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Multivariate Data Analysis of Organochlorines and Brominated Flame Retardants in Baltic Sea Salmon (*Salmo salar*)

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Abstract

This report contains information about contaminants in salmon caught in November in the Baltic Sea, the year 2000. Concentrations of numerous types of organochlorines (OCs) and brominated flame retardants (BFRs) in the salmons have been analyzed and studied using multivariate data analysis. The report have four aims and the first aim is to determine the concentrations, variations and patterns of pollutants. The second aim is to see if there are any differences in contaminant pattern between the genders. The third aim is to look at concentrations of pollutants eventual correlations to biological factors of the fish (*e.g.* length, weight, condition factors and/or fat content). The last aim is to investigate if the concentrations of OCs and BFRs co-varied with each other, if concentrations of OCs can be used to calculate BFRs and *vice versa*.

DDE was the contaminant that reached the highest concentrations in both males and females, with higher concentration then ∑PCB. The pollutants showed different patterns in male and females, meaning that there is a difference in the contaminant patterns between the genders. Several containments had significantly higher levels in females than in males. Regarding the groupings of the contaminants when analyzing the contaminant concentration data with principal component analysis several groups were formed, one with all BFRs and OCs (excluding dioxins and furans, and “dioxin-like” polychlorinated bipenhyls (DL-PCBs)), one group consisted of the DL-PCBs and the last was one more loosely formed group with the dioxins and furans. The groupings show that the contaminants within the same group have the same exposure routes, chemical reactivity, bioavailability, distribution, biotransformation, and/or excretion thus co-varying to a high degree. The result shows that females have significant higher lipid content than males. The concentration of BFRs and OCs co-varied with each other a linear regression for instance between BDE47 and CB101, concentrations showed a r^2 of > 0.92 and a p-value of < 0.0001.

Sammanfattning

Den här rapporten innehåller information om lax som är infångad i november i Östersjön år 2000. Olika typer av organokloriner (OK) och bromerande flamskyddsmedel (BFM) har blivit analyserade och studerade med hjälp av multivariat dataanalys. Rapporten är uppbyggd kring fyra frågeställningar, varav den första frågan rör koncentrationer, variationer och mönster i miljögifterna. Den andra är att undersöka om det finns det skillnader mellan könen. Den tredje frågan handlar om det finns samband mellan miljögifter och laxarnas biologiska faktorer t ex längd, vikt och fetthalt. Den sista frågeställningen undersöker om koncentrationerna av BFM och OK samvarierar med varandra, om koncentrationen av BFM kan räknas ut med hjälp av koncentrationen av OK och vice versa. DDE är det miljögift som når de högsta koncentrationerna både i honor och i hanar med högre koncentration än ∑PCB. Miljögifterna har olika koncentrationer i honor och hanar, vilket betyder att där är skillnad i kontaminantmönstret mellan honor och hanar. Flera miljögifter hade högre nivåer i honor än hanar. Vid principalkomponentanalys av alla föroreningars koncentrationer i laxarna skapades grupperingar med olika miljögifter; en med BFM och OK (exkluderat dioxiner, furaner och ”dioxinliknande” polyklorerade bifenyler (DL-PCBer)), en grupp med DL-PCBer och en mer löst formad grupp med dioxiner och furaner. Denna gruppering indikerar att vissa ämnen inom samma grupp har samma exponeringsvägar, kemisk reaktivitet, biotillgänglighet, biotransformation och/eller exkretion, vilket leder till en hög grad av kovarians. Honorna har signifikant högre fetthalt än hanarna. Koncentrationerna av BFM och OK kovarierade med en varandra; linjär regression till exempel mellan BDE47 och CB101 visar ett r^2värde > 0.92 och ett p-värde < 0.0001.

Contents

Abbreviations

BFR	Brominated flame retardants
BFM	Bromerade flamskyddmedel (in Swedish)
DDD	Dichlorodiphenyldichloroethane
DDE	Dichloroethylene
DDT	Dichlorodiphenyltrichloro-ethane
FR	Flame retardant
HBCD	Hexabromocyclododecane
MVDA	Multivariate data analysis
OC	Organochlorines
OK	Oragnokloriner (in Swedish)
PBB	Polybrominated biphenyls
PBDE	Polybrominated diphenyl ethers
PCA	Principal component analysis
PCB	Polychlorinated bipenhyls
PLS	Partial least squares regression projection to latent structures
POP	Persitant organic pollutants
TBBPA	Tetrabromobisphenol-A
TCDD	Tetrachlorodibenzodioxin
TEF	Toxic equivalency factors
TEQ	Toxic equivalency quotient
VIP	Variable influences on projection

Introduction

The Baltic Sea has during the last century been contaminated with various pollutants through the activities of man; via eutrophication [1] and industry [2]. In the Baltic Sea the pollutants get incorporated in the food chain and affects living organisms [3]. Some persistent contaminants biomagnify to top predators [4] and can reach high levels in piscivorous fish like the salmon. Salmon serve as an important food source and the Swedish Food Administration recommends that one eat fish three times a week [5, 6], because of its nutritional value *e.g.* that fish contains long chains of essential omega-3 fatty acids [6]. Knowing current pollutant levels is of great importance, for example when giving food recommendations to the public or specific risk groups like pregnant women. Pollution may lead to severe damages in an already threatened environment like the Baltic Sea [7] and basic data regarding levels and trends as well as effects caused by pollutants are needed. Especially before one can start to regulate the use of certain chemicals.

This report is about salmon caught in the Baltic Sea and is focusing on four different aims. The first aim is to look at the contaminants analyzed in the salmon muscle; to determine concentrations, to discern variations and patterns among different pollutants. The second aim is to see if there are any differences between the genders. The third aim is to look at concentrations of pollutants and their correlation to biological factors of the fish *e.g.* length, weight, and/or lipid content. The fourth aim is to investigate if the concentrations of organochlorines (OCs) and brominated flame retardants (BFRs) co-varied with each other, if concentrations of OCs can be used to calculate BFRs and *vice versa*.

Baltic Sea

The Baltic Sea is the largest sea with brackish water in the world. The sea consist of several basins with various depth and the only communication with the North Sea is through the narrow and shallow Öresund and the Belt Sea [8]. The Baltic Sea drainage area includes 14 densely populated and industrial countries, where about 90 million people live. The Sea contains both hard- and soft-bottoms, with bladder wrack *Fucus vesiculosus* and the blue mussel *Mytilus edulis* as the dominant species of hard-bottoms and the Baltic macoma *Macoma balthica* as a dominant species of the soft-bottom. The salinity is declining from the south towards the north, leading to a rapid decrease in the biomass and number of species towards the north [3]. The severe ecosystem in the Baltic Sea leads to high physiological stress, causing increased sensitivity to pollutants [9].

Salmon (Salmo salar)

The salmon was named *Salmo salar* by Linné in the year 1758. The salmon in the Baltic Sea is hatched in several different rivers, lives there for a few years and then transforms from spawn to smolt and emerge to the Baltic Sea or in the lake Vänern with its surrounding waters. Vänern is a large fresh water lake in Sweden. In the rivers young salmons is characterized by 8-10 blue-green dots along the sides with red dots in between. As an adult the salmon lives either in the sea or in Vänern. The adult salmon has a grey-silverish color, with black x-shaped or circle dots above the collateral line. Before spawning the males get a colorful costume and the lower jaw transforms into a hook, while the females get a less colorful costume. Maximum weight and length for salmon is 35-40 kg and 130-150 cm respectively. At the end of 1990 there were 40 rivers in Sweden with an annual natural reproduction of wild salmon. Compensation rearing of salmon (spawn and smolt) has been performed in numerous waters to improve the populations. The human influence have the last decades been a severe treat to the wild salmon *eg*. development of hydropower, pollution and changes in the biotope. Since the 1800-century wild populations has disappeared from smaller waters and in the 2000-century also from larger. An ongoing exploitation of rivers may lead to the disappearance of even more populations. Recreation of spawning- and growth areas have lead to an improvement in the reproduction situation and a weak increase in salmon spawning has been seen. More improvements for the salmon are planned and the development of the salmon population growth will be monitored. The salmon is classified as endangered by the Swedish Species Information Center, for more information see www.artdata.slu.se [10]. Today, all Swedish salmon contain pollutants to such a degree that salmon meat exceeds the limiting value set by the EU, which is a TEQ of 8 pg/g (ww) for all dioxins and DL-PCBs. The Swedish Food Administration now recommend the females and children (both boys and girls) only consume wild caught salmon from the Baltic Sea or lake Vänern 2-3 times/ year.

Contaminants

All the contaminants in this report are chlorinated or brominated organic substances so called organochlorines (OCs) and brominated flame retardants (BFRs). Among the OCs some compounds are classified as persistent organic pollutants (POPs). To deal with these kind of compounds the Stockholm Convention of Persistant Organic Pollutations adopted a text on the 22 May 2001, which later was entered into force the 17 May 2004 [11]. All compounds listed as POPs share four properties; they are highly toxic, accumulate in fat tissue, they have the ability to travel long distance in air and water and they are persistent. Visit www.pops.int for further information. In this report the following compounds, which also are in the list of “The first 12 POPs”, are included; DDT, dioxins, furans, HCB and PCBs [12].

Organochlorines

From the mid- 1940s OCs agents were used widely in a numbers of various aspects *e.g.* agriculture, forestry and to control insect pests. Some OCs make up an efficient group of insecticides because of the chemical structure; chemical stability, lipid solubility, slow rate of biotransformation and degradation. These properties lead to persistence in the nature, and an accumulation of concentration and possible biomagnification within various food chains [13].

DDT, DDE, DDD and PCB

DDT (dichlorodiphenyltrichloro-ethane) was first synthesized by Zeidler in 1874 and it was rediscovered when searching for an insecticide against clothes moths and carpet beetles [14]. The use of DDT has many advantages; it is extremly toxic to insects but less toxic to other animals, it has a low production cost, it is persistent thus continuing its insecticidal properties for a very long time. In history as well as today in many developing countries DDT has been used for control of malaria and other insect-borne diseases. DDE (dichloroethylene) is a metabolite of DDT, resulting from the loss of one chlor and one hydrogen atom (see Figure 1). It doesn’t serve as an insecticide like DDT because of its low toxicity to insects. DDE is the most common chlorinated hydrocarbon in the sea and in marine organisms, as a result of metabolism of DDT. DDD (dichlorodiphenyldichloroerthane) is another metabolite of DDT. DDD has been used as an insecticide because it has lower toxicity to fish than DDT. Due to its chemical and physical characteristics it can be excreted by organisms and rarely accumulates, like DDE [8].

Dr D.A. Ratcliff showed in 1967 that DDT causes thinning of eggshells, resulting in reproductive failure. This caused the declining in the white-tailed eagle (*Haliaeetus albicilla*) population in Sweden, and after the prohibition of DDT and PCB it took over ten years before the eagle population started to recover [15]. *p,p*’DDT and *p,p*’DDE display oestrogenic activity and areas contaminated with these substances have declining animal populations. For instance, the alligator populations in Florida show sexual abnormalities and have eggs that fail to hatch. The alligators had low levels of DDE (0.01 ppm) not enough for causing toxic effects but enough to disrupt the endocrine system [3].

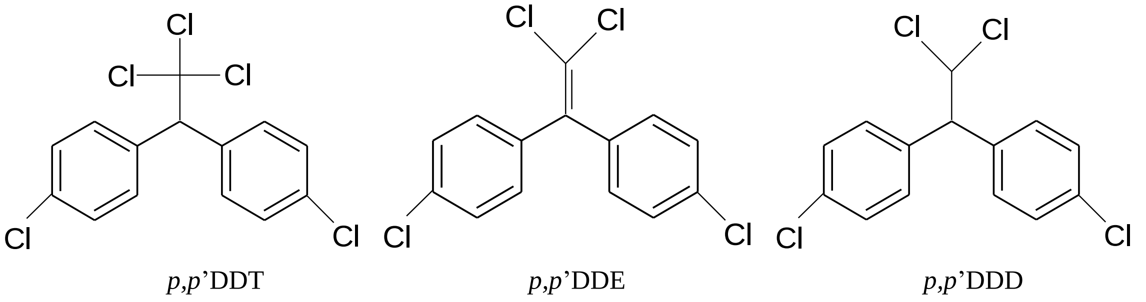


Figure 1. Chemical structure of *p,p*'DDT, *p,p*'DDE and *p,p*'DDD.

