring muscle and guillemot egg, the variation between the 10 resulting BMF_{RSRGM} values (from the ten runs with same number of iterations) showed similar patterns.

The coefficient of variation (CV%) rapidly declined with an increase in number of iterations, from a CV% of 57% between the 10 runs with 3 iterations to 0.5% or below between the 10 runs with 10 000 iterations or more (Figure 9a).

When using a higher number of iterations, the resulting BMF_{RSRGM} converge to one consistent value (equal to BMF_{GM}). Accordingly, when using a higher number of iterations, the resulting GMSD and AMSD converge to one consistent value (shown for GMSD in Figure 9b).
Figure 9. Effect of increasing the number of iterations on the calculated biomagnification factor (BMFRSRGM) as well as on the size of the geometric mean standard deviation (GMSD) based on CB153 concentration in herring (*Clupea harengus*) muscle and guillemot (*Uria aalge*) egg, from the Baltic Sea, 1996-1999. (a) Each dot represents the resulting value; BMFRSRGM after one run with 3, 5, 10…10 000 iterations/run. Horizontal line represents the BMFRSRGM for CB153 after 50 000 iterations. Increasing number of iterations leads to decreasing coefficients of variation, from ~60% (with 3 iterations) to ~0.5% (with 10 000 iterations). (b) Each triangle represents the resulting standard deviation for the BMFRSRGM after one run with 3, 5, 10…10 000 iterations/run. Horizontal line represents the geometric mean standard deviation (GMSD) for CB153 after 50 000 iterations.
Modeling Biomagnification Processes

One important issue to consider when modeling biomagnification processes is the wild animals’ diet as the exact composition is unknown. In the present study, the BMFs are based on data from herring, although the diet of guillemot is a mix, mainly of herring and sprat (10,18,230,231). A number of studies has, however, shown that the contaminant levels in herring and sprat are remarkably similar (19,20,23). Due to differences in growth rate, a sprat of a given size might be older and have higher concentrations of contaminants than a herring of the same size (20), and therefore, it is possible to overestimate BMFs for the step herring to guillemot if the guillemot diet was to a large extent comprised of old sprat. Ideally, for future research, I would therefore seek to calculate BMFs using contaminant data from the guillemots’ actual feed collected from the guillemots’ crop or stomach.

The data from herring used in article III originate from muscle. I assume that the contaminant composition of herring muscle reflects the composition of the whole body burden. To my knowledge, a comparison between concentration in herring muscle and concentration in whole fish is not available in the literature. However, unpublished data from the Swedish National Food Administration (NFA) that I have analyzed showed that the concentrations of contaminants in herring and sprat muscle as compared to the concentration in the whole body are similar when expressed on a lipid weight basis.

By using animals from several locations within the predators foraging area, a better representation of the prey contaminant content is gained. As the concentration of any given contaminant in both prey and predator varies due to, e.g. age, feed, location, as well as season of sampling, I believe that it is not possible to denote a “true” BMF for any contaminant based on data from wild living organisms. To make reliable comparisons with other calculations of BMFs possible, rigorously defined background information concerning predator and prey, as well as denoted variances for the BMFs, are needed.

Uncertainty and Variability

Recently, the U.S. EPA recommended probabilistic modeling approaches to characterize uncertainty and variability in ecological risk assessment (170). A method generating an estimate of the variation for the BMFs such as the RSR method that I present here is in line with this recommendation. When dealing with error propagation of bioaccumulation factors, many existing models are based on one or a few analyzed contaminant/s from one single individual or pooled sample, or from a few individuals, and generation of new, larger distributions for the contaminant concentrations is done by using Monte Carlo techniques (166,232) or Markov Chain Monte Carlo techniques (169). The difference between BMF_{MIN} and BMF_{MAX} can be close to 100-fold.
(Figure 4 and III) due to the individual variation in concentrations between samples. Analyzed contaminant levels in a population of living animals tend to be skewed (Figure 4, p. 23) with a few animals with more than 10 times higher contaminant levels as compared to the population mean. Here I illustrate (Figure 9, p. 35) that the accuracy of the BMF calculations is closely related to the number of observations. With low numbers of iterations, where 3 iterations simulate 3 individual samples, the calculated BMF for CB153 could be lower than 10 or as high as ~60. The high CV% between the runs with few iterations show that presence or the absence of outliers has a great impact on the resulting BMFs. This also demonstrates how uncertainties in the BMFs (e.g., BMF_{RSRGM} with its GMSD) could be reduced with larger sampling programs.

