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Abstract

There are multiple goals in this study it is partly a method comparison study in environmental toxicology were different extraction methods and cleanup methods for quantitative determination of POPs (Persistent organic pollutants) will be discussed. There will also be a method validation part to this study were a new GPC column will be validated using an existing GPC cleanup method and using a standard reference material for chlorinated pesticides and comparing samples run over the GPC column with samples analyzed with existing methods for quantification of POPs. The last goal of the study will be to investigate if there is a correlation between the fatty acid lipid profile and POPs in fish samples.

4 different fish samples 3 cod liver samples and 1 salmon fillet were selected and they were homogenized together with sodium sulphate. These 4 different homogenates were the basis for all the samples. 8 samples (2 parallels of each fish) underwent cold column extraction and GPC/alumina oxide cleanup. 8 samples (2 parallels of each fish) underwent cold column extraction and sulfuric acid/silica cleanup. And 4 samples (1 of each fish) underwent ultrasonic bath (USB) extractions and GPC/alumina oxide cleanup. All the samples were analyzed for PCBs and chlorinated pesticides. Fatty acid lipid profiles were also determined by derivatisation of the fatty acids (FA) into fatty acid methyl esters (FAMEs) and analyzing them. The relative amounts were used to create the lipid profile.

Comparison between ultrasonic bath extraction and cold column extractions were done and the results of the cold column extractions showed better recoveries than the USB extractions, while an USB extraction gives more uncertainties in the method due to lower recoveries and some cross contaminations in the method.

Comparison of the sulfuric acid cleanup and the GPC cleanup showed that both methods give good results with good recoveries. But the acid cleanup degenerate and destroy some of the chlorinated pesticides makes the GPC cleanup method preferable in analysis of chlorinated pesticides.

The Waters Envirogel GPC cleanup column were used in different experiments and the results showed good and reproducible results with good recoveries. And a comparison with a certified standard reference material, 1588b, showed that the chlorinated pesticides were close to their given literature values.

There were created a lipid profile containing 8 fatty acids and their relative amounts, this were compared to calculated POPs from the same samples. The lipid profile and the calculated POPs were analyzed against each other using a PLS 2 algorithm. This analysis showed no clear correlation between the relative amounts of FA and any of the POPs, except for γ-HCH that were the only compound that might be effected by the different amounts of FA.

1. Introduction

1.1 Persistent organic pollutants (POPs)

Persistent organic pollutants are a group of compounds that are known for their persistents in the environment and their bioaccumulation in wildlife and toxicity. The POPs bioaccumulate through the food web, and there is a risk of causing adverse effects to human health and the environment. The Stockholm convention therefore identified an initial 12 compounds that were a priority, “the dirty dozen”. The twelve were aldrin, chlordane, DDT, dieldrin, endrin heptachlor, hexachlorbenzene, mirex, toxaphene, PCBs and polychlorinated dioxins and polychlorinated furans. There has been progress in the work of analyzing for POPs and new and rapid methods for analytical screening of 23 persistent organic pollutants (POPs) in human plasma have been developed. [1]

POPs are also known for their long range atmospheric transport, this means these contaminants are not contained in small areas where they are created but they migrate with the ocean currents and or wind flow. They usually migrate towards the poles in a way that has been nicknamed “grasshopping”. [2]

Air surface exchange of POPs occurs in response to changes in the temperature. Semi-volatile compounds can participate in repeated air-surface exchange events and as a consequence, move from one area of the globe to another. Under high temperatures the volatilization rates are higher and the POPs are released into the air in a gas phase. Under low temperatures gas phase POPs can condensate back to the earth’s surface, or it can return back to the earth’s surface as wet depositions. The rates of re-release from the earth’s surface will be slower in colder Polar areas or cold high altitude places. POPs usually tends to accumulate in these places. [3]

POPs are very persistent in the environments because they have long half lives in soil, air and biota. They are also a lipophilic group of chemicals and in aquatic systems they are drawn towards solids and in particular organic matter like lipids. In organism they are therefore stored in the fatty tissues, where the metabolism usually are slow the different POPs may bioaccumulate in food chains. [4]

After the Stockholm convention many of the POPs were banned from commercial use, but because of the long half-lives of the POPs additional years of monitoring are necessary to increase the knowledge of temporal trends. This will allow further studies and efforts to reduce global emissions and how this may be affected by climate variability and possible changes in contaminant pathways. Despite the fact that many of the POPs have been banned for a long period and reductions of concentrations of some legacy POPs, like PCBs, they are still present in top predators in the marine food web in such high concentrations that they can affect the health of wildlife and humans. [5]

There are many harmful effects of POPs and the clearest evidence of effects has been in birds and marine mammals. Many organochlorines, notably DDE, a metabolic breakdown product of DDT, can affect eggshell thickness in birds. [6] Other harmful effects like reproductive impairment in seals from the Baltic Sea have been shown. [7] There have also been shown reproductive impairments for Beluga whales in the St. Lawrence Seaway, Canada that have been linked to POPs especially PCBs. [8]

1.2 Lipids

Carboxylic acids are generally denoted “fatty acids” due to their natural occurrence in oils, waxes and fats from animals and plants. The fatty acid chains are long and aliphatic, and may be saturated or unsaturated. The aliphatic chains are the basic units of lipids, and fatty acids are therefore usually found as triglycerides. To analyze triglycerides one has to convert them to methyl esters, due to their volatile nature. Esters are organic compounds that are formed when an alcohol and an acid react.

The most used method for isolation and purification of total lipids from animal tissue are the folch method. This is an old method that still are in use, this method utilize a homogenizing technique were you homogenize the tissue with 2:1 chloroform:methanol and filtering the homogenate to extract the lipids. [9]

Gas chromatography is the most suitable and most used method to analyze fatty acids and the compositions of fat. This method has many scientific uses, but a common factor is that the samples have to be volatile for the analysis to be successful. Fatty acids are reactive, but not very volatile and many of the acids are to polar to be analyzed on a GC. The corresponding methyl esters are volatile because they are less polar, and this can be used in the GC analysis as the esters are easy to elute through a columns. [10]

Esterification shown in figure 1 is the most popular method for derivatisation of carboxylic acids, and these acids can be converted to esters by mixing an alcohol with an acid. This reaction leads to the formation of 67% of the ester, as the equilibrium constant is about 4. To enhance the formation and maximize the formation of the ester one can remove water, use an excess of alcohol or one can remove the ester during the reaction. [10]

Figure 1 – Esterification

There are also possible with microwave assisted derivatisation for the determination of monocarboxylic, dicarboxylic and tricarboxylic acids in water. [11]

Other methods that can be used is to extract the desired fatty acids from the matrix with an organic solvent, and the extracted fatty acids are saponified (by acid or base hydrolysis) to form free fatty acids, which may be methylated to form fatty acid methyl esters. [12]

Table 1 shows the fatty acid to be used as standards and comparisons in the assignment. The table shows IUPAC name and trivial name, number of carbon atoms and number of double bonds.