PCBs (polychlorinated bipenhyls) have been used since the 1930s as flame retardants in electric equipment, in paints and in plastics as it is resistant to chemical attacks. The number of chlorine atoms at one PCB-molecule varies from one to ten and these can be differently positioned on the two phenyl rings (Figure 4) giving 209 possible so called congeners. A rising concern about environmental damage from chlorinated hydrocarbon pesticides affected the use of PCBs as well. A reduction of manufacturing of PCB started in 1970 and by the mid-1980s most members of the European Union had stopped the production. But even though the manufacture has been restricted today, the concentrations are still high in the environment [8].

PCB has affected several species in the Baltic Sea. Mammals all over the world like seals, sea lions and otters have had declining populations. It is suggested that the high levels of PCB in seals is responsible for a failure of reproduction. There was an accident in Japan were rice oil became contaminated with PCB which caused darkening of the skin in humans, enlargement of hair follicles and eruptions of the skin resembling acne. Similar symptoms have also been observed in workers in Japan, and their symptoms disappeared when the use of PCBs ceased. Exposure to PCB and *p,p*'DDE from consumption of fat fish from the Baltic Sea has shown to effect human sperm quality [16].

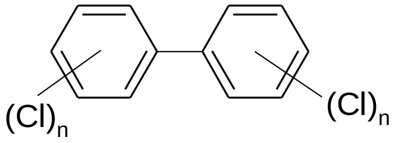
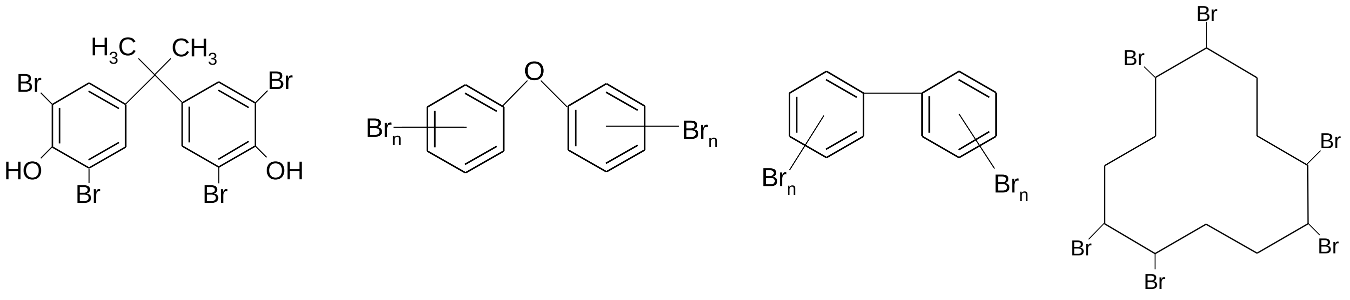


Figure 2. Chemical structure of PCB.

Brominated Flame Retardants

BFRs are an umbrella term for organic compounds which contains bromine and prevent the spreading of fire and increase the time for a fire to ignite. They are used for example in electronic equipment, textiles, construction materials and furniture. The use has increased dramatically over the last decades [17]. BFRs are an umbrella term for organic compounds which contains bromine. Some groups of BFRs are; TBBPA (Tetrabromobisphenol-A), HBCD (Hexabromocyclododecane), PBDEs (Polybrominated diphenyl ethers) and PBB (Polybrominated biphenyls) (see Figure 3).

Concerns, has risen because of the BFRs persistence, bioaccumulation and toxicity, in human and animals. Due to the industrial use, BFR have been released in to the surrounding environment, mainly via equipment that has been treated with BFR. BFR can now be found everywhere in water, sediment, animals and human tissue. BFR is lipophilic and accumulates in the bodies' fat tissue [18]. PBDE have a biomagnification potential in the food chain in the Baltic Sea ecosystem [11]. Major exposure routes to human are dietary intake, dust inhalation and occupational exposure. Uptake via food are of great importance, especially consumption of meat, fish and dairy products. Fish is of great concern, due to high levels of PBDE [19]. There is only a limit of studies made on toxicity to humans. One study showed higher-than-normal prevalence of primary hypothyroidism and a reduction on conducting velocities in sensory and motor neurons. Hypothyroidism is a disease caused by insufficient production of thyroid hormones [18]. Viberg *et al.* ,2004, have showed that PBDE can cause a behavioural neurotoxic effect and affect cholinergic receptors in mice [20].



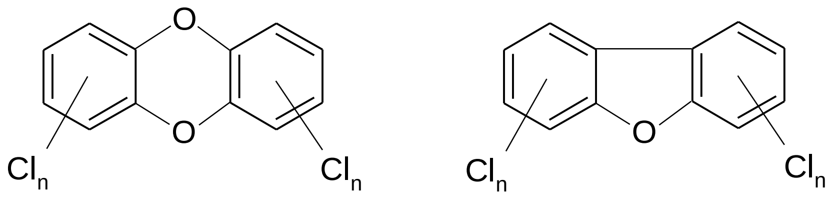
TBBPA PBDE PBB HBCD

Figure 3. Chemical structure of TBBPA, PBDE, PBB and HBCD.

Dioxins and furans

Dioxins (PCDD) and furans (PCDF) consist of two groups; chlorinated dioxins (75 congeners) containing one to eight chlorine atoms, were the congener TCDD (2,3,7,8-tetrachlorodibenzodioxin) is of greatest interest due to its high toxicity. The second group, chlorinated dibenzofurans has a similar structure but contains 135 congener (Figure 4). Dioxins are a side product in the wood processing industry and when producing herbicides. They are extremely toxic, physically and chemically stable and soluble in organic solvent, fat and oil. These characters makes dioxins and furans an important group to eliminate, and some of the sources has been reduced or eliminated [8].

Evidence of dioxins being damaging to humans, is rather inconclusive. One accident, when there was an explosion in a pesticide factory, and the surroundings became showered with dioxin, lead to chloracne, minor but reversible nerve damage, and some impaired liver functions. Studies have been made to reveal a link between dioxins and soft tissue sarcomas, but this cancer type has been rare and so far the link hasn't been confirmed [3].



Dioxin (PCDD) Furan (PCDF)

Figure 4. Chemical structure of dioxins and furans.

TEF & TEQ

Toxic equivalency factors (TEF) is a measurement of toxicity for dioxin-like compounds. In this report several compounds are included that have dioxinlike modes of action, both dioxins, furans and also “dioxin-like” PCBs (DL-PCBs). For a compound to be included in the TEF concept these criteria must been reached [21]:

- show a structure relationship to the PCDDs and PCDFs
- bind to the aryl hydrocarbon receptor (AhR)
- elicit Ah-receptor-mediated biochemical and toxic response
- be persistent and accumulate in the food chain

TEF-values are used by the World Health Organisation (WHO) as a method to evaluate toxicities of mixtures consisting of dioxins and furans as well as DL-PCBs.As 2,3,7,8-TCDD is one of the most studied and also one of the most toxic congener, it therefore has a TEF-value set to one. Then the other dioxins/furans and DL-PCBs are given TEF-values that show their respective toxicity in relation to TCDD. TEF-values is a useful tool for determine risks from mixtures of dioxin compounds. Toxic equivalency quotient (TEQ) is a measurement were the concentration of each compound is taken into account multiplied with its TEF-value. TEQ-value is calculated according to this formula [22]:

$$TEQ = \sum_{n} c_i * TEF$$

TEQ=value for toxicity of a mixture of compounds
n=numbers of compounds
c_i=concentration of each compound
TEF=a value for toxicity for each compound taken from WHO [21]

Aims

The report contains several aims:

- The first aim is to look at the contaminants; to determine concentrations, to discern variations and patterns among different pollutants.

- The second aim is to determine if there are any differences between the genders.

- The third aim is to look at concentrations of pollutants and their correlation to biological factors of the fish *e.g.* length, weight and/or lipid content.

- The fourth aim is to investigate if the concentrations of OCs and BFRs co-varied with each other, if concentrations of OCs can be used to calculate BFRs and *vice versa*.

Material and Methods

Salmon

In this report a total of 17 salmons (*Salmon salar*) are included that were caught in the Baltic Sea, in November the year 2000, near Gotland will be used. The salmons' biological variables (see Table 1) were; smolt age, sex, body weight (BW), body length (excluding tail fin (BL) and including tail fin (TBL), separately), condition factor (Cond. F.), liver weight (LW), liver somatic index (LSI), brain weight (BrW) and lipid content (F%). Condition factors were calculated as BW/TBL^3. Liver somatic index is calculated as LW/BW*100. The origin of the fish, either wild or reared was noted.

Table 1. Biological data (mean ± st.dev, min - max) for salmon (*Salmo salar*) (n=17) caught in the Baltic Sea, the year 2000. BW= body weight, BL=Body length excluding tail fin, TBL= Total body length including tail fin, Cond. F.= Condition factor (BW/TBL^3), LW=Liver Weight, LSI=Liver Somatic Index (LW/BW*100), BrW=Brain Weight and F%=Lipid content.

			Females n=10	**Males** n=7	**P-value** Female vs. male[1]
Smolt age[2]	year	Mean ± St Dev	2.20 ± 0.42	2.00 ± 0.0	ns
		Min – Max	2.00 – 3.00	2.00 – 2.00	
BW	g	Mean ± St Dev	4159 ± 461.3	3728±593.5	ns
		Min – Max	3328 – 4960	2945 - 4561	
BL	cm	Mean ± St Dev	62.25 ± 2.05	60.93 ± 4.99	ns
		Min – Max	58.00 – 64.00	54.00 – 68.00	
TBL	cm	Mean ± St Dev	71.35 ± 2.36	69.36 ± 4.42	ns
		Min – Max	66.00 – 74.00	62.00 – 76.00	
Cond. F.	g/cm^3	Mean ± St Dev	1.143 ± 0.03	1.11 ± 0.08	ns
		Min - Max	0.99 – 1.28	1.00 – 1.24	
LW	g	Mean ± St Dev	50.40 ± 7.89	52.71 ± 15.33	ns
		Min - Max	39.00 – 63.00	31.00 – 73.00	
LSI	%	Mean ± St Dev	1.22 ± 0.16	1.41 ± 0.31	ns
		Min - Max	0.96 – 1.43	1.05 – 1.89	
BrW[2]	g	Mean ± St Dev	0.80 ± 0.055	0.69 ± 0.13	ns
		Min - Max	0.73 – 0.88	0.46 – 0.83	
F%	%	Mean ± St Dev	12.61 ± 3.95	7.93 ± 3.74	0.027
		Min - Max	5.95 – 18.40	4.76 – 14.37	

[1] t-test

[2] n=15 for BrW and Smolt age

Contaminant analysis

The following OCs and BFRs were analysed (dioxins, furans and DL-PCBs are presented in table 2); trans-nona chlor (t-n Chlor), 2,2-bis(4-chlorophenyl)-1,1,1-tri-chloroethane (*p,p'*-DDT) and its'metabolites *p,p'*-DDE and *p,p'*-DDD, polychlorinated biphenyls (PCBs) with the congeners'; 2,4,4´ tri-CB (CB28), 2,2´,5,5´ tetra-CB (CB52), 2,2´,4,5,5´penta-CB (CB101), 2,3,3',4,4'-penta-CB (CB105), 2,3',4,4',5-penta-CB (CB118), 2,2´,3,4,4´,5´hexa-CB (CB138), 2,2´, 4,4´,5,5´hexa-CB (CB153), 2,3,3',4,4',5-hexa-CB (CB156), 2,2´,3,4,4´,5,5´hepta-CB (CB-180), hexachlorocyclohexane (isomers α-, β-, and γ-HCH), and hexachlorobenzene (HCB). The BFRs were; hexabromocyclododecane (HBCD) and the polybrominated diphenyl ethers (PBDEs): 2,2´,4,4´tetra-BDE (BDE47), 2,2´,4,4´,5 penta-BDE (BDE99), 2,2´,4,4´,6 penta-BDE (BDE100), 2,2´,4,4´,5,5´ hexa-BDE (BDE153), and 2,2´,4,4´,5,6´ hexa- BDE (BDE154). ∑HCH was calculated as the sum of α-HCH, β-HCH and γ-HCH concentrations, ∑DDT was calculated as the sum of *p,p*'DDT, *p,p*'DDE and *p,p*'DDD concentrations, ∑PCB as the sum of ICES 7 marker PCBs': CB28, CB52, CB101, CB118, CB138, CB153 and CB180 concentrations and ∑PBDE as the sum of BDE cogeners BDE47, BDE99, BDE100, BDE153 and BDE154. The contaminants are presented both in lipid weight (lw) and wet weight (ww) and if that not specified concentrations are always given as wet weight. Analysed PCDD/DF and DL-PCBs, their abbreviations and their corresponding TEF values are presented separately in Table 2.