The geometric mean for the different BMFs, the old BMF_{GM} and the new BMF_{RSRGM}, does not differ from each other when I used 50 000 iterations or more. It is important to note that when performed correctly, these two values are identical. The main advantage with the RSR method is that it gives an estimate of the variation.

Why Differential BMFs?

Results presented (in III) show considerable variations in BMF_{RSR} between different contaminants (Table 3, p. 38). For instance, there were large differences in the BMFs between the three HCH isomers. The BMF_{RSRGM} (with ±SD range) for αHCH was 1.38 (1.08 - 1.75), for βHCH was 18.6 (12.7 – 27.3), and for γHCH was 2.10 (1.82 – 2.43). The logK_{ow} for α-, β-, and γHCH are similar: 3.80, 3.78, and 3.72, respectively (233).

The six chlorine atoms have different orientations, axial (a) or equatorial (e):

\[ \alpha\text{HCH in } aaaaee, \]
\[ \beta\text{HCH in } eeeeee, \]
\[ \gamma\text{HCH in } aaaeee. \]

These differences lead to differences in properties, and that βHCH has all chlorines in the e-direction seems to increase the stability and biomagnification of this isomer as compared to the others (72,74,234-236). For a more thorough discussion regarding what I believe are latent causes behind these differences in biomagnification factors, see the discussion in article (IV).
Article IV

QSBMR - Quantitative Structure Biomagnification Relationships: Physicochemical and structural descriptors important for the biomagnification of organochlorines and brominated flame retardants.

The aim was to establish a model as an aid to predict the biomagnification of contaminants as well as to explore what descriptor or combination of descriptors that were important for contaminant biomagnification. Included in the modeling were OCs and BFRs. One important find was that all the included BFRs biomagnified with BMFRSRGM between 2.5 (1.3-4.8) for BDE100 to 9.1 (5.1-16) for HBCD (Table 3) (237).

Table 3. Biomagnification factors (BMF) calculated with the RSR method using the concentration of contaminants in herring (Clupea harengus) muscle from Landsort and Ut langugan (n=72), and Guillemot (Uria aalge) egg from Stora Karlsö (n=30) from the Baltic Sea 1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>BMF&lt;sub&gt;RSRGM&lt;/sub&gt;</th>
<th>-SD</th>
<th>+SD</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;p&gt;&lt;sub&gt;p&lt;/sub&gt;,&lt;sub&gt;p&lt;/sub&gt;' DDE</td>
<td>57.1</td>
<td>28.8</td>
<td>113.1</td>
<td>yes</td>
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<tr>
<td>CB118</td>
<td>42.5</td>
<td>22.3</td>
<td>80.8</td>
<td>yes</td>
</tr>
<tr>
<td>&lt;p&gt;&lt;sub&gt;Σ&lt;/sub&gt;DDE</td>
<td>36</td>
<td>19.4</td>
<td>66.9</td>
<td>yes</td>
</tr>
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<td>HCB</td>
<td>33.8</td>
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<td>59.2</td>
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<td>12.1</td>
<td>50.4</td>
<td>yes</td>
</tr>
<tr>
<td>&lt;p&gt;&lt;sub&gt;Σ&lt;/sub&gt;PCB</td>
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<td>12.5</td>
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<td>tHCH</td>
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<td>12.7</td>
<td>27.3</td>
<td>yes</td>
</tr>
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<tr>
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<td>5.1</td>
<td>16.1</td>
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<tr>
<td>BDE154&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>2.6</td>
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</tr>
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</tr>
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<tr>
<td>BDE100&lt;sup&gt;6&lt;/sup&gt;</td>
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<td>4.8</td>
<td>yes</td>
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<td>γHCH&lt;sup&gt;9&lt;/sup&gt;</td>
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<td>1.82</td>
<td>2.4</td>
<td>-</td>
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<td>CB52&lt;sup&gt;8&lt;/sup&gt;</td>
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<td>αHCH&lt;sup&gt;9&lt;/sup&gt;</td>
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<td>0.84</td>
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<td>0.42</td>
<td>0.22</td>
<td>0.79</td>
<td>no</td>
</tr>
</tbody>
</table>