Table 1, common fatty acids

Trivial name	[1]Number of C	IUPAC name
Myristic acid	14:0	Tetradecanoic acid
Palmitic acid	16:0	Hexadecanoic acid
Stearic acid	18:0	Octadecanoic acid
Oleic acid	18:1 (9*c*)	9-Octadecenoic acid
Linoleic acid	18:2 (9*c*, 12*c*)	9,12-Octadecadienoic acid
Linolenic acid	18:3 (9*c*, 12*c*, 15*c*)	9,12,15-Octadecatrienoic acid

1.3 Aim of the study

There are multiple goals in this study it is partly a method comparison study in environmental toxicology were different extraction methods and cleanup methods for quantitative determination of POPs. Different extraction methods and cleanup methods will be done and the methods and results will be compared and discussed.

There will also be a method validation part to this study were a new GPC column will be validated using an existing GPC cleanup method and using a standard reference material for chlorinated pesticides. The certified standard reference material 1588b will be used because of the relevant chlorinated pesticides it contains. [13] The SRM will be analyzed and the results will determine if the GPC column can be used for cleanup of samples containing POPs.

The last goal of the study will be to investigate if there is a correlation between the fatty acid lipid profile and POPs in fish samples. This will be done by multivariate data analysis. There is not much literature on the subject, but levels of omega-3 and omega- 6 fatty acids and lipid-adjusted concentrations of PCBs, dioxins, toxaphene and dieldrin have been determined in famed and wild salmon, this study shows that farmed Atlantic salmon had a greater level of total lipids and a significantly higher contaminant level than wild pacific salmon. [14]

Another study shows that Atlantic salmon accumulate high levels of contaminants such as polychlorinated biphenyls (PCBs) in their lipids during the adult growth phase spent at sea. The lipids are later utilized during migration for swimming and biological adaptations. [15]

2. Method

2.1 Quantitative determination of POPs in marine biota

The methods for the quantitative determination of POPs in biota are performed according to the NILU-SOP for determination of persistent organic compounds. [16]

The internal standard and the recovery standard with concentrations are shown in appendix 1.

Samples

Samples

Four different fish samples were chosen for this experiment due to their easy availability and relevant sampling places. The four fish samples were:

11/2386: A composite sample of cod livers from 10 fish, collected in Etnefjorden.
11/2387: A composite sample of cod livers from 16 fish, collected in Drammensfjorden (Husteinbukta).
11/2388: A composite sample of cod livers, collected in Drammensfjorden (Engersand - Berget).
11/2389: Farmed salmon fillet, intended for commercial use.

Sample preparation

The biota sample (fish) is collected and frozen. Before homogenization the samples has to be temperate to room temperature. A known amount of sample is weighed and transferred to a blender, also a known amount of sodium sulphate was added before the samples got mixed well. More sodium sulphate was added to ensure that the sample mix was dry and free of lumps. The samples got mixed in a blender, and then put in a freezer to freeze-dry for approximately one hour to ensure that the samples were free of water. Table 1 shows the masses of the fish sample and the total amount of homogenate after the mixing and the freeze-drying was complete.

Table 1. The weighed amount of fish sample and the homogenate after the Na_2SO_4 was added to the fish samples, and the samples fat percentage.

Sample	amount fish sample (g)	amount homogenat (g)	fat %
11/2386	6,0	55,5	58,1
11/2387	5,9	55,8	49,0
11/2388	6,1	55	48,5
11/2389	9,0	127,4	20*

* Fat % estimated from common fat% of farmed salmon

Extractions

Two different extraction methods were performed, cold column extraction and ultrasonic extraction.

Cold column extraction

The homogenate (sample/Na_2SO_4) was weighed in so the total amount of fat was approximately 0,25 grams (so not to overload the GPC column), the calculations was done with the information given in table 1. The column was packed with the weighed in sample/Na_2SO_4 and more Na_2SO_4 to a total amount of 50 grams. 20 µl of internal standard POP I (34.10) was added to the packing material prior to the elution. The packed column was eluted with 150 ml of cyclohexane:ethyl acetate into a TurboVap glass. 1 drop of isooctane was added to the eluate before the extract was evaporated to a volume of 0,5 ml in a TurboVap. In order to minimize loss of analytes, the TurboVap glass was rinsed twice; with cyclohexane/ethylacetate (1:1). Pure Na2SO4 was used as a blank sample.

Ultra-sonic bath extraction (USB)

The homogenate (a total amount of 0,25g of fat) was transferred to a beaker and 50 ml of a cyclohexane/acetone mixture (3:1) and 20 µl of internal standard POP I (34.10). The beaker is placed in an ultrasonic bath for 15 minutes. The extract (liquid phase) was transferred to a TurboVap glass and the extraction procedure was repeated two more times. The volume of the extract (150 ml) was reduced to approximately 10 ml in a turbovap and the extract was transferred to 10 ml centrifugation glass. The liquid phase was centrifuged at 2000 rpm for approximately 5 minutes to get rid of solid material. The liquid phase was transferred to a TurboVap glass and the volume was evaporated to 0,5 ml with the TurboVap. In order to minimize loss of analytes, the TurboVap glass was rinsed twice; with cyclohexane/ethyl acetate (1:1). Pure Na_2SO_4 was used as a blank sample.

Clean-up

Sulfuric acid treatment and silica chromatography

The sample solvent was changed from cyclohexane/ethyl acetate (1:1) to hexane before the sample extracts was transferred to a centrifuge glasses and the volume was adjusted to 2 ml n-hexane. Approximately 2 ml concentrated sulfuric acid was added and the samples were mixed on a whirl mixer. The samples was left to react for approximately 15 hours, the hexane fraction was transferred to another centrifuge glass before the acid rest was rinsed with 1 ml n-hexane 2 times. There was not a clear phase separation so the samples were centrifuged at 2000 rpm for 5 minutes. The acid treatment was repeated 2 more times were the reaction times were reduced. The total extract volume was reduced to approximately 0,5 ml.

A glass column was dry packed with 6 g activated silica and a top layer of 2 cm Na_2SO_4. The sample extract was transferred to the column and eluted with 30 ml 10 % cleaned diethyl ether. The sample is collected and the volume is reduced to 0,5 ml and the solvent was changed to isooctane.

The sample is transferred to analysis glass and concentrated to 100 µl. 10µl recovery standard PG (2.33.11) was added after the sample volume was concentrated to 100 µl. The samples are now ready for analysis

Gel permeation chromatography (GPC) and alumina column

Two different GPC columns were used in this cleanup step, a column packed with Bio-Beads S-X3 Beads (200-400 mesh) was used in the cleanup of the samples from the USB extractions and a new Waters Envirogel GPC cleanup column (100 Å pore size, with a nominal particle size of 15 µm) pre packed, used to clean the samples from the cold column extractions.

Both columns was calibrated with a 5 ml solution of cyclohexane/ethyl acetate (1:1) containing the components in the concentrations shown in table 2.

Table 2. Components and their concentrations in a cyclohexane/ethyl acetate (1:1) solution used as a GPC calibration standard

Component	Amount (mg/L)
coconutfat	600
2,4 dinitrophenol	0,35
Sulfur	1

After the calibrations the samples are injected on the GPC system and the fraction that contain POPs are collected. On the samples that were injected on the new GPC column also the lipid fraction were collected for the determination of a lipid profile.

A glass column is filled with n-hexane and 30 g alumina oxide deactivated with 5 % ddH_2O. The alox is let to sediment before a 2 cm layer of Na_2SO_4 is added on the top of the column. The excess n-hexane is removed before 50 ml n-hexane is used to clean the system. The sample extract solved in hexane is added gently to the top of the column, the faucet was closed immediately after the sample extract drops into the Na_2SO_4 layer. The column is eluted with 2 different solvents, first 50 ml n- hexane, then 35 ml tert-butyl-methyl-ether:n-hexane (1:1). The sample is collected and the volume is reduced to 0,5 ml and the solvent was changed to isooctane.