The chemical analyses was carried out by Swedish Museum of Natural History (for the organochlorines, excluding dioxins and furans), at Applied Enviromental Science (ITM) at Stockholm University (for the brominated flame retardants) and at Enviromental Chemistry at Umeå University (for the dioxins, furans and DL-PCBs).

Table 2. Analyzed dioxins, furans and DL-PCBs, their abbreviation and TEF-values for contaminants included in this report.

Analyzed substances	Abbreviations	TEF[1]
Chlorinated dibenzo-*p*-dioxins		
2,3,7,8-TCDD	TCDD	1
1,2,3,7,8-PeCDD	PeCDD	1
1,2,3,4,7,8-HxCDD	HxCDD1	0.1
1,2,3,6,7,8-HxCDD	HxCDD2	0.1
1,2,3,7,8,9-HxCDD	HxCDD3	0.1
1,2,3,4,6,7,8-HpCDD	HpCDD	0.01
OCDD	OCDD	0.0003
Chlorinated dibenzofurans		
2,3,7,8-TCDF	TCDF	0.1
1,2,3,7,8-PeCDF	PeCDF1	0.03
2,3,4,7,8-PeCDF	PeCDF2	0.3
1,2,3,4,7,8-HxCDF	HxCDF1	0.1
1,2,3,6,7,8-HxCDF	HxCDF2	0.1
2,3,4,6,7,8- HxCDF	HxCDF3	0.1
1,2,3,7,8,9- HxCDF	HxCDF4	0.1
1,2,3,4,6,7,8-HpCDF	HpCDF1	0.01
1,2,3,4,7,8,9-HpCDF	HpCDF2	0.01
OCDF	OCDF	0.0003
Non-*ortho*-PCBs		
3,3´,4,4´-tetraCB	CB77	0.0001
3,4,4´,5-tetraCB	CB81	0.0001
3,3´,4,4´,5-pentaCB	CB126	0.00003
3,3',4,4',5,5'-hexaCB	CB169	0.03

[1] Values based on the article by Van den Berg *et al*. 2006 [21].

Statistics

Basic Statistics

For statistic regarding the biological variables and concentrations of OCs and BFRs the software GraphPad Prism 5.01 [23] was used. This included calculations of the geometric mean (GM), 95% confidence interval (lower and upper), arithmetic mean values (Mean), standard deviation (St. Dev.), minimum (Min), maximum (Max), correlation analysis (Pearson), un-paired two-tailed t-test, Kolmogorov-Smirnov normality test, D'Agostino and Pearson omnibus normality test and Shapiro-Wilk normality test. The significance level was set to 0.05 for all the tests.

Multivariate statistics

Multivariate data analysis (MVDA) is a useful tool when handling data which has three or more variables, *i.e.* columns for each individual animal with measured or analysed values.

Two types of MVDA have been used; principal component analysis (PCA) and partial least-squares projection to latent structures (PLS). MVDA were performed using the software SIMCA-P +11 [24] and for all MVDA a significance level of 0.05 was used.

PCA was used to illustrate the data and to discover groupings in the data among the contaminants as well as grouping according to gender. 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 denote an acceptable model when analyzing biological data [25].

PLS was used to determine whether there were a significant relationship between biological factors and contaminants. PLS was also used to investigate if the concentrations of OCs and BFRs co-varied with each other. PLS is an extension of multiple linear regressions similar to PCA but it is used to model the relationship between two matrixes, Y and X, that can both be multidimensional.

Results

Concentrations of OCs and BFRs

Concentrations as geometric mean (GM) ± 95% confidence interval (-Cl and+Cl) in salmon are presentented in Table 3 and Table 4. The contaminants with the highest concentration in both females and males is *p,p*'DDE with a concentration almost as high as ∑PCB. When looking at the sum of different contaminants they will be arranged as followed: ∑DDT>∑PCB>∑HCH>∑PBDE both when considering lipid weight, wet weight and females and males separately.

Table 3. Concentrations (ng/g) as geometric mean (GM) with lower and upper 95% confidence interval (-Cl) and (+Cl) of organochlorines and brominated flame retardants in salmon (*Salmo salar*) (females n=10 and males n=7) muscle from the Baltic Sea, near Gotland, the year 2000. For abbreviations see Materials and Methods.

		Females n=10 ng/g			**Males** n=7 ng/g		
		GM	-Cl	+Cl	GM	-Cl	+Cl
αHCH	Lipid weight	10.69	10.34	11.05	10.55	9.863	11.28
	Wet weight	1.284	0.954	1.729	0.8154	0.558	1.192
βHCH	Lipid weight	16.28	15.61	16.97	15.51	14.20	16.94
	Wet weight	1.940	1.473	2.555	1.215	0.823	1.793
γHCH	Lipid weight	8.082	7.685	8.499	7.849	7.491	8.224
	Wet weight	0.975	0.769	1.237	0.605	0.411	0.891
∑HCH[1]	**Lipid weight**	**35.05**	**33.64**	**36.52**	**33.91**	**31.55**	**36.44**
	Wet weight	**4.199**	**3.200**	**5.521**	**2.635**	**1.792**	**3.876**
HCB	Lipid weight	24.73	21.75	28.11	24.27	21.68	27.16
	Wet weight	3.019	2.178	4.185	1.844	1.330	2.556
t-n Chlor	Lipid weight	19.65	17.19	22.47	22.50	17.87	28.31
	Wet weight	2.407	1.792	3.233	1.670	1.192	2.340
CB28	Lipid weight	3.120	2.639	3.689	2.479	1.906	3.226
	Wet weight	0.378	0.286	0.498	0.195	0.140	0.273
CB52[2]	Lipid weight	15.29	13.26	17.64	14.61	12.22	17.47
	Wet weight	1.983	1.667	2.357	1.107	0.806	1.521
CB77[3]	Lipid weight	1.041	0.848	1.278	0.892	0.565	1.408
	Wet weight	0.119	0.761	0.186	0.069	0.025	0.189
CB81[3]	Lipid weigh	0.016	0.012	0.023	0.015	0.010	0.022
	Wet weight	0.002	0.001	0.003	0.012	0.001	0.003
CB101	Lipid weight	58.54	51.09	67.08	58.42	47.56	71.77
	Wet weight	7.119	5.338	9.495	4.449	3.301	5.996
CB105	Lipid weight	22.10	19.56	24.96	21.66	17.06	27.51
	Wet weight	2.678	2.177	3.296	1.660	1.160	2.374
CB118	Lipid weight	60.87	53.95	68.68	57.65	46.07	72.15
	Wet weight	7.377	5.858	9.290	4.437	3.212	6.129
CB126[3]	Lipid weight	0.451	0.360	0.564	0.420	0.303	0.581
	Wet weight	0.051	0.032	0.081	0.033	0.013	0.079
CB138	Lipid weight	113.6	100.3	128.6	122.5	98.63	152.3
	Wet weight	13.74	10.89	17.33	9.298	6.681	12.94
CB153	Lipid weight	143.8	127.2	162.6	158.3	127.9	195.8
	Wet weight	17.46	13.58	22.44	11.93	8.751	16.27
CB156	Lipid weight	0.008	0.007	0.009	0.008	0.007	0.011
	Wet weight	0.001	0.001	0.001	0.001	nd	0.001
CB169[3]	Lipid weight	0.075	0.060	0.095	0.087	0.058	0.130
	Wet weight	0.009	0.005	0.014	0.007	0.003	0.013

CB180	Lipid weight	41.38	36.28	47.19	50.21	37.98	66.38
	Wet weight	5.006	3.934	6.371	3.753	2.687	5.242
∑PCB[4]	**Lipid weight**	**436.6**	**384.7**	**495.5**	**464.2**	**372.3**	**579.1**
	Wet weight	**70.52**	**41.55**	**67.78**	**35.17**	**25.58**	**48.37**
p,p’DDE	Lipid weight	364.4	308.3	430.6	368.9	305.3	445.6
	Wet weight	45.05	32.75	61.96	27.52	20.60	36.77
p,p’DDD	Lipid weight	154.9	128.7	186.5	126.1	85.03	186.9
	Wet weight	19.21	14.23	25.94	9.634	6.527	14.22
p,p’DDT	Lipid weight	77.19	62.03	96.06	73.53	55.00	98.29
	Wet weight	9.568	6.620	13.83	5.512	3.933	7.726
∑DDT[5]	**Lipid weight**	**596.5**	**499.0**	**713.2**	**568.53**	**445.3**	**730.8**
	Wet weight	**73.83**	**53.60**	**101.7**	**42.666**	**31.11**	**58.72**
PBDE47	Lipid weight	20.26	16.90	24.28	19.92	17.09	23.23
	Wet weight	2.506	1.801	3.487	1.491	1.094	2.033
PBDE99	Lipid weight	3.745	2.931	4.784	3.432	2.603	4.526
	Wet weight	0.466	0.325	0.666	0.2577	0.186	0.357
PBDE100	Lipid weight	3.357	2.823	3.991	3.710	3.073	4.479
	Wet weight	0.411	0.306	0.554	0.2765	0.205	0.373
PBDE153	Lipid weight	0.616	0.491	0.775	0.6396	0.473	0.865
	Wet weight	0.076	0.056	0.104	0.04761	0.036	0.063
PBDE154	Lipid weight	0.651	0.523	0.812	0.7493	0.571	0.983
	Wet weight	0.079	0.059	0.107	0.05608	0.044	0.071
∑PBDE[6]	**Lipid weight**	**28.63**	**23.67**	**34.64**	**28.451**	**23.81**	**34.08**
	Wet weight	**3.539**	**2.546**	**4.919**	**2.1289**	**1.565**	**2.898**
HBCD	Lipid weight	15.20	12.60	18.33	13.86	10.55	18.20
	Wet weight	1.872	1.343	2.609	1.052	0.800	1.384

[1] ∑HCH= sum of αHCH, βHCH and γHCH concentrations.

[2] n=9 for females

[3] n=6 for females and n=5 for males

[4] ∑PCB= sum of ICES marker PCBs: CB28, CB52, CB101, CB118, CB138, CB153 and CB180

[5] ∑DDT= sum of *p,p*’DDT, *p,p*’DDE and *p,p*’DDD

[6] ∑PBDE= sum of BDE congeners: BDE47, BDE99, BDE100, BDE153 and BDE154

Figure 5-6 illustrate the concentrations of chlorinated contaminants, female and male respectively. In Figure 5 all the analyzed OCs are shown though the DL-PCB cogeners are shown in Figure 6 seperatly. All the contaminants are shown on both lipid (A) and wet weight (B) basis in the different graphs. When considering PCBs on wet weight basis there is a significant different in concentration in some of the contaminants between females and males

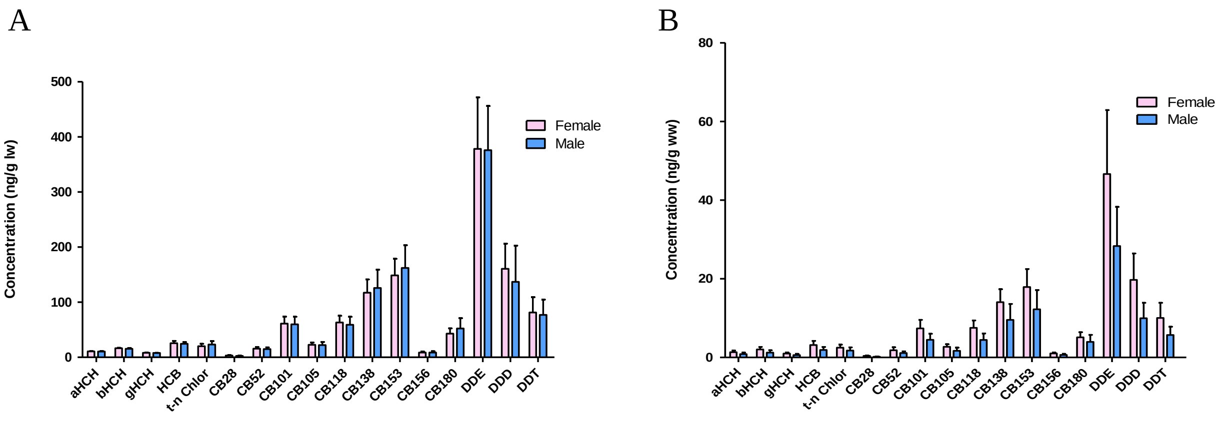


Figure 5. Concentration (ng/g) of clorinated contaminats in A lipid weight and B wet weight in salmon (*S. salar*) (females n= 10 and males n=7) muscle from the Baltic Sea, the year 2000.