<sup>1</sup>The animal material for OCs was collected between 1996 and 1999 and for BFRs between 2000 and 2002.
<sup>2</sup>BMFRSRGM calculated with “randomly sampled ratios” (RSR) method using 50 000 iterations, resulting in geometric mean (GM) BMFRSR ± GMSD
<sup>3</sup>-SD is the lower limit and +SD the upper limit. The lower limit was calculated as BMFRSR / GMSD.
<sup>4</sup>+SD; the upper limit was calculated as BMFRSR * GMSD.
<sup>5</sup>BM; Yes= Biomagnification (BM) of contaminant with BMF significantly (p<0.05) higher than 1, No=No BM of contaminant, BMF not significantly higher than 1.
<sup>6</sup>ΣPCB = sum of ICES 7 marker PCBs: IUPAC no. CB28, CB52, CB101, CB118, CB138, CB153 and CB180
<sup>7</sup>n= 20 for BDE 154 and BDE100.
<sup>8</sup>ΣPBDE = sum of BDE congeners no BDE47, BDE99, BDE100, BDE153 and BDE154.
<sup>9</sup>Eventual BM cannot be determined, too high level of quantification (LOQ) for γ-HCH in guillemot egg.
<sup>9</sup>The level of quantification was used as the concentration in guillemot.
Model 1

A PLS with BMFs for the OCs and BFRs as the Y response, and ~100 descriptors as the X matrix gave a model ($R^2_X = 0.73$, $R^2_Y = 0.87$ and $Q^2 = 0.63$, three components) that showed that the generated descriptors are important for the biomagnification of the contaminants (for coefficients see the Supporting Information (IV)). Worth noticing is that there is not just a single structure but more than 20 descriptors together that are important, i.e., show significant positive or negative correlation to the BMFs.

Several descriptors do not have significant influence. In this QSBMR, log-$K_{ow}$, the often discussed and used descriptor as a determinant of a contaminants biomagnification, showed only a small, non-significant, positive influence on the BMF. This is further illustrated in Figure 10, that shows that BMF and log$K_{ow}$ are not significantly correlated (Pearson’s correlation; $P = 0.62$, $r^2 = 0.01$).

I believe that log$K_{ow}$ is an important variable for the bioavailability of contaminants. For the contaminants included in this QSBMR there are not large enough differences in log$K_{ow}$ to lead to any significant differences in bioavailability.

Figure 10. Persistent environmental pollutants log$K_{ow}$ versus their calculated biomagnification factors (BMF) (see Materials and Methods in IV). Correlation test (Pearson) $P = 0.62$, $r^2 = 0.01$.

It can be seen (Figure 11) that Model 1 had high ability to predict a contaminants BMF. However, for CB101 the model calculates a higher BMF (~12) then the one actually observed (0.46).
Model 2

BMFs for PCBs as the Y response and the descriptors for the PCBs as the X matrix gave a model (R²X= 0.83, R²Y= 0.87 and Q²= 0.58, two components). Figure 12 show that this model’s ability to calculate the BMF for the PCBs is high meaning that the generated descriptors are important for the biomagnification of the PCBs, e.g., the positions of the chlorine atoms on the biphenyl rings. This has also been discussed in (III). It is known that the elimination of PCBs is hindered by chlorines in the meta and para positions (238) and that metabolism is favored by adjacent hydrogen atoms in the meta and para positions (239). However, CB101 might have some additional property/ies that is not covered by the descriptors that lead to its “non-biomagnification”, or it might be a result of an unbalanced model. A training set chosen with the help of MVD and SMD would lessen this type of problem (see below).

Figure 12. OBS/PRED plot showing observed biomagnification factor (BMF) versus calculated BMF based on a PLS model (R²X= 0.83, R²Y= 0.87 and Q²= 0.58, two components) between PCBs’ BMF and their descriptors.
Model 3

The model with the BMF for the BFRs as Y, and the descriptors for the BFRs as X (R²X= 0.68, R²Y= 0.88 and Q²= 0.41, two components) showed lower Q² than the previous model that included only PCBs. This indicates that the model including only the BFRs is more unbalanced than the one including the PCBs. Figure 13 show that this model’s ability to calculate the BMF for the BFRs is still high meaning that the generated descriptors are important for the biomagnification of the BFRs.