The sample is transferred to analysis glass and concentrated to 100 µl. 10µl recovery standard PG (2.33.11) was added after the sample volume was concentrated to 100 µl. The samples are now ready for analysis.

Analysis

The analysis was done on a GC-MS (Gas chromatography–mass spectrometry) instrument, with a selected ion monitoring program. The temperature programs and SIM programs for the POP analysis are shown in appendix 2.

2.2 Determination of lipid profile

The method for the determination of the fatty acid lipid profile is taken from the UMB-SOP. [10]

50mg metallic Na was weighed and along with 10mL methanol, it was transferred to a 50mL Erlenmeyer flask. No solids can be present in the analysis, so it was observed that all the Na was dissolved. 1-2 drops (approximately 10mg) of the GPC fat fraction was dissolved in 2mL hexane, and then 1 ml of the methanolate solution (5 mg/ml) was added to the hexane solution. The mixture was shaken well for approximately 10 minutes before centrifugation. After 10 minutes on the centrifuge, the samples were blended well and there was a phase separation. 1mL of this hexane phase, the upper layer, was dissolved to a total of 3mL hexane in a vial.

Analysis

1,0 µL of the sample was injected on the GCMS. A sample of hexane was used as a blank reference. Parameters for the GC analysis can be found in Appendix 2.

The fatty acid methyl esters were identified by library search with the MS software and comparison with the standard R38 which contains 37 FAMEs.

3 Results

Comparison of ultrasonic bath extraction against cold column extraction

4 samples underwent the ultrasonic bath extraction one of each fish sample and 8 samples underwent the cold column extractions two of each fish sample. After the extractions all the samples underwent GPC and alumina oxide cleanup. And all the samples underwent identical analysis. Blank samples (Na_2SO_4) underwent the same sample preparations as the fish samples.

The calculated values and recoveries of PCBs, DDTs, HCHs and chlorinated pesticides of the samples that underwent the USB extractions are shown in appendix 3 and 4. The calculated values and recoveries of the same components that underwent the cold column extraction are shown in appendix 5 and 6.

Comparison of sulfuric acid and silica cleanup against GPC and alox cleanup

16 samples underwent cold column extractions and then half of the extracted samples underwent sulfuric acid and silica chromatography cleanup while the other half underwent GPC and alumina oxide chromatography cleanup. Blank samples (Na_2SO_4) also underwent the same sample preparations.

The calculated values and recoveries of PCBs, DDTs, HCHs and chlorinated pesticides for the samples that underwent the sulfuric acid and silica cleanup are shown in appendix 5 and 6. While the samples that underwent the GPC and alumina oxide cleanup are shown in appendix 7 and 8.

Validation of the GPC method with the Waters Envirogel GPC cleanup column

To validate the GPC method with the new GPC column, the calculated values of POPs from the GPC method were compared with the calculated values of POPs from the sulfuric acid cleanup method. This is further explained in the chapter comparison of sulfuric acid and silica cleanup against GPC and alox cleanup

In addition a certified standard reference material 1588b was analyzed with the GPC method and the results are shown in table???? The calculated values of the chlorinated pesticides can be compared with known literature values of these pesticides. [13]

Table. Calculated and literature values of chlorinated pesticides.

Compound	**SRM 1588b**		**SRM Literature value**	
	Consentration	Recovery	Consentration	uncertainty
Structure	ng/g	%	ng/g	±
Dieldrin	128	83	156	4
Heptachlor-exo-epoxide	17	98	30	1,9
cis-Chlordane	161	75	186	22
Oxy-chlordane	34,6	80	37,5	4,5
trans-Nonachlor	224	71	222	10
cis-Nonachlor	128	27	92,4	3
HCB	136	58	163	16
Mirex	8,69	93	11,8	0,6

Comparison between chlorinated environmental toxins and the relative amounts of fatty acid lipid profile

Due to difficulties identifying the different FAMEs, it was decided to create a FAME lipid profile from the FAMEs that were identified in all samples, this will also make the multivariate analysis more accurate and relevant to for the identified FAMEs. The FAMEs that were chosen were also among the most abundant and therefore the analysis should be relevant on a general basis.

Table. Relative amounts of the fatty acid lipid profile of the 8 samples.

	C14:0	C16:0	C16:1 c9	C18:0	C18:1 t9	C18:1 c9	c20:1 c11	22:6 c4,7,10,13,16,19
2386A	0,068	0,274	0,074	0,051	0,411	0,072	0,041	0,010
2386B	0,074	0,279	0,078	0,047	0,412	0,063	0,036	0,011
2387A	0,108	0,362	0,110	0,043	0,275	0,049	0,043	0,010
2387B	0,108	0,335	0,110	0,049	0,277	0,056	0,053	0,012
2388A	0,096	0,364	0,113	0,047	0,285	0,051	0,024	0,020
2388B	0,105	0,361	0,120	0,044	0,278	0,052	0,023	0,018
2389A	0,065	0,197	0,061	0,040	0,482	0,038	0,072	0,045
2389B	0,065	0,200	0,059	0,037	0,488	0,037	0,070	0,045

The lipid fraction was collected through the GPC experiments so the calculated POPs used for the multivariate data analyzes also comes from the same samples. The samples have therefor undergone the same sample preparation. The calculated amounts of POPs are shown in table24525. The software used for the multivariate data analyzes is Portable Unscrambler 9.7.

It was decided to use regression analysis with the PLS 2 algorithm to determine if there is a correlation between the fatty acids and the different POPs. Where the fatty acids are the X-variables and the POPs are the Y-variables. Due to the fact that the calculated concentrations from some of the chlorinated pesticides were outliers they were excluded from the regression analyzes. Figure ???shows the result of the regression analysis.