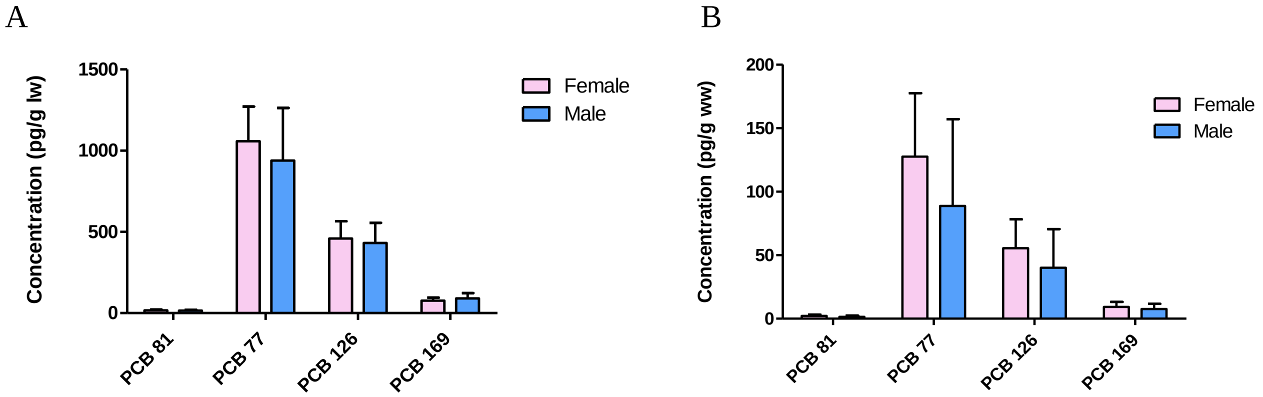


Figure 6. Concentration (pg/g) of clorinated contaminats in A, lipid weight and B, wet weight in salmon (*S. salar*) (n=6 for females and n=5 for males) muscle from the Baltic Sea, the year 2000.

The brominated contaminants (Figure 7) show a different contaminants pattern in males and females, were there are higher levels of some brominated contaminants in females than in males when considering concentrations on a wet weight basis (see also page 19).

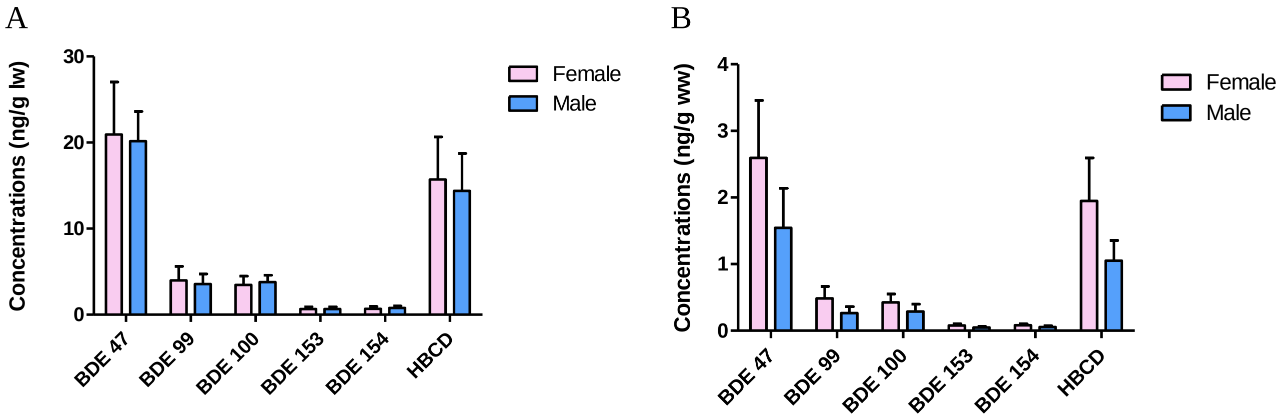


Figure 7. Concentration (ng/g) of brominated contaminants in A, lipid weigh and B, wet weight in salmon (*S. salar*) muscle (females n=10 and males n=7) from the Baltic Sea, the year 2000.

Concentration (GM ± 95% Cl) for salmon (*S. salar*) muscle for dioxins and furans are presented in table 4. The contaminant with the highest concentration is TCDF, with a concentration more than ten times higher than that of TCDD.

Tabel 4. Concentrations (geometric mean (GM) with 95% confidence interval (-Cl and +Cl)) of dioxions and furans in salmon (*S. salar*) (females n=6 and males n=5) muscle caught in the Baltic Sea, the year 2000. For abbreviations, see Material and Methods.

		Females n=6 pg/g			**Males** n=5 pg/g		
		GM	-Cl	+Cl	GM	-Cl	+Cl
TCDF	Lipid weight	42.93	36.49	50.52	41.03	32.50	51.81
	Wet weight	3.483	2.584	4.694	3.525	2.498	4.975
TCDD	Lipid weight	3.410	2.814	4.133	3.326	2.440	4.534
	Wet weight	0.277	0.210	0.364	0.285	0.217	0.374
PeCDF1	Lipid weight	6.490	5.284	7.973	6.906	5.209	9.157
	Wet weight	0.546	0.4139	0.719	0.566	0.393	0.814

PeCDF2	Lipid weight	34.81	29.02	41.75	41.95	25.83	68.14
	Wet weight	2.903	2.183	3.859	3.388	2.419	4.745
PeCDD	Lipid weight	5.937	4.944	7.130	6.762	4.869	9.389
	Wet weight	0.486	0.374	0.632	0.560	0.425	0.737
HxCDF1	Lipid weight	0.896	0.663	1.209	0.941	0.705	1.256
	Wet weight	0.075	0.060	0.095	0.077	0.054	0.109
HxCDF2	Lipid weight	1.132	0.791	1.620	1.330	0.965	1.834
	Wet weight	0.102	0.077	0.134	0.101	0.063	0.163
HxCDF3	Lipid weight	0.892	0.670	1.189	1.022	0.770	1.355
	Wet weight	0.077	0.055	0.106	0.081	0.055	0.120
HxCDF4	Lipid weight	0.160	0.094	0.273	0.143	0.104	0.196
	Wet weight	0.013	0.008	0.022	0.012	0.011	0.013
HxCDD1	Lipid weight	0.236	0.177	0.314	0.244	0.194	0.308
	Wet weight	0.020	0.016	0.025	0.020	0.017	0.023
HxCDD2	Lipid weight	2.193	1.781	2.701	2.700	1.813	4.022
	Wet weight	0.183	0.143	0.236	0.217	0.159	0.297
HxCDD3	Lipid weight	0.219	0.160	0.299	0.328	0.183	0.589
	Wet weight	0.017	0.010	0.026	0.028	0.020	0.038
HpCDF1	Lipid weight	0.164	0.121	0.223	0.166	0.122	0.226
	Wet weight	0.013	0.011	0.016	0.014	0.013	0.015
HpCDF2	Lipid weight	0.189	0.139	0.258	0.198	0.145	0.272
	Wet weight	0.015	0.013	0.018	0.017	0.015	0.018
HpCDD	Lipid weight	0.285	0.209	0.388	0.338	0.253	0.452
	Wet weight	0.023	0.020	0.028	0.028	0.022	0.035
OCDF	Lipid weight	0.264	0.198	0.354	0.279	0.211	0.370
	Wet weight	0.021	0.018	0.026	0.024	0.022	0.026
OCDD	Lipid weight	1.907	1.313	2.767	2.045	1.366	3.059
	Wet weight	0.146	0.108	0.198	0.181	0.1588	0.205

Furans and dioxins are illustrated in Figure 8, female and male respectively. All the contaminants are shown both on lipid and wet weight basis.

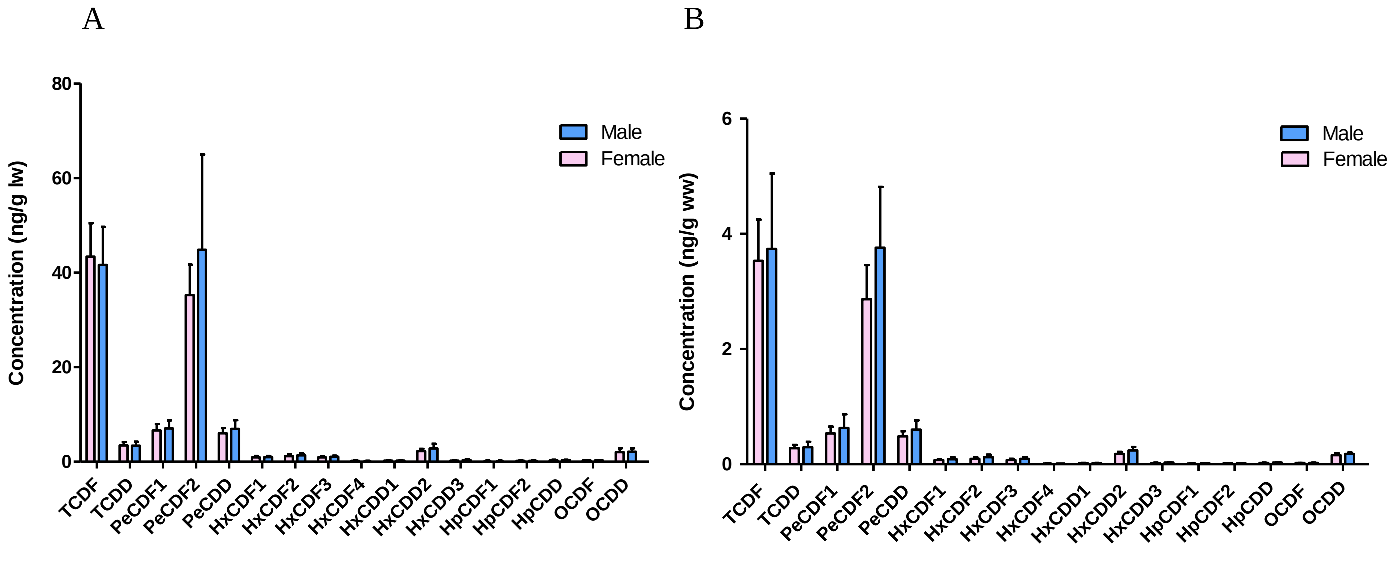


Figure 8. Concentration (pg/g) of furans and dioxions in A lipid weight and B wet weight in salmon muscle (*S. salar*) (females n=10 and males n=7) from the Baltic Sea, the year 2000.

The PCA analysis ($R^2\mathbf{X}$ = 0.849 and Q^2 = 0.58, three components) reveals the formation of two groups, males and females (Figure 9A). Even though there is some overlap between the two genders, this indicates a difference in contaminant patterns between females and males. Figure 9B show a formation of four different groups of contaminants. One homogenous group of brominated flame retardants and “ordinary” organochlorines (excluding furans and dioxins, and DL-PCBs). The rest of the contaminants are divided into three groups one with the DL-PCBs, and two other groups with the lower chlorinated dioxins and furans and one with the higher chlorinated ones. Alternative these may create a more “loosly” formed group with all dioxins and furans.