![Figure 13](image)

When relating the BMFs for the contaminants to their respective descriptors the general pattern for the contaminants that biomagnify are that they have higher values in several descriptors and lower values in several others. All variables with a VIP (variables influence on projection) higher than ~1 are important in the model (240). After combining the VIP with the variables’ coefficient (that can be positive or negative), the variables with a large positive influence on BMF could be identified, as well as those with a large negative influence. For instance, the EV (Ellipsoidal Volume), DMX (Dipole Moment X Component (Whole Molecule)), binary fingerprint in 3'4' position, Kier 11 (Kier Chi4 (path/cluster) index) and number of halogens, were all positive, e.g., an increase in DMX is correlated to an increase in biomagnification.

The reverse can be said about variables with negative coefficients. An increase in e.g., VHF (Heat of formation), VTE (Total Energy), HA (Number of H-bond acceptors), DMZ (Dipole Moment Z Component (Whole Molecule)) LUMO (lowest unoccupied molecular orbital) and HOMO (highest occupied molecular orbital) have negative influence on BMF, an increase leads to lower BMF. I believe that these descriptors summarize several important properties in the contaminants that favors or counteract biomagnification. To be a candidate for biomagnification a substance
has to be bioavailable and not easily eliminated. It is probable that the QSBMR include important descriptors that in combination determine properties that lead to the biomagnification of a contaminant. As usual with computer generated descriptors the interpretation of them to a specific structural feature is not always straightforward. However, for estimating BMF the generated QSBMR models are still useful as a tool for predicting the BMF for untested contaminants.

Validation and future modeling

There are numerous articles that report concentration data as e.g. ΣPCB, ΣDDT, ΣPCDD/PCDF and ΣHCH. Though, by using the sum of all analyzed congeners/isomers and then calculating BMFs, differences between the various congeners/isomers’ BMFs are not possible to discern. This is a serious disadvantage as congener/isomer specific data is crucial when performing QSBMR in order to gain a deeper insight into underlying mechanisms favoring or counteracting the biomagnification of contaminants.

I based the QSBMR models on BMF calculated on data from the Swedish monitoring program. The number of analyzed contaminants (~20) is fairly large but the composition of contaminants is not optimal which can lead to an unbalanced model. This article must therefore be regarded as an initial attempt to investigate if the existing monitoring data, together with the generated structures, could be used for QSBMR.

To evaluate a model with contaminants not included in the model making is very important. The training set of this article, the 7 contaminants chosen with the help of MVD (CB101, CB118, CB153, CB180, BDE47, BDE153 and BDE154) was used to establish a model. The BMF for the test set (7 contaminants left out of the model) were then predicted by the model. The OBS/PRED plot for the test set (Figure 14) showed that there was a high R² (0.65) between predicted and observed BMFs.

Most of the contaminants that biomagnify were also predicted by the model to have high BMFs. A noteworthy exception is HBCD that was predicted to have a BMF of ~1 when in reality HBCD biomagnify ~9 times between herring and guillemot. This indicates again (as with CB101) that the QSBMR can be improved.
As this study was based on the contaminants included in the Swedish monitoring program the selection of the training set was restrained. One important step shown in the present work is the need to establish good training sets. Therefore, in order to accomplish a general QSBMR with high predictive power, a training set, selected with statistical molecular design (SMD) (200,241-243) together with the contaminants found in nature should be used. Such a training set spanning the chemical variation space is hereby suggested (Table 4). It includes 16 PCBs and/or 16 PBDEs that have their Cl respectively Br atoms in as varied positions as possible. This can be performed in a similar way for other compounds.

Table 4. Training set selected with the use of SMD for PCBs or PBDEs, (IUPAC nomenclature).

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</table>

After designing a training set it is important to determine the response (e.g., BMF in feeding experiments). Such a QSBMR will be useful for in silico predictions of the biomagnification of new, not yet investigated, compounds and as an aid in risk assessments.
Sammanfattning på Svenska

Jag har arbetat med persistenta organiska miljöföroreningar i strömming (*Clupea harengus*) och sillgrissla (*Uria aalge*) från Östersjön. För sillgrissla har jag arbetat med både vuxna fåglar och ägg. De miljöföroreningar som ingår i avhandlingen är dels klorerade organiska ämnen (OCs): diklorodifenyltrikloroetaner (DDTs), polyklorerade bifenyler (PCBs), hexaklorobensen (HCB), och hexaklorocyklohexan (HCHs); dels bromerade flamskyddsmedel (BFRs): polybromerade difenyletrar (PBDEs) och hexabromocyklododekan (HBCD). Ämnet med högst koncentration i både strömming och sillgrissla var DDT-metaboliten \(p,p'\)-DDE, vars koncentration i sillgrissleägg var 18200 (17000 – 19600) ng/g lipid vikt (lw) (som geometriskt medelvärde (GM) med 95% konfidensintervall (CI)). Den BFR som visade högst koncentration i äggen var HBCD med 140 (120 – 160) ng/g lw (GM, 95% CI).