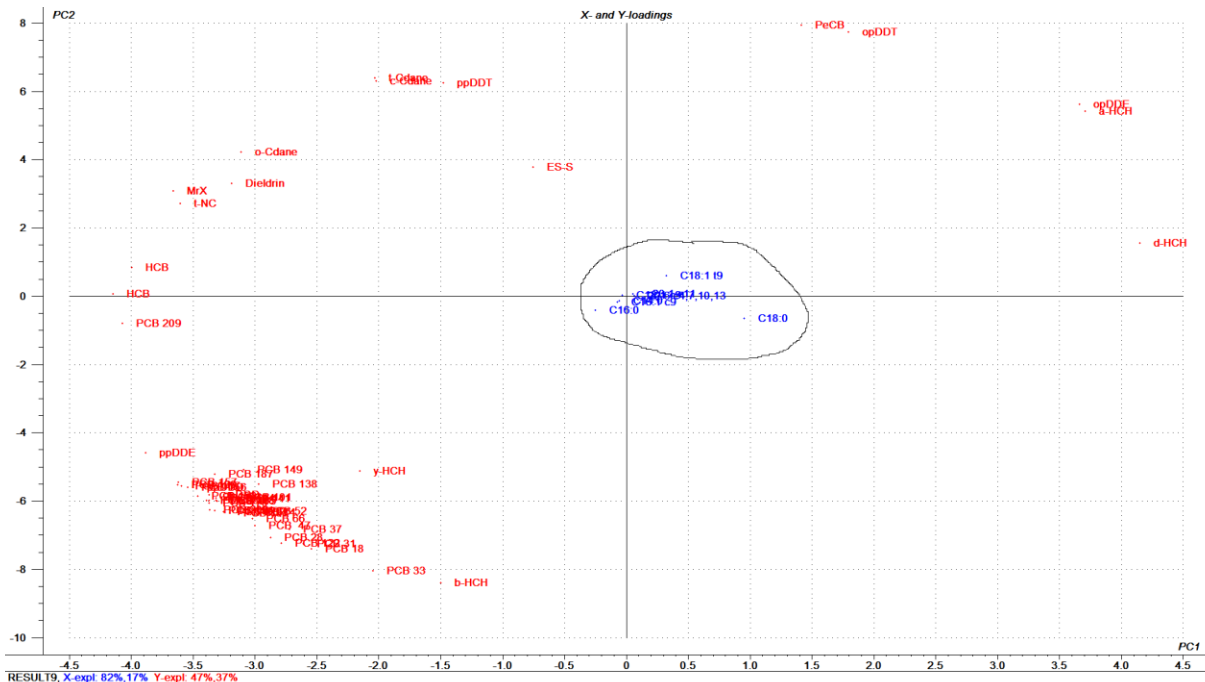

Figure ?? Score plot from the regression analysis.

4 Discussion

Comparison of ultrasonic bath extraction against cold column extraction

Ultrasonic bath extraction is an easy and fast way to extract organic material from a biological sample. Compared to cold column extraction USB is the fastest extraction but it requires more equipment, like a centrifuge to remove particulate matter and a sonicator. From the calculated values of PCBs in appendix 4,6 there is a clear indication that the sample that have been extracted with the USB method has a lower recovery rate than the samples that have been extracted with the cold column method. On average the recovery rates of the samples extracted with the USB method are 5-15 % lower.

For the chlorinated pesticides, appendix 5,7, the indication is the same. The USB samples have on average 10-20 % lower recovery rates than the GPC samples.

The blank USB sample contained PCBs and some chlorinated pesticides this indicates that it has been some cross contaminations between samples. This gives the results from the USB extractions higher uncertainties.

The loss of analyte and internal standard are probably occurring when the samples are in the sonicator, the generated extra heat from the sound waves and the sound waves them self may cause the compounds to evaporate. Another probable cause for lower recoveries is that some target compounds remains in the solids, and are therefore not transferred into the organic phase for further sample preparation.

This means that less of the target compounds and internal standard is lost during the sample preparations with the cold column extractions then the USB. The uncertainties in the calculations for the USB extractions are higher due to that some of the IS has slightly different properties then the target compound. Cold column extractions are a much softer extraction method but the calculated values from the cold column extractions and the USB extractions are still in the same range as each other.

Comparison of sulfuric acid and silica cleanup against GPC and alumina oxide cleanup

The cleanup method that is the fastest is clearly the GPC method where one sample can be done within an hour whereas one sample done with the sulfuric acid cleanup method will take approximately a day. But on the other hand you can only run one sample at a time with the GPC, unless you got more columns, pumps, etc. While you don't have the equipment requirements with the sulfuric acid method and can do sample preparation on multiple samples at a time. The biggest problem with the sulfuric acid treatment is that some of the chlorinated pesticides like dieldrin, aldrin, isodrin, endrin, endosulfan and trifluralin are not acid stable so they will during the acid treatment degenerate and be lost, from the results in appendix 5, on these compounds the recovery rates are zero or close to zero so the calculated results cannot be trusted. To quantify these compounds it is necessary to use the GPC method.

Silica and alumina oxide chromatography are an additional cleanup step where the stationary phase withholds contaminants and lets the target compounds thru. Both methods are similar the differences are different stationary phases and different eluents. The alumina oxide will also withhold organic material that got true the GPC cleanup step.

There is hard to find some general differences in the calculated values of the POPs and their recoveries from the sulfuric acid cleanup and the GPC cleanup. With only two parallels there is hard to find a statistical differences in the results. If the compounds that are not acid stable are excluded the methods gives close to the same results in calculated values and their recoveries. Both the sulfuric acid cleanup and the GPC cleanup are approved methods in laboratories that do environmental pollutants analysis, so the methods themselves are good and reliable. The choice of method is dependent on what compounds that will be analyzed and what equipment that are available.

Validation of the GPC method with the Waters Envirogel GPC cleanup column

To validate the GPC method with the new Envirogel GPC cleanup column 8 samples were analyzed for PCBs and chlorinated pesticides with an established GPC method. The results were compared with the results of the same samples that underwent sulfuric acid cleanup. This has been discussed in the previous chapter. This discussion led to that there were hard to find general differences in the results while the overall amounts of the PCBs and chlorinated pesticides were valid and generated good reproducible results. Some strange amounts occurred in the calculated results but these are attributed to human errors not the GPC column or method.

There were also analyzed a certified standard reference material, 1588b, that were compared to the given values in the certification. This sample was only analyzed for chlorinated pesticides because the GPC method is mainly used when analyzing for these compounds. The calculated amounts are lower than the given literature value, except for trans-nonachlor. This might be because of a systematic error in the analysis, but since two of the compounds are within the range of the uncertainty this is highly unlikely. More likely is that the accuracy of the analysis is lower than the uncertainties of the literature values. This means that the calculated values of the SRM are close to the literature value.

To investigate the accuracy of the analysis I would recommend analyzing more SRM to get idea of the accuracy. This would also help to get a statistical overview, and then we could comment further on if the calculated results with their standard deviations are within range of the given literature values.

Comparison between chlorinated environmental toxins and the relative amounts of fatty acid lipid profile

The lipid profile were created from the lipid fraction collected from the GPC, due to the fact that the GPC separate molecules from size the big lipid molecules are among the first molecules to be separated. The collected fat fraction were analyzed for methylated into fatty acid methyl esters and analyzed on the GC-MS.

A standard R38 containing the 37 most common FAMEs and through the library search option in the MS software, the FAMEs were identified. But due to strong matrix effects and contaminations causing noise in the chromatogram only 8 FAMEs form the lipid profile. This is because it was impossible to separate some of the peaks in the chromatogram and for some peaks it was hard to identify the compound without a doubt. The 8 FAMEs were selected due to their presence and positive identification in all the samples. The FAMEs had to be in all the samples so the multivariate data analysis could be as accurate as possible in determining if the relative amounts of the different FA had an impact on the amount of POPs. The selected FAMEs were also among the most abundant and the multivariate data analysis and should therefore be valid for the general FA content in the four fish samples.