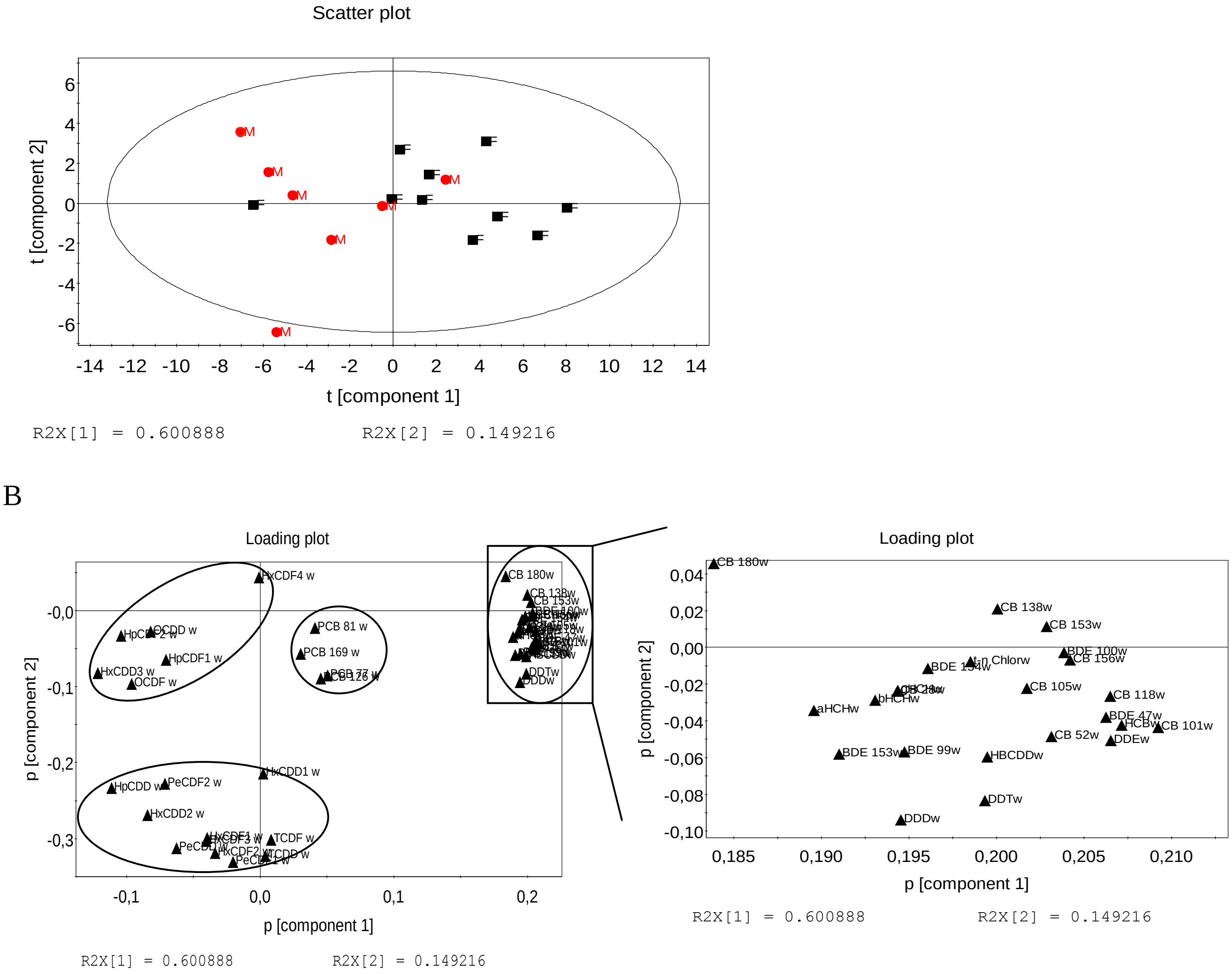


Figure 9. Principal component analysis (PCA) including the concentrations of contaminants (wet weight) in salmon (*S. salar*) muscle from Gotland, Baltic Sea, (females n=10 males n=7). PCA model (R^2**X** = 0.849 and Q^2 = 0.58, three components), (a) scatter plot with the two classes, males (M) (dots) and females (F) (sqaures) (b) loading plot. For abbreviations, see Materials and Methods.

The PLS analysis (R^2**X** = 0.591, R^2**Y**=0.382 and Q^2 = 0.219, one component) with female and male as **Y** and all the contaminants (wet weight) as **X,** show that there is a positive correlation between higher concentration of several of the contaminants and being a female (Figure 10).

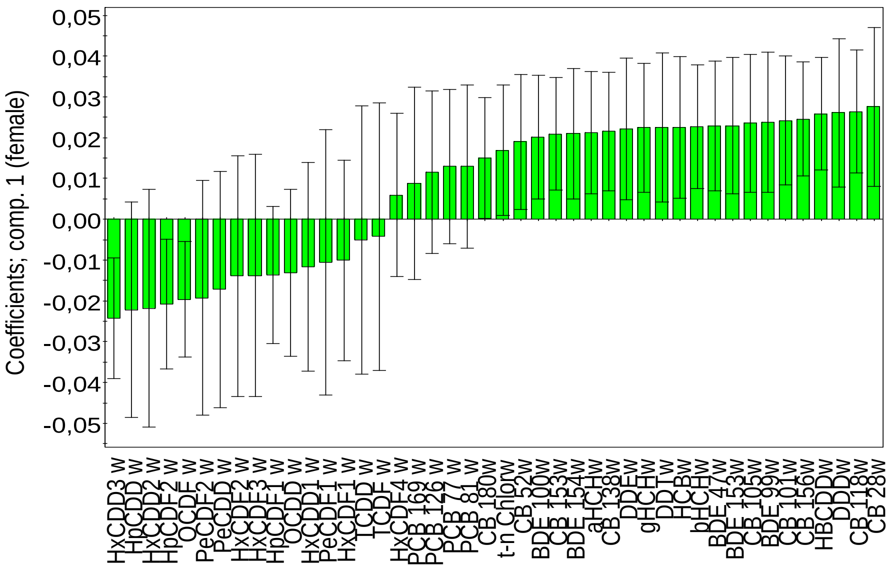


Figure 10. Coefficient plot with 95% Cl for the respective variables for PLS model (R^2**X** = 0.591, R^2**Y**=0.382 and Q^2 = 0.219, one component) between gender (here females) and concentrations of organochlorines and brominated flame retardants in salmon (*S. salar*) muscle (n=17) from the Baltic Sea. For abbreviations see Materials and Method.

There are significant differences between some of the contaminants between female and male, see Figure 11-14. Females has a significant (one-way t-test) higher values of *p,p*'DDD (p-value 0.0158), *p,p*'DDT (p-value 0.0369) (Figure 11), CB101 (p-value=0.0396), CB118 (p-value=0.0222) (Figure 12), BDE47 (p-value= 0.0499), BDE99 (p-value=0.0306) (Figure 13) and HBCD (p-value=0.0130) (Figure 14). There was no significant difference in TEQ-values between males and females. The following contaminants are included in the TEQ calculation; all dioxions, furans and DL-PCBs (those contaminants in Table 2).

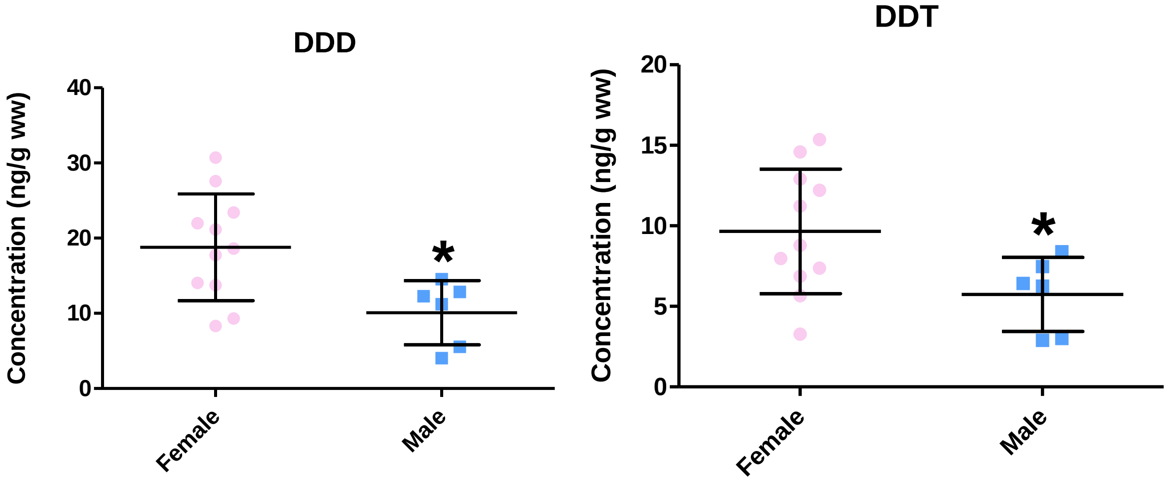


Figure 11. Concentration of DDD and DDT (ng/g wet weight) of salmon (*S. salar*) (females n=10 and males n=7) muscle from the Baltic Sea, the year 2000. T-test with p-value= 0.0158 and 0.0396 for DDD and DDT and respectively. The lines represent mean ± SD. The dots and squares represent the individual values for the female and male, respectively.

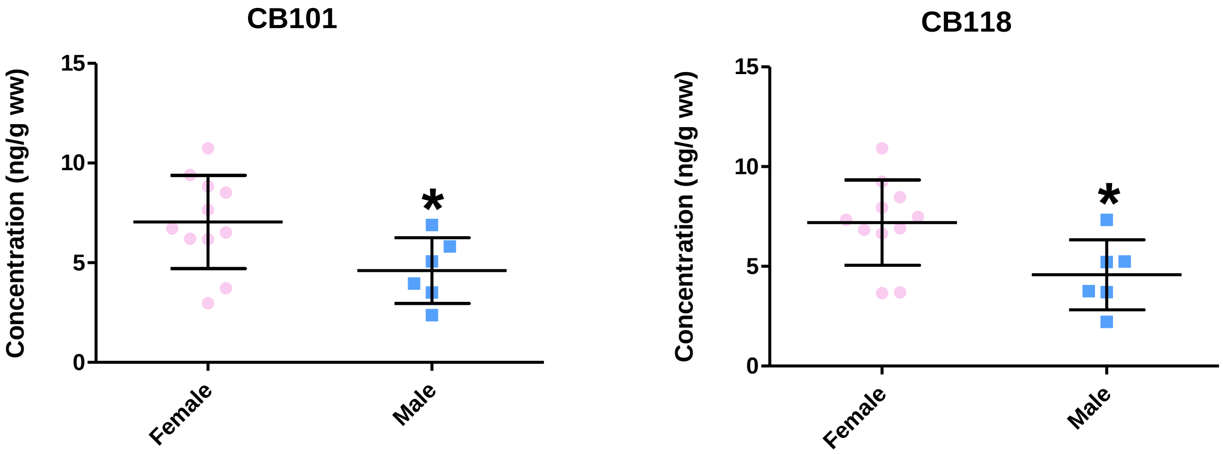


Figure 12. Concentration of and CB101 and CB118 (ng/g wet weight) of salmon (*S. salar*) (females n=17 and males n=10) muscle from the Baltic Sea, the year 2000. T-test with p-value= 0.0396 and 0.0222 for CB101 and CB118 respectively. The lines represent mean ± SD. The dots and squares represent the individual values for the female and male, respectively.

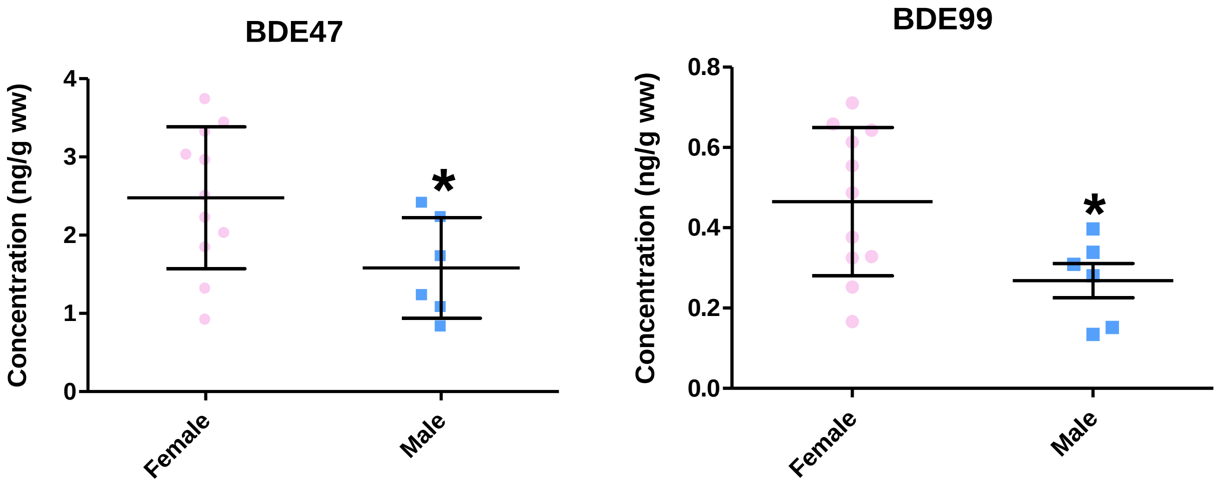


Figure 13. Concentration of BDE47 and BDE99 (ng/g wet weight) of salmon (*S. salar*) (females n=10 and males n=7) muscle from the Baltic Sea, the year 2000. T-test with p-value= 0.0499 and 0.0306 for BDE47 and BDE99 respectively. The lines represent mean ± SD. The dots and squares represent the individual values for the female and male, respectively.