För den statistiska analysen använde jag följande multivariata dataanalystekniker: principalkomponentanalys (PCA), ”partial least squares regression” (PLS), ”soft independent modelling of class analogy” (SIMCA) och PLS-diskriminantanalys (PLS-DA). När jag modellerade korrelationer mellan kontaminantmönstret och djurens eller äggen biologiska variabler fann jag bl. a. signifikanta negativa korrelationer mellan äggens vikt och deras koncentration av HCB och \(p,p'\)-DDE. Vidare fann jag att koncentrationerna av OCs och av BFRs i djuren eller äggen samvarierade på så vis att koncentrationerna av BFRs kunde beräknas från provets koncentration av OCs och *vice versa*. En PLS-modell beräknade koncentrationen av BFRs i individen från dess koncentration av OCs med t.ex. \(R^2_Y \sim 0.5\) för HBCD och \(\sim 0.9\) för BDE47. En PLS-DA-modell visade att några av miljöföroreningar (t. ex. HBCD) hade signifikant högre koncentration i sillgrissleägg än i muskelvävnad från vuxna sillgrisslor.

I denna avhandling presenterar jag en ny metod för att beräkna biomagnifikationsfaktorer (BMFs). Med denna metod, som jag kallar ”randomly sampled ratios” (RSR), kan BMFs beräknas och resultatet anges med ett mått på variansen. Ämnen som biomagnifierade, d.v.s. som har en BMF\_\text{RSR} signifikant högre än 1, var \(p,p'\)-DDE, CB118, HCB, CB138, CB180, CB153, \(\beta\)-HCH, och CB28. Ämnen som ej biomagnifierade var \(p,p'\)-DDT, \(p,p'\)-DDD, \(\alpha\)-HCH, CB101, och CB52. Ett viktigt resultat var att alla BFRs biomagnifierade.

Slutligen modellerade jag ”Quantitative Structure Biomagnification Relationships”, QSBMR, för att undersöka vilka fysikalisk-kemiska egenskaper och andra strukturella deskriptorer som är gemensamma för ämnen som biomagnifierar. Jag modellerade ämnenas respektive BMF vs \(~100\) deskriptorer och resultaten visade att fler än 20 deskriptorer var viktiga för biomagnifikationen av ämnena mellan strömmning och sillgrissla. Denna modell kommer att kunna vara till hjälp vid riskbedömningen av ett ämne.
Personal note

My work with this project has now come to one sort of an end; it has most certainly not reached The End. This project, as I suppose that most other projects do, contains a multitude of part-problems. I have solved some, I have initiated the solving of some, I have begun to realise some, and I am sure I have not yet realised that some problems even exists.

When I started with the project I honestly believed that I would ask some specific questions, that I would work on finding answers to those questions and, that I would end up with a finished project. Now, at this somewhat end I can honestly say that I have worked diligently. But I can not say that I have answered all questions that a project of this complexity give rise to. This conclusion is strangely enough very satisfying to me.

William Wordsworth, the British Poet Laureate, once wrote the poem Peter Bell, a tale in verse (244). In that poem there are some lines that have stuck in my memory…

A primrose by a river’s brim

A yellow primrose was to him,

And it was nothing more.

Here Wordsworth writes about a person that was a non-observer. I do not belong in that category. I am a biologist, an environmental toxicologist and I live in a world that is a wonderful and complex, sometimes incomprehensible, thing. My curiosity and strife to ask questions and to determine answers, to unravel “truths”, will continue to have a never-ending supply of special, interesting and funny projects to work with. I feel awe when I realise the complexity of the project I have been working with, I feel gratitude that I have had the privilege to work with solving some of my initial questions, I feel pride in my articles and in this thesis, and I am excited about continuing with research, finding more questions and ways to answer them.

Thank’s for that!  🎉
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