Some of the chlorinated pesticides were excluded from the multivariate data analysis because they were acting like outliers and disturbed the analysis, we can clearly see that something are wrong with the results of some of the compounds. This can be because of poor detection of the signals in the chromatograms, and or low signal-to-noise ratio. If we look at the results for Tox-32 and Tox-42a especially the error is so big that it can only be a gross error caused by a human error. When we excluded the outliers and analyzed our remaining PCBs and chlorinated pesticides against the FA lipid profile, with a PLS 2 algorithm. We got the score plot shown in figure ??? This score plot shows that all of the FA is centered inside the circle, the PCBs and chlorinated pesticides are forming almost a circle around the FA center. The PCBs are all gathered in a small area in the lower left square, the exception is PCB 209 who is alone. PCB 209 is the biggest PCB and it is deca chlorinated if this is the reason it is the only PCB that stands out or that that the FA have an effect on the amount of PCB 209 is unsure. The rest of the PCBs are gathered in a small place this indicates that the FA profile have the same effect on all the PCBs. The rest of the chlorinated pesticides are circled around the FA in the center in approximately the same distance. This indicates that the FA has the same or none effect on the amounts of PCBs and chlorinated pesticides. The exceptions are Endosulfan-sulphate and γ-HCH. If we look at the amounts of Endosulfan-sulphate the amounts varies much even among parallels, and therefore it is highly likely that Endosulfan-sulphate is placed closer to the FA because the compound are an outlier. γ-HCH on the other hand seems to have good values and the difference between parallels seems to be low. This can indicate a direct link between the amount of γ-HCH and the FA. It is tempting to conclude that the amount of FA correlates with the amount of γ-HCH. But if this was true we should be able to see a small connection between the FA and the other HCH because of their similar structures.

In this study the correlations between the relative amounts of FA and the amounts of POPs have been investigated. In further studies it might be relevant to investigate if other types of lipids like phospholipids or glycerols among other have an effect on the amount of POPs in the lipids. This is because we know that POPs are stored in the lipids and we found no connection between the relative amounts of the FA and POPs. So there might be a connection between POPs and other types of lipids.

5 Conclusion

Comparison between ultrasonic bath extraction and cold column extractions were done and the results of the cold column extractions showed better recoveries than the USB extractions, while an USB extraction gives more uncertainties in the method due to lower recoveries and some cross contaminations in the method. This makes the cold column extraction preferable to USB extractions.

Comparison of the sulfuric acid cleanup and the GPC cleanup showed that both methods give good results with good recoveries. But due to the fact that the acid cleanup degenerate and destroy some of the chlorinated pesticides and that the GPC method is faster and much less labor intensive then the sulfuric acid counterpart. I would say that in most cases the GPC method is preferable to the sulfuric acid method.

The Waters Envirogel GPC cleanup column were used in different experiments and the results showed good and reproducible results with good recoveries. And a comparison with a certified standard reference material, 1588b, showed that the chlorinated pesticides were close to their given literature values. This indicates that the established GPC method works well with the new Waters Envirogel GPC column.

There were created a lipid profile containing 8 fatty acids and their relative amounts from fish samples. This was linked together with the determined amount of POPs that were found in the same fish samples that were used to create to FA lipid profile. There were used multivariate data analyses with the algorithm PLS 2 to investigate if there were any correlations between the relative amounts of FA and the selected POPs. The analyses showed that there were no clear correlation between the relative amounts of FA and any of the POPs, except for γ-HCH that were the only compound that might be effected by the different amounts of FA. But more experiments are needed to conclude certainly.

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16. Determination of persistent organic compounds, NILU-O-2, SOP, valid from 12.05.2011

Appendix

Appendix 1 Standards

Recovery standard PG (2.33.11) and internal standard (POPI 34.10)

PG (2.33.11) glass.

Laget:19.08.2011

DATO +	GRUNNSTANDARD:	KONSENTRASJON:	UTTAK:	VEKT:	KORR.UTTAK:	KONSENTRASJON:
INT:		Grunnstandard ng/µ	µl	47,1578	µl	pg/µl
	PG.1.08.09		1400	48,1421	1424	
	1,2,3,4 TCN	3,51				100,02
				81,69370	48,555	
	FORTYNNET TIL:	50	ml			
	LØSEMIDDEL:	Isooctan	Tetthet: 0,691			
	GODKJENT:			PÅ BAKGRUNN AV:		

POP I. (34.10) glass

Benyttes som internstandard til PCB-, DDT/HCH- og pest/multi- analyser

GRUNNSTANDARD:	KONSENTRASJON:	VEKT:	UTTAK:	KONSENTRASJON:	absolutt verdi
Komponent	Grunnstandard ng/µl	47,8393	µl	pg/µl	
13C.PCB-mix (44.09)		50,2852	3411		
13C MBP- 28	3,397			223,5	4,47
13C MBP- 52	3,228			212,4	4,25
13C MBP- 101	3,362			221,2	4,42
13C MBP- 105	3,336			219,4	4,39
13C MBP- 114	3,336			219,4	4,39
13C MBP- 118	3,338			219,6	4,39
13C MBP- 123	3,288			216,3	4,33
13C MBP- 138	3,362			221,2	4,42
13C MBP- 153	3,305			217,4	4,35
13C MBP- 156	3,354			220,7	4,41
13C MBP- 157	3,338			219,6	4,39
13C MBP- 167	3,331			219,1	4,38
13C MBP- 180	3,375			222,0	4,44
13C MBP- 189	3,304			217,3	4,35
13C MBP- 209	3,349			220,4	4,41
13C α-HCH (46.09)	100	50,6495	506	975,8	19,52
13C β-HCH (40.04)	100	50,7370	111	213,6	4,27
13C γ-HCH (47.09)	100	51,0885	490	945,4	18,91
13C p.p.DDE (39.08)	100	51,2020	158	304,0	6,08
13C p.p. DDT(39.08)	100	51,3147	157	301,9	6,04
13C PeCB (1.46.09)	10	51,6631	505	97,4	1,95
13C HCB (1.46.09)	10	51,9902	474	91,4	1,83
13C Hp.Cl.epoxid (46.09)	100	52,3542	508	979,1	19,58
13C tr.Nonachlor (46.09)	100	52,4704	162	312,5	6,25
13C tr.Chlordane (03.09)	100	52,6582	262	505,1	10,10
13C Dieldrin (en ampulle)	100	53,5628	1262	2433,1	48,66
13C Mirex (46.09)	100	53,7662	284	547,1	10,94
13C Endosulfan I (en ampulle)	100	54,6643	1253	2415,7	48,31
13C Endosulfan II (en ampulle)	100	55,5569	1245	2400,9	48,02
13C Endosulfan Sulfate (en ampulle)	100	56,4596	1259	2428,0	48,56
13C Trifluralin (en ampulle)	100	57,3616	1258	2426,1	48,52
13C Cis-NonaChlor (en ampulle)	100	58,2625	1256	2423,2	48,46
13C Aldrin (en ampulle)	100	59,1603	1252	2414,8	48,30
13C Endrin (en ampulle)	100	60,0531	1245	2401,4	48,03
13C Oxychlordane (en ampulle)	100	60,9405	1238	2386,9	47,74
13C Isodrin (en ampulle)	100	61,8257	1235	2381,0	47,62
13C Cis-Chlordane (en ampulle)	100	62,7221	1250	2411,1	48,22
13C Delta-BHC (en ampulle)	100	63,6105	1239	2389,6	47,79
13C HeptaChlor (en ampulle)	100	64,5039	1246	2403,0	48,06
		84,1361			
FORTYNNET TIL:	52	ml			
LØSEMIDDEL:	Nonan:iso-oktan(23:27)	Tetthet: 0,70			
GODKJENT:		PÅ BAKGRUNN AV:			