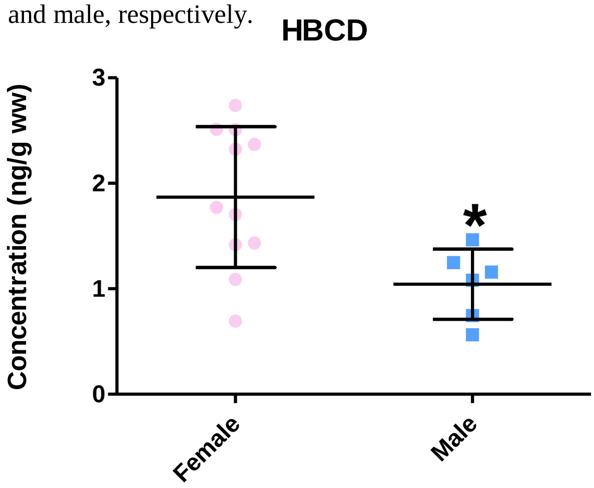


Figure 14. Concentration of HBCD (ng/g wet weight) of salmon (*S. salar*) (females n=10 and males n=7) muscle from the Baltic Sea, the year 2000. T-test with p-value= 0.0130. The lines represent mean ± SD. The dots and squares represent the individual values for the female and male, respectively.

Differences due to gender

The biological variables (mean ± St. Dev, min - max) for salmon are presented in Table 1. Univariate statistics testing female vs. male for each biological variable separately showed no significant differences between the sexes except for lipid content (Table 1 and Figure 15). The lipid content is significant higher (p-value= 0.02) in females than in males.

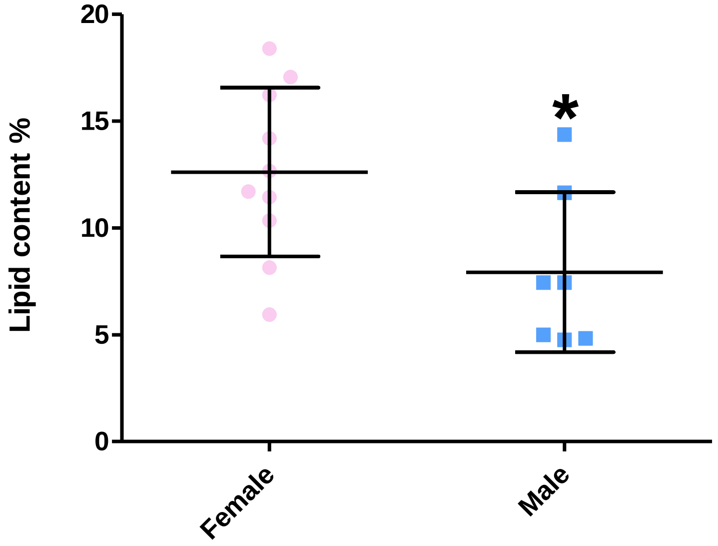


Figure 15. Graph with the lipid content as % in salmon (*S. salar*) muscle (females n=10 and males n=7) from the Baltic Sea, near Gotland, the year 2000. T-test with P-value= 0.023. The lines represent mean ± SD. The dots and squares represent the individual values for the female and male, respectively.

Relationship between biological variables and the contaminants

The only biological variable that co-varied with the contaminant concentrations was the lipid content. The PLS ($R^2\mathbf{X} = 0.691$, $R^2\mathbf{Y} = 0.939$, $Q^2 = 0.791$) model in Figure 16 show that the lipid content has a positive relationship with a number of PCBs *e.g.* CB138, t-n chlor and CB180 as well as with αHCH, βHCH and γHCH. Figure 17 show a linear regression between lipid content and t-n Chlor, CB138 and CB180, all with p-values < 0.0001. The regression coeifficients are: 0.8155, 0.8284, 0.7055 for t-n Chlor, CB138 and CB180 respectively.

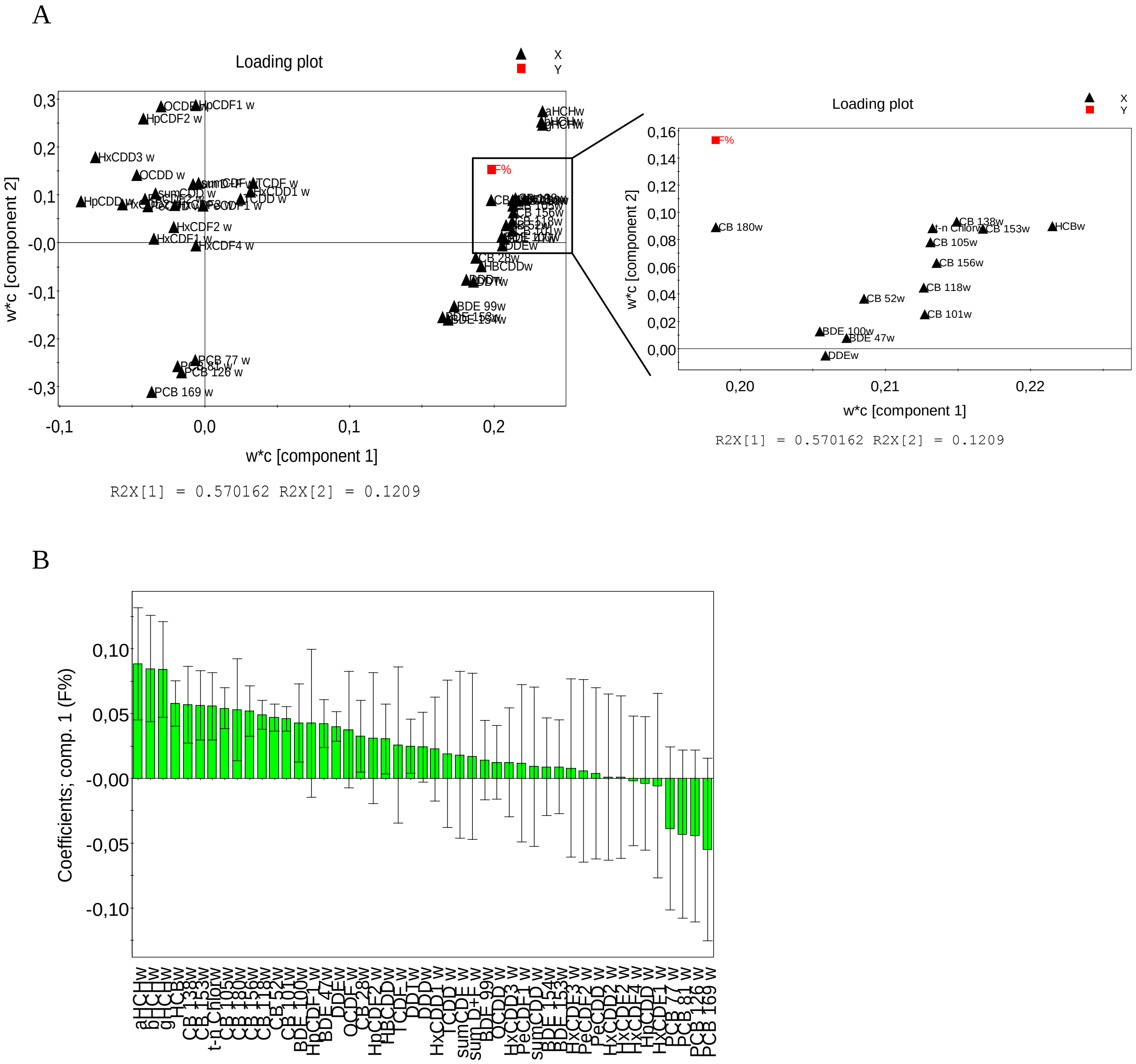


Figure 16. Partial least squares regression to latent structures (PLS) model ($R^2\mathbf{X} = 0.691$, $R^2\mathbf{Y} = 0.939$, $Q^2 = 0.791$, two components) between fat content (F%) and concentrations of contaminants (ng/g ww) in salmon (*Salmo salar*) muscle from the Baltic Sea, the year 2000 (n=17). (A) Loading plot (B) Coefficent plot with lipid content (F%) as **Y** and all contaminants on wet weight basis as **X** . For abbreviations see Material and Method.

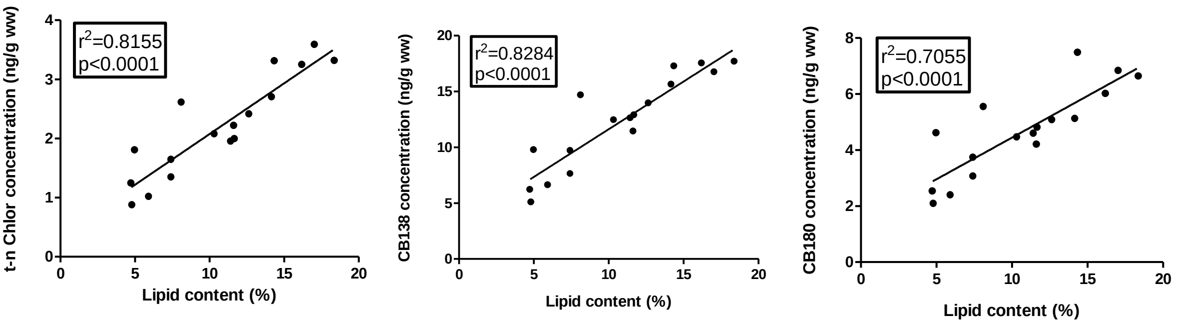


Figure 17. The relationship between lipid content (%) and t-n chlor, CB138 and CB180 in salmon (*S.salar*) muscle (n=17) caught in the Baltic Sea, near Gotland, the year 2000, p-value < 0.0001, r^2=0.82, 0.83, 0.71 for t-n Chlor, CB138 and CB180 respectively.

Figure 16 also shows that there is no correlation between several PCDD/DF and the lipid content *e.g.* HpCDF2 and OCDF. To further illustrate this figure 18 show a linear regression between lipid content and HpCDF2 and OCDF, p-value= 0.561 and 0.681 for HpCDF2 and OCDF respectively. R^2= 0.04 and 0.02 for HpCDF2 and OCDF respectively.

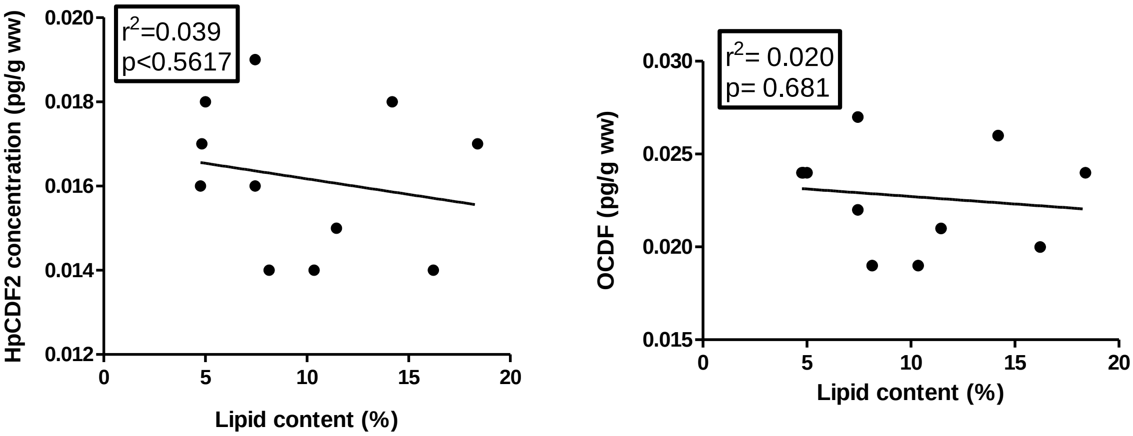


Figure 18. The relationship between lipid content (%) and HpCDF2 and OCDF in salmon (*S. salar*) muscle (n=17), caught in the Baltic Sea, near Gotland, the year 2000, p-value= 0.56 and 0.68, r^2=0.04, and 0.02 for HpCDF2 and OCDF respectively.