Appendix 2 The temperature programs and SIM programs for the POP analysis

Metode	Komponenter	Stasjonærfase	Lengde [m]	Indre diameter [mm]	Filmtykkelse [µm]	Gasshastighet [mL/min]	Inj.temperatur [°C]	Temp 1 [°C]	Tid 1 [min]	Ramp 1 [°C/min]	Temp 2 [°C]	Tid 2 [min]	Ramp 2 [°C/min]	Temp 3 [°C]	Tid 3 [min]	Ramp 3 [°C/min]	Temp 4 [°C]	Tid 4 [min]
O-2	DDT	HP-1	25	0,2	0,25	1	220	60	2	20	270	4						
	PCB	HT-8	50	0,22	0,15	1	280	90	2	25	170	0	3	300	3			
	Pest.	Ultra-II	25	0,2	0,11	0,9	260	70	2	20	170	3	5	240	0	30	280	5

gasskromatograf kombinert med høyoppløsende massespektrometer VG AutoSpec, MICROMASS, Wythenshawe Manchester, England (forkort.: Autospec) bestående av:

- HP 5890 gasskromatograf
- Agilent 6890/6890N gasskromatograf
- split/splitless injektor
- HP 7683B/CTC analytics autosampler
- VG GC/MS interface med locksubstans inlet system
- VG AutoSpec tresektor høyoppløsende massespektrometer med EI- og NCI-ionekilde
- Personal workstation 500/1000 med OPUS MS-Software-system eller PC med XP og Masslynx

1 µL splittless

SIM-program for DDT-komponenter:

SIM-funksjon	Isomergruppe	^{12}C-Masse 1	^{12}C-Masse 2	^{13}C-Masse 1	^{13}C-Masse 2
1	TCN	263,907	265,904		
	PFTBA	218,986			
	DDE	246,000	247,997	258,041	260,038
	DDD	235,008	237,005		
	DDT	235,008	237,005		
	DDT(kontroll)	246,000	247,997		

SIM-program for PCB-komponenter:

SIM-funksjon	Isomergruppe	^{12}C-Masse 1	^{12}C-Masse 2	^{13}C-Masse 1	^{13}C-Masse 2
1	Pentaklorbenzen	249,8491	251,8462	255,8693	257,8663
	HCB	283,8102	285,8072	293,8244	295,8214
	PFK	292,9825			

2	TCN	263,9067	265,9038		
	TrCB	255,9613	257,9584	268,0016	269,9986
	TeCB	289,9224	291,9194	301,9226	303,9597
	PFK	280,9825			
3	PeCB	325,8804	327,8775	337,9207	339,9177
	HxCB	359,8415	361,8385	371,8817	373,8788
	PFK	330,9792			
4	HxCB	359,8415	361,8385	371,8817	373,8788
	HpCB	393,8025	395,7995	405,8428	407,8398
	OcCB	427,7635	429,7606		
	PFK	380,9760			
5	NoCB	461,7246	463,7217		
	DeCB	497,6867	499,6798	509,7229	511,7199
	PFK	480,9697			

Veiledende SIM-program for POC:

SIM-funksjon	Isomergruppe	Masse 1	Masse 2
1	13C-HCB	289,8303	291,8273
	HCB	283,8102	285,8072
	D-Trifluralin	349,1972	350,2001
	Trifluralin	335,1093	336,1122
2	13C-Heptaklor	309,8983	311,8954
	Heptaklor	299,8648	301,8618
	Klorden	303,8961	305,8931
3	TCN	263,9067	265,9038
	13C-Aldrin/Isodrin	341,9520	343,9490
	Aldrin/Isodrin	329,9117	331,9088
4	13C-Heptaklor-exo-epoksyd	397,8466	399,8437
	Heptaklorepoksyd, endo/exo	387,8131	389,8101
	13C-Oxyklordan	431,8076	433,8047
	Oxyklordan	421,7741	423,7711
5	13C-Trans/Cis-klordan	417,8284	419,8254
	Trans/Cis-klordan	407,7948	409,7919
	13C-Endosulfan I	414,8441	416,8412
	Endosulfan I	405,8139	407,8110
	13C-Trans-Nonaklor	451,7894	453,7864
	Trans-Nonaklor	441,7558	443,7529
6	13C-Dieldrin	391,9079	393,9050
	Dieldrin	379,8677	381,8647
	TOX-26	376,8573	378,8544
7	13C-Endosulfan II	414,8441	416,8412
	Endosulfan II	405,8139	407,8110
	13C-Cis-Nonaklor	451,7894	453,7864
	Cis-Nonaklor	441,7558	443,7529
	TOX-32	377,8651	379,8621
8	13-C Endosulfan-sulfat	394,8624	396,8594
	Endosulfan-sulfat	385,8322	387,8292
	TOX 40, 41, 42a, 44	376,8573	378,8544
9	13C-Mirex	411,7814	413,7785
	Mirex	401,7479	403,7449
	TOX-50	410,8183	412,8154
	TOX-62	374,8417	376,8387

Appendix 3

Table. The calculated concentrations and recovery's of PCBs from the samples that went through ultrasonic bath extractions and GPC/alox cleanup.

Compound		**11/2386 USB**		**11/2387 USB**		**11/2388 USB**		**11/2389 USB**	
		Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery
Structure	IUPAC-no	ng/g	%	ng/g	%	ng/g	%	ng/g	%
PeCB		0,64	25	0,51	27	0,55	24	0,5	21
HCB		8,84	34	9,8	35	7,71	33	4,7	29
2,2',5-TriCB	18	0,26		2,19		0,2		0,24	
2,4,4'-TriCB	28	1,77	55	12,1	56	1,49	48	0,67	48
2,4',5-TriCB	31	0,54		4,41		0,44		0,49	
2',3,4-TriCB	33	0,13		0,72		0,1		0,17	
3,4,4'-TriCB	37	0,02		0,25		0,02		0,02	
Sum-TriCB		3,58		27,9		2,95		2,17	
2,2',4,4'-TetCB	47	2		16,6		1,76		0,6	
2,2',5,5'-TetCB	52	4,02	59	28,7	60	3,45	51	1,92	51
2,3',4,4'-TetCB	66	4,64		40		4,15		1,04	
2,4,4',5-TetCB	74	3,03		21,6		2,69		0,64	
Sum-TetCB		13,9		110		12,2		4,38	
2,2',4,4',5-PenCB	99	10,9		59,9		9,68		2,1	
2,2',4,5,5'-PenCB	101	12,5	66	73	72	10,8	56	3,67	58
2,3,3',4,4'-PenCB	105	6,64	72	32,7	82	5,83	60	0,9	64
2,3,4,4',5-PenCB	114	0,59	68	2,77	77	0,53	57	0,1	62
2,3',4,4',5-PenCB	118	20,3	70	88,3	81	17,6	59	2,79	64
2'3,3',4,5-PenCB	122	0,02		0,14		0,02		<0,01	
2',3,4,4',5-PenCB	123	0,31	71	1,46	79	0,27	59	0,04	63
Sum-PenCB		51,2		258		44,7		9,59	
2,2',3,3',4,4'-HexCB	128	5,72		27,9		5,13		0,85	
2,2',3,4,4',5'-HexCB	138	36,1	72	158	85	31,7	59	4,53	64
2,2',3,4,5,5'-HexCB	141	1,96		12		1,76		0,5	
2,2',3,4',5',6-HexCB	149	5,43		30,8		4,95		3,04	
2,2',4,4',5,5'-HexCB	153	69,2	75	242	81	59,9	60	6,81	63
2,3,3',4,4',5-HexCB	156	3,16	70	10,8	82	2,72	60	0,28	68
2,3,3',4,4',5'-HexCB	157	0,72	70	2,25	83	0,61	60	0,07	67
2,3',4,4',5,5'-HexCB	167	2,05	70	6,42	82	1,82	59	0,17	66
Sum-HexCB		124		491		109		16,3	
2,2',3,3',4,4',5-HepCB	170	5,63		23,7		4,98		0,61	
2,2',3,4,4',5,5'-HepCB	180	17,4	68	71,1	81	15,2	59	1,78	66
2,2',3,4,4',5',6-HepCB	183	3,48		18,4		3,01		0,38	
2,2',3,4',5,5',6-HepCB	187	7,04		32,4		6,05		1,72	
2,3,3',4,4',5,5'-HepCB	189	0,37	69	1,1	78	0,33	59	0,02	65
Sum-HepCB		33,9		147		29,5		4,52	
2,2',3,3',4,4',5,5'-OctCB	194	2,04		8,27		1,72		0,19	
2,2',3,3',4,4',5,5',6-NonCB	206	1,07		3,86		0,89		0,09	
DecaCB	209	1,31	67	1,37	73	1,13	54	0,05	59
Sum 7 PCB		161		674		140		22,2	
Sum PCB		231		1046		202		37,3	

Appendix 4.

Table. The calculated concentrations and recovery's of HCHs, DDTs and chlorinated pesticides from the samples that went through ultrasonic bath extractions and GPC/alox cleanup.

Compound		11/2386 USB		11/2387 USB		11/2388 USB		11/2389 USB	
		Consentratio	Recovery	Consentratio	Recovery	Consentratio	Recovery	Consentratio	Recovery
Structure		ng/g	%	ng/g	%	ng/g	%	ng/g	%
	a-HCH	0,64	47	0,5	47	0,53	43	0,65	40
	b-HCH	0,46	59	0,67	60	0,34	53	0,41	53
	y-HCH	0,31	56	0,43	57	0,25	50	0,3	48
	d-HCH	0,04	27	0,02	37	0,04	29	0,02	37
	o,p'-DDE	0,29		0,13		0,25		0,41	
	p,p'-DDE	112	71	256	76	101	60	16,9	60
	o,p'-DDD	1,3		1,56		0,91		1,66	
	p,p'-DDD	24,2		51,5		18,4		5,99	
	o,p'-DDT	1,2		2,08		0,98		0,7	
	p,p'-DDT	11,9	57	31	74	10,6	60	2,57	62
	Sum DDT	151		343		132		27,3	
Dieldrin		13,7	76	12,5	77	12,2	63	6,59	61
Aldrin		0,01	59	0,01	60	0,01	55	0,01	55
Isodrin		0,01	62	0,02	64	0,03	54	0,01	53
Endrin		0,56	73	0,47	75	0,54	61	0,3	60
Heptachlor-exo-epoxide		0,83	71	1,2	69	2,78	58	1,21	55
Heptachlor-endo-epoxide		0,32		0,46		0,72		0,26	
trans-Chlordane		0,62	71	0,64	73	0,54	59	0,45	59
cis-Chlordane		5,57	71	4,84	74	4,8	61	2,9	59
Oxy-chlordane		4,58	66	3,07	65	3,87	59	0,84	55
Chlordene		0,17		0,14		0,16		0,09	
Heptachlor		0,06	57	0,05	55	0,05	50	0,03	46
trans-Nonachlor		15,9	69	13,9	69	14,1	59	4,06	55
cis-Nonachlor		7,12	91	5,62	104	6,26	62	1,81	67
Tox-26		5,91		3,19		4,78		2,99	
Tox-32		1,92		1,15		3,16		2,76	
Tox-40 + Tox-41		2,88		1,27		3,03		3,05	
Tox-42a		0,16		0,02		0,06		0,97	
Tox-44		10,5		3,91		11		9,73	
Tox-50		7,19		3,98		7,48		5	
Tox-62		0,73		28,9		2		0,73	
Endosulfan-I		0,05	67	0,02	68	0,04	58	0,13	55
Endosulfan-II		0,01	0	0,01	0	1,6	1	5,88	0
Endosulfan-sulphate		0,01	0	0,01	0	2,76	0	0,01	0
HCB		8,26	36	8,79	41	7,11	36	4,37	33
Trifluralin		0,01	0	3,27	9	0,81	9	0,77	4
Mirex		1,44	73	0,97	78	1,32	62	0,18	60

Appendix 5

Table. The calculated concentrations and recoveries of PCBs from the samples that went through cold column extractions and sulfuric acid/silica cleanup.