The relationships between OCs and BFRs

Figures 19-23 show result from the PLS model ($R^2\mathbf{X} = 0.549$, $R^2\mathbf{Y} = 0.924$, $Q^2= 0.882$, one component) that shows the relationship between several brominated contaminants and chlorinated contaminants. The variable influences on projection (VIP) plots on the left show the variables of importance for calculation of BDE47, BDE99, BDE100, BDE153 and HBCD, respectively. A linear regression, showing the relationship between one brominated contaminant and one chlorinated, can be seen in Figures 18-22 on the right. The linear regressions all have a p-value < 0.0001 and $r^2 > 0.92$.

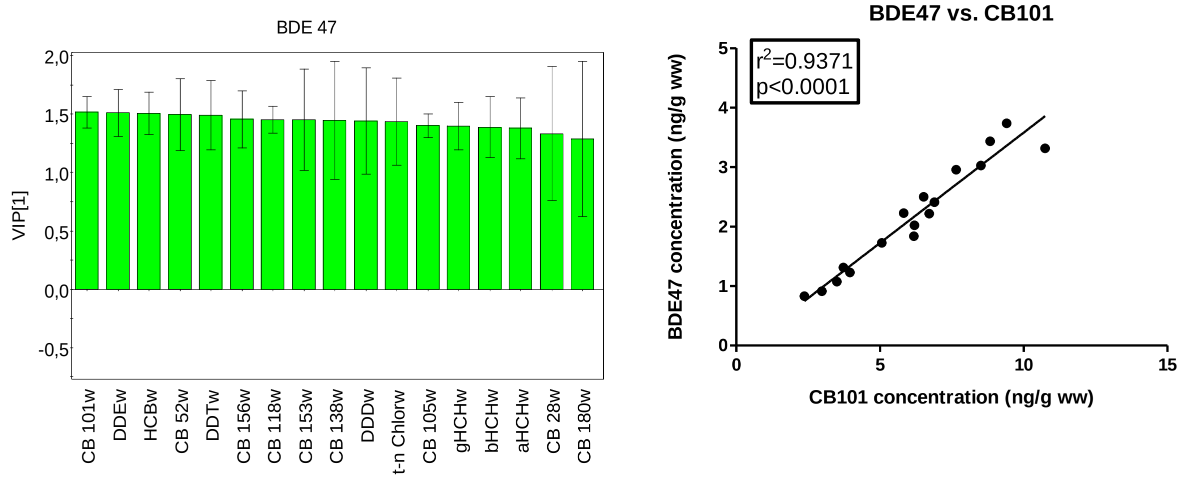


Figure 19. PLS model ($R^2\mathbf{X} = 0.549$, $R^2\mathbf{Y} = 0.924$, $Q^2=0.882$, one component) between the concentration of organochlorines (OCs) as **X** and BDE47 as **Y**. Contaminants in salmon (*S. salar*) muscle from the Baltic Sea, the year 2000 (females n=10 and males n=7). Varibles of importance (VIP) on the left showing the respective variables importance for calculation of BDE47 concentration. Linear regression on the right with PBDE vs. CB 101 ($p < 0.0001$, $r^2= 0.9371$). For abbreviations see Material and Methods.

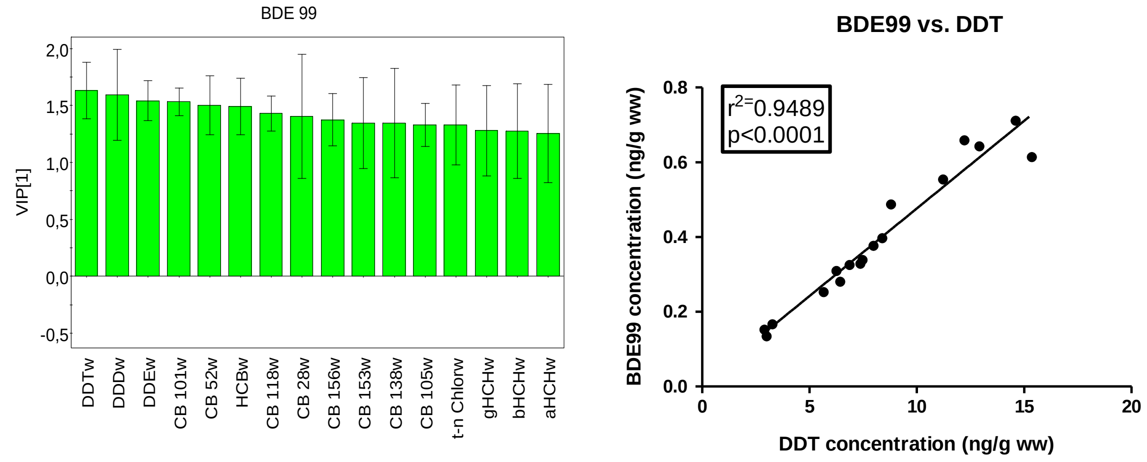


Figure 20. PLS model ($R^2\mathbf{X} =0.548$, $R^2\mathbf{Y} = 0.803$ $Q^2= 0.741$, one component) between the concentration of organochlorines (OCs) as **X** and BDE99 as **Y**. Contaminants in salmon (*S. salar*) muscle from the Baltic Sea, the year 2000 (females n=10 and males n=7). Varibles of importance (VIP) on the left showing the respective variables importance for calculation of BDE 99 concentration. Linear regression on the right with BDE99 vs. DDT ($p < 0.000,1$ $r^2= 0.9489$). For abbreviations see Material and Methods.

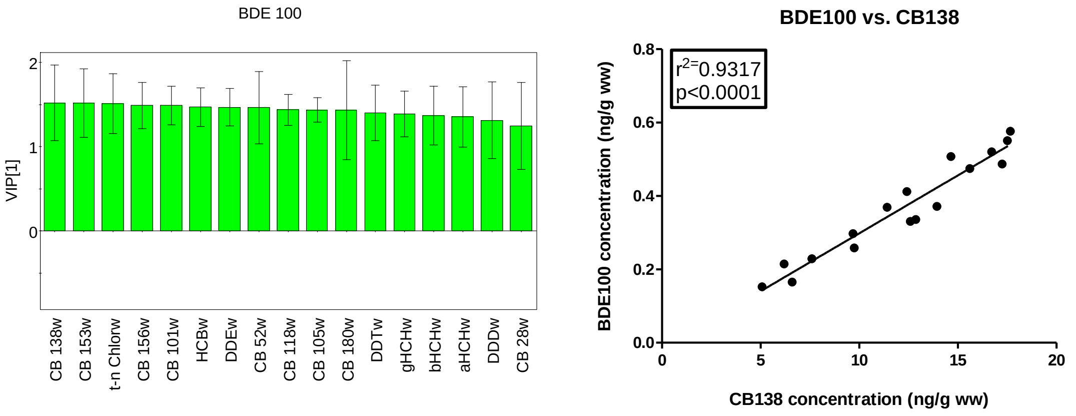


Figure 21. PLS model (R^2**X** = 0.55, R^2**Y** = 0.91 Q^2= 0.82, one component) between the concentration of organochlorines (OCs) as **X** and BDE100 as **Y**. Contaminants in salmon (*S. salar*) muscle from the Baltic Sea, the year 2000 (females n=10 and males n=7). Varibles of importance (VIP) on the left showing the respective variables importance for calculation of BDE100 concentration. Linear regression on the right with BDE100 vs. CB138 ($p < 0.0001$, r^2= 0.93). For abbreviations see Material and Methods.

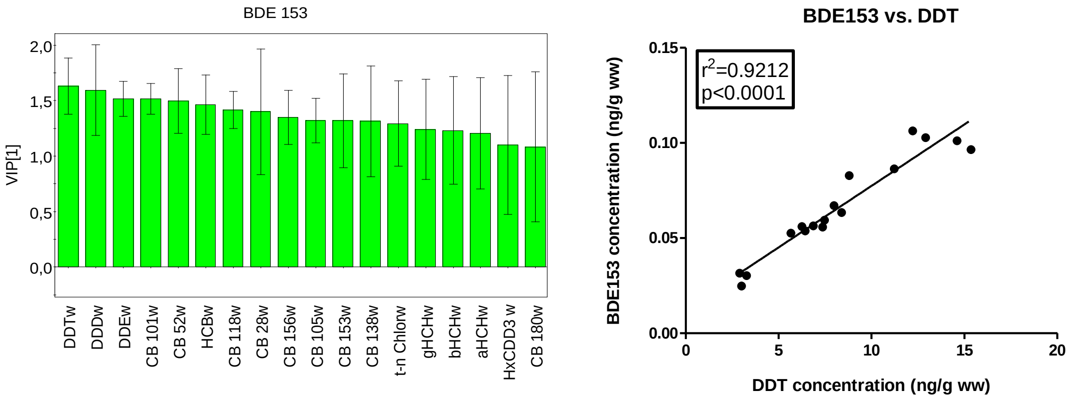


Figure 22. PLS model (R^2**X** = 0.55, R^2**Y** = 0.78 Q^2= 0.72, one component) between the concentration of organochlorines (OCs) as **X** and BDE153 as **Y**. Contaminants in salmon (*S. salar*) muscle from the Baltic Sea, the year 2000 (females n=10 and males n=7). Varibles of importance (VIP) on the left showing the respective variables importance for calculation of BDE153 concentration. Linear regression on the right with BDE153 vs. DDT ($p < 0.000,1$ r^2= 0.92). For abbreviations see Material and Methods.

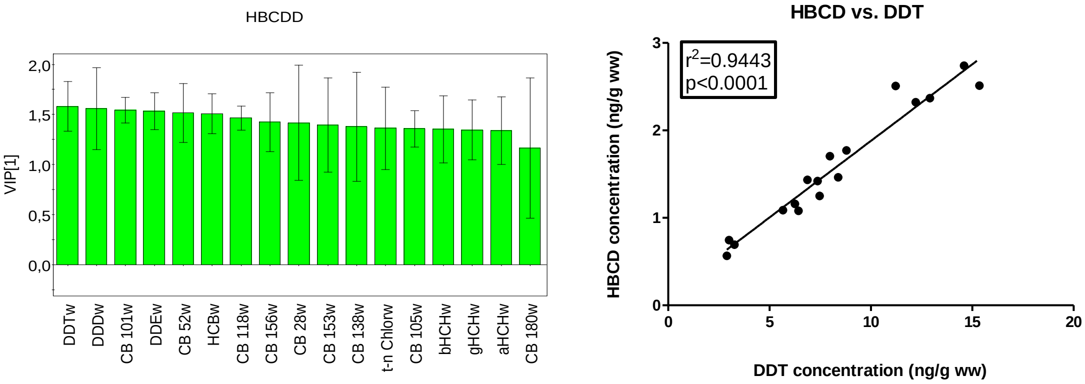


Figure 23. PLS model (R^2**X** = 0.55, R^2**Y** = 0.85 Q^2= 0.80, one component) between the concentration of organochlorines (OCs) as **X** and HBCD as **Y**. Contaminants in salmon (*S. salar*) muscle from the Baltic Sea, the year 2000 (females n=10 and males n=7). Varibles of importance (VIP) on the left showing the respective variables importance for calculation of HBCD concentration. Linear regression on the right with HBCD vs. DDT ($p < 0.0001$, r^2= 0.94). For abbreviations see Material and Methods.

Discussion

The first aim was to look at the contaminants; to determine concentrations, to discern variations and patterns among different pollutants. The sum of concentrations arranged from the highest to the lowest looks as followed; ∑DDT>∑PCB>∑HCH>∑PBDE. The contaminants with the highest sum are the compounds and their metabolites which have been used longest by man. DDT and PCB has been used almost 80 years [8, 13] while PBDE is a rather new FR and wasn´t established as a new major chemical FR until the year 1978 [19]. The use of OCs and increasing use of BFRs will probably lead to a change in the sum of the contaminants as well as their relative order. Szlinder-Richert *et al.* [26] showed in their article regarding contaminants in *e.g.* salmon from the Polish Baltic Sea a level of ∑HCH (~ 20 ng/g lw) that is lower than the level presented in this report (~ 34-35 ng/g lw). The article also showed a downward trend from ~23 to ~15 between the years 2003 and 2006. The salmons in this report are from 2000, so there might be a general downward trend in concentrations of HCH, which correspond well with the result from Szlinder-Richert *et al*. The sum of DDT in Szlinder-Richert *et al*. article varied between 1700 (ng/g lw) to 777 (ng/g lw) between the year 2003 and 2006. The concentrations of ∑DDT show a large fluctuate between the years; no clear trends can be seen. These concentrations compared to the 585 (ng/g lw) in the present article is a little bit higher which is interesting since DDT has been prohibited 40 years [15] meaning that DDT levels should decline. The high DDT concentration might be an indication that DDT is still in use in Poland or in other eastern-european countries. This show that DDT is still a treat to the environment, due to its slow degradation, and that DDT has to be monitored even though it has been prohibited. All the values in Szlinder-Richert *et al.* article is presented as arithmetic mean compared to the concentrations in this article which is presented as geometric mean. Arithmetic mean has always a higher values than geometric mean when calculated on the same data since “outliers” with high concentrations affect the arithmetic mean stronger than when calculating the geometric mean. In geometric mean calculations outliers doesn’t have the same dramatic influence of the result. Figure 9B indicate that contaminants with similar exposure routes, chemical reactivity, bioavailability, distribution, biotransformation, and/or excretion form different groups with strong co-variation between the contaminants within each group. There are one homogenous group of brominated flame retardants and “ordinary” organochlorines (excluding furans and dioxins, and DL-PCBs). The rest of the contaminants are divided into three groups one with the DL-PCBs, and two other groups with lower and higher chlorinated dioxins and furans, respectively. This strong co-variation is the reason why the OC concentration could be used to calculate the individual’s BFR concentrations with such high accuracy ($r^2>0.92$) (see aim 4).

The second aim was to see if there are any differences between the genders. There is a significantly difference between the genders when considering lipid content (Figure 15). The significantly higher lipid content in females may cause a higher risk for bioaccumulation of POPs, since one of the properties for POPs is an accumulation in fat tissue. In this report this is confirmed by the results showing several contaminants which have significant higher levels in female salmon when considering the contaminant concentrations on a wet weight basis. Wet weight is also of greatest interest when dealing with salmon as a food source. These results indicate that further investigations that separates between females and males are of great interest. This is also of interest for salmon farming since it has been shown earlier that reared salmon has greater lipid content than wild salmon [27]. Would the human exposure to contaminants from salmon farming be lower with a different sex ratio, favoring males or leaner fish? In the report several contaminants has been presented which has significant higher values in females than in males. But even though there was a difference between the genders, regarding many contaminants, there were no significant differences between the genders when considering the TEQ-values on wet weight basis. Also, males seem to have proportionally higher concentrations of dioxins and furans than females (Figure 10). The scatter plot reveals information regarding the grouping between individuals. This gives a good feed-back system, indicating that one individual classified as a female, actually could be a male (since the contaminant pattern remind more of a male than a female). Unfortunately this can’t be controlled in this study. The gonads should perhaps have been investigated using a microscope instead of a stereomicroscope that was used here with much lower resolution.

The third aim is to look at concentrations of pollutants and their correlation to biological factors of the fish *e.g.* length, weight and/or lipid content. There was only one biological factor that correlated with the contaminants, and that was the lipid content (see Figure 16). There are some important factors to take into concern when trying to understand why the lipid content is co-varying with many contaminants; the administration, distribution, metabolism and excretion, also known as the ADME of the contaminants. The administration between the animals is probably almost the same, though a fatter individual may have had more food. The distribution of contaminants vary a lot, contaminants can be accumulated in the fat, in the organs or in the blood. The contaminants can travel around in the body or have a specific target organ or tissue where it stays. The metabolism of contaminants can vary both between different compounds but also between individuals. A healthy and young individual probably have a faster metabolism, than an old sick individual. When considering pollutants it is not unusual to find individuals which have a higher level of contaminants than what is the mean even extremely high concentrations can be found in few individuals (~10-15 times the mean) [28]. Excretion of compounds is dependent on its chemical properties, a water soluble compounds is rather easily excreted by the kidneys via the urine. When a fat soluble compounds can be incorporated in the body´s fat tissue as well as being excreted via the liver and the bile and for fish, the compound can be excreted via gills. Some of the substances which are not correlated with lipid content are classified as POPs *e.g.*the dioxins, furans and DL-PCBs.

The fourth aim is to investigate if the concentrations of OCs and BFRs co-varied with each other, if concentrations of OCs can be used to calculate BFRs and *vice versa*. The result showed a strong correlation between brominated containments and chlorinated containments in the Baltic Sea salmon.This relationship between BFRs and OCs has been shown before in adult guillemot (*Uria aalge*), see Supporting Information to Lundstedt-Enkel *et al*., 2005 [29]. This suggests that these contaminants have similar ADME routes in the animals. The high r^2 and low p-values mean that the co-variation between these pollutions is strong. If some of the OCs can be calculated from the levels of BFRs, it could be of great economic interest. Saving some of the cost for sample analysis and using the model to calculate levels instead. If more reports included the relationships between BFRs and OCs reliable models for calculations of OCs could be done rather easily. This OCs-BFRs model will have to be validated at close intervals as the general levels of OCs is declining while the general level of BFRs especially for HBCD are increasing.

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References

1. Elmgren, R., Man´s Imapct on the Ecosystem of the Baltic Sea: Energy Flows Today and at the Turn of the century. *Ambio*, **1989**. 18(6): p. 326-332.
2. Lehtinen, K.-J., M. Notini, J. Mattson, and L. Landner, Disappearance of Bladder-Wrack (Fucus vesiculosus L.) in the Baltic Sea: Relation to Pulp-Mill Chlorate. *Ambio*, **1988**. 17: p. 387-393.
3. Kautsky, L. and N. Kautsky, The Baltic Sea, Including Bothnian Sea and Bothnian Bay, in *Seas at the Millennium: An Environmental Evaluation* 2000.
4. Dietz, R., F. Riget, M. Cleemann, A. Aarkrog, P. Johansen, and J.C. Hansen, Comparison of contaminants from different trophic levels and ecosystems. *The Science of The Total Environment*, **2000**. 245(1-3): p. 221-231.
5. Wulf, B., L. Niels, P. Agnes, A. Antti, F. Mikael, T. Inga, A. Jan, A. Sigmund, M. Helle, and P. Jan, Nordic Nutrition Recommendations 2004 - integrating nutrition and physical activity. *Scandinavian Journal of Nutrition*, **2004**. 48: p. 178-187.
6. National Food Administration, [Accessed June 2008], www.slv.se
7. WWF, Breathless Coastal Seas WWF Briefing Paper: Dead Ocean Zones - a global Problem of the 21st century. **2008**
8. Clark, R.B., *Marine Pollution*. Fitfh ed. 2001, New York: Oxford University Press Inc.
9. Tedengren, M., M. Arnér, and N. Kautsky, Ecophysiology and stress response of marine and brackish water *Gammarus* species (Crustacea, Amphipopda) to changes in salinity and exposure to cadmium and diesel-oil. *Marine Ecology Progress Series*, **1988**. 47: p. 107-116.
10. Jansson, H., P. Nyberg, and A. Johlander, Faktablad: *Salmo Salar* - gullspångslax. **2006** *In Swedish.*
11. Lundstedt-Enkel, K., P.M. Lek, and J. Örberg, QSBMR - Quantitative Structure Biomagnification Relationships: Physiochemical and Structural Descriptors Important for the Biomagnification of Organochlorines and Brominated Flame Retardants *Journal of Chemometrics*, **2006**. 20(8-10): p. 392 -401.
12. United Nations Environment Programme., Ridding the World of POPs: A Guide to the Stockholm Convention on Persistant Organic Pollutants. **2005**.
13. Ecobichon, J.D., Toxic Effects of Pesticides, in *Casarett and Doull's Toxicology: The Basic Science of Poisons*, C.D. Klaassen, Editor. 2001, McGraw-Hill. p. 763-772.
14. Ecobichon, J.D., Toxic Effects of Pestcides, in *Casarett and Doull's Toxicology: The Basic Science of Poisons*, C.D. Klaassen, Editor. 1996, McGraw-Hill.
15. Helander, B., Faktablad: *Haliaeetus albicilla* – havsörn. **2006** *In Swedish*
16. Rignell-Hydbom, A., L. Rylander, A. Giwercman, B.A.G. Jönsson, C. Lindh, P. Eleuteri, M. Rescia, G. Leter, E. Cordelli, M. Spano, and L. Hagmar, Exposure to

PCBs and p,p′-DDE and Human Sperm Chromatin Integrity. *Environmental Health Perspectives*, **2005**. 113: p. 175-179.

17. Birnbaum, L.S. and D.F. Staskal, Brominated Flame Retardants: Cause for Concern? *Environ Health Perspectives*, **2004**. 112: p. 9-17.
18. Darnerud, P.O., E. Gunnar S., J. Torkell, L. Poul B., and V. Matti, Polybrominated diphenyl ethers: Occurrence, Dietary Exposure, and Toxicology. *Environmental Health Perspect*, **2001**. 109.
19. Vonderheide, A.P., K.E. Mueller, J. Meija, and G.L. Welsh, Polybrominated diphenyl ethers: Causes for concern and knowledge gaps regarding environmental distribution, fate and toxicity. *Science of The Total Environment*.
20. Viberg, H., A. Fredriksson, and P. Eriksson, Neonatal exposure to the brominated flame-retardant, 2,2',4,4',5-pentabromodiphenyl ether, decreases cholinergic nicotinic receptors in hippocampus and affects spontaneous behaviour in the adult mouse. *Environmental Toxicology and Pharmacology*, **2004**. 17(2): p. 61-65.
21. Van den Berg, M., L.S. Birnbaum, M. Denison, M. De Vito, W. Farland, M. Feeley, H. Fiedler, H. Hakansson, A. Hanberg, L. Haws, M. Rose, S. Safe, D. Schrenk, C. Tohyama, A. Tritscher, J. Tuomisto, M. Tysklind, N. Walker, and R.E. Peterson, The 2005 World Health Organization Reevaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-Like Compounds. Toxicological Sciences **2006**. p. 223-241.
22. American Chemistry Council, [Accessed August 2008], http://www.dioxinfacts.org/
23. GraphPadPrism, Version 4.03, GraphPad software, 2003, www.graphpad.com
24. SIMCA-P, Version 10.0.4. Umetrics, Umeå, Sweden, 2004, www.umetrics.com
25. Lundstedt, T., E. Seifert, E. Abramo, B. Thelin, Å. Nyström, J. Petterson, and R. Bergman, Experimental Design and Optimization. *Chemometrics and Intelligent Laboratory Systems*, **1998**. 42: p. 3-40.
26. Szlinder-Richert, J., I. Barska, J. Mazerski, and Z. Usydus, Organochlorine pesticides in fish from the southern Baltic Sea: Levels, bioaccumulation features and temporal trends during the 1995-2006 period. *Marine Pollution Bulletin*, **2008**. 56(5): p. 927-940.
27. Bergström, E., Effect of natural and artificial diets on seasonal changes in fatty acid composition and total body lipid content of wild and hatchery-reared Atlantic salmon (*Salmo salar* L.) parr-smolt. *Aquaculture*, **1989**. 82(1-4): p. 205-217.
28. Bignert, A., S. Danielsson, A. Strandmark, E. Nyberg, L. Asplund, U. Eriksson, U. Berger, A. Wilander, and P. Haglund, Sakrapport: Metaller och organiska miljögifter i marin biota, trend- och områdesövervakning Överenskommelse 212 0714, dnr 721-4235-07Mm. **2008**.
29. Lundstedt-Enkel, K., A.K. Johansson, M. Tysklind, L. Asplund, K. Nylund, M. Olsson, and J. Örberg, Multivariate Data Analyses of Chlorinated and Brominated Contaminants and Biological Characteristics in Adult Guillemot (Uria aalge) from the Baltic Sea. *Environmental Science and Technology*, **2005**. 39(22): p. 8630-8637.