Compound		11/2386 acid A		11/2386 acid B		11/2387 acid A		11/2387 acid B		11/2388 acid A		11/2388 acid B		11/2389 acid A		11/2389 acid B	
		Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery
Structure	IUPAC-no	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%
PeCB		0,69	25	0,67	27	0,56	21	0,6	25	0,54	25	0,48	20	0,57	20	0,6	19
HCB		8,96	38	8,65	42	10	33	10,6	39	8,68	38	7,64	33	5,22	33	5,31	29
2,2',5-TriCB	18	0,24		0,24		2,25		2,47		2,06		1,81		0,28		0,28	
2,4,4'-TriCB	28	1,84	60	1,8	66	12,5	53	13,3	59	11,1	59	9,68	50	0,72	50	74	45
2,4',5-TriCB	31	0,55		0,53		4,58		4,97		3,95		3,48		0,58		0,59	
2',3,4-TriCB	33	0,12		0,11		0,7		0,74		0,74		0,64		0,18		0,18	
3,4,4'-TriCB	37	0,02		0,02		0,21		0,23		0,15		0,13		0,02		0,02	
Sum-TriCB		3,6		3,54		28,4		30,5		25,5		22,3		2,42		2,49	
2,2',4,4'-TetCB	47	2,09		2,07		17		18,2		13,3		11,6		0,66		0,7	
2,2',5,5'-TetCB	52	4,3	61	4,18	68	29,8	55	31,8	60	20,8	62	18,4	53	2,26	55	2,3	47
2,3',4,4'-TetCB	66	4,86		4,87		40,9		43,3		30,8		26,8		1,07		1,17	
2,4,4',5-TetCB	74	3,17		3,18		22,8		24,1		16,5		14,4		0,67		0,74	
Sum-TetCB		14,7		14,5		113		120		83,4		72,9		4,84		5,12	
2,2',4,4',5-PenCB	99	11,7		11,4		63,6		68,2		48,3		41,7		2,49		2,52	
2,2',4,5,5'-PenCB	101	12,5	68	12,3	78	74	67	78,9	72	48,2	73	41,7	64	4,19	60	4,24	55
2,3,3',4,4'-PenCB	105	6,72	78	6,53	88	33,4	83	36	83	24,3	85	21	75	1,03	60	1,04	60
2,3,4,4',5-PenCB	114	0,61	78	0,59	89	2,8	79	3,03	80	2,06	81	1,78	72	0,11	63	0,11	60
2,3',4,4',5-PenCB	118	20,2	78	19,9	89	90,4	80	96,6	82	66,1	84	57,3	74	3,14	63	3,017	62
2'3,3',4,5-PenCB	122	0,02		0,02		0,14		0,15		0,11		0,09		<0,01		<0,01	
2',3,4,4',5-PenCB	123	0,3	78	0,3	89	1,51	80	1,61	81	1,19	83	1,03	73	0,04	64	0,05	61
Sum-PenCB		52,1		51		266		284		190		165		11		11,1	
2,2',3,3',4,4'-HexCB	128	5,56		5,45		26,6		29		19,7		17		0,84		0,88	
2,2',3,4,4',5'-HexCB	138	36,8	80	36,2	91	161	89	173	88	120	90	104	81	5,2	65	5,2	65
2,2',3,4,5,5'-HexCB	141	2,19		2,07		13		13,8		8,59		7,25		0,54		0,57	
2,2',3,4',5',6-HexCB	149	5,82		5,57		30,9		34		16,5		13,9		3,24		3,29	
2,2',4,4',5,5'-HexCB	153	67	78	63,2	91	241	80	238	79	190	86	162	79	7,98	70	8,07	65
2,3,3',4,4',5-HexCB	156	3,17	98	3,1	108	11,4	100	12,2	93	8,55	106	7,42	97	0,32	79	0,33	79
2,3,3',4,4',5'-HexCB	157	0,72	97	0,7	104	2,42	101	2,56	95	1,78	106	1,54	96	0,07	71	0,08	74
2,3',4,4',5,5'-HexCB	167	2,09	93	2,05	101	6,66	101	7,1	95	5,2	102	4,5	90	0,2	73	0,2	74
Sum-HexCB		123		119		493		509		370		318		18,4		18,6	
2,2',3,3',4,4',5-HepCB	170	5,92		5,75		24,8		26		19,6		17,2		0,66		0,7	
2,2',3,4,4',5,5'-HepCB	180	17,5	92	17,1	103	73,8	96	78,8	89	56,3	102	49,3	95	2,03	77	2,06	76
2,2',3,4,4',5',6-HepCB	183	3,16		3,1		18,1		20,3		14		11,7		0,43		0,44	
2,2',3,4',5,5',6-HepCB	187	6,46		6,27		31,2		34,5		18,1		15,6		1,92		1,94	
2,3,3',4,4',5,5'-HepCB	189	0,37	96	0,37	110	1,2	94	1,26	84	0,94	101	0,81	96	0,03	81	0,03	81
Sum-HepCB		33,4		32,6		149		161		109		94,7		5,08		5,16	
2,2',3,3',4,4',5,5'-OctCB	194	2,04		1,93		8,92		9,67		7,44		6,34		0,21		0,21	
2,2',3,3',4,4',5,5',6-NonCB	206	1,15		1,08		4,37		4,67		3,81		3,28		0,11		0,11	
DecaCB	209	1,31	93	1,32	103	1,43	98	1,51	92	1,27	99	1,09	90	0,06	70	0,06	76
Sum 7 PCB		160		155		682		710		512		443		25,5		25,8	
Sum PCB		232		225		1064		1121		790		683		42,1		42,9	

Appendix 6.

Table. The calculated concentrations and recoveries of HCHs, DDT's and chlorinated pesticides from the samples that went through cold column extractions and sulfuric acid/silica cleanup.

Compound	11/2386 syre A		11/2386 syre B		11/2387 syre A		11/2387 syre B		11/2388 syre A		11/2388 syre B		11/2389 syre A		11/2389 syre B	
	Consentra	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery
Structure	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%
a-HCH	0,62	42	0,58	48	0,52	32	0,53	33	0,52	40	0,43	34	0,79	27	0,79	21
b-HCH	0,5	41	0,47	47	0,8	38	0,86	41	0,74	42	0,66	33	0,53	21	0,55	21
y-HCH	0,36	41	0,32	49	0,44	45	0,49	50	0,45	50	0,38	41	0,34	33	0,35	28
d-HCH	0,02	34	0,02	41	0,02	35	0,06	35	0,02	40	0,03	30	0,02	18	0,02	17
o,p'-DDE	0,42		0,37		0,14		0,16		0,12		0,1		0,49		0,49	
p,p'-DDE	124	53	120	62	251	59	256	59	226	65	204	58	20,4	63	20,5	55
o,p'-DDD	1,15		1,03		1,54		1,62		1,37		1,14		0,66		0,58	
p,p'-DDD	22,2		21		53,2		55,2		41,1		35,5		6,06		5,6	
o,p'-DDT	1,14		1,13		1,95		1,08		0,55		1,17		0,84		0,76	
p,p'-DDT	12,5	59	12,3	71	34,7	70	36	71	18,4	73	16,3	63	2,87	61	2,96	65
Sum DDT	162		156		343		350		288		258		31,3		30,9	
Dieldrin	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0
Aldrin	0,02	7	0,23	1	0,05	3	2,91	0	0,03	8	0,07	4	0,04	3	0,03	3
Isodrin	0,64	1	5,23	0	2,59	0	8,39	0	0,96	1	1,06	1	0,93	0	1,06	0
Endrin	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0
Heptachlor-exo-epoxide	2,66	5	4,58	5	6,11	4	4,4	4	9,77	6	12,2	4	0,01	0	0,01	0
Heptachlor-endo-epoxide	1,02		1,75		2,34		1,69		3,73		4,67		0,01		0,01	
trans-Chlordane	0,66	63	0,63	77	0,72	58	0,71	62	0,56	73	0,48	60	0,49	51	0,51	54
cis-Chlordane	5,53	66	5,3	83	5,05	58	5,28	64	3,63	76	3,12	62	3,21	52	3,27	55
Oxy-chlordane	4,35	62	4,14	71	3,09	54	3,27	56	2,4	68	2,16	52	0,85	49	0,93	50
Chlordene	0,05		0,1		0,06		0,06		0,1		0,14		0,06		0,04	
Heptachlor	0,02	55	0,03	64	0,02	48	0,04	51	0,04	55	0,06	46	0,02	46	0,01	43
trans-Nonachlor	16,6	65	16,2	79	14,1	65	14,7	69	9,89	75	8,64	63	4,39	60	4,58	58
cis-Nonachlor	7,51	73	6,83	76	6,24	62	6,6	71	4,12	82	3,28	77	2,08	54	2,58	51
Tox-26	6,15		6		3,02		3,09		2,08		1,86		2,93		3,15	
Tox-32	0,83		1,48		0,72		1,17		1,91		1,26		1,36		0,88	
Tox-40 + Tox-41	3,21		3,69		1,92		1,74		1,24		0,91		3,3		3,73	
Tox-42a	0,2		0,21		0,03		0,02		0,02		0,02		1,12		1,42	
Tox-44	12,1		14,2		5,36		4,87		3,89		5,49		10,9		12,4	
Tox-50	8,95		10,5		6,76		5,92		3,87		3,02		6,53		7,21	
Tox-62	0,4		1,05		0,64		40,2		0,79		61,3		2,36		4,1	
Endosulfan-I	13,6	0	6,96	0	0,01	0	0,01	0	16,7	0	30,1	1	9,53	0	8,03	0
Endosulfan-II	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0
Endosulfan-sulphate	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0
HCB	7,83	41	7,58	51	9,27	34	9,76	43	7,98	40	7,2	35	4,91	36	4,76	37
Trifluralin	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0	0,01	0
Mirex	1,71	72	1,66	85	1,13	82	1,17	80	0,86	85	0,75	72	0,19	68	0,2	67

Appendix 7.

Table. The calculated concentrations and recovery's of PCB's from the samples that went through cold column extractions and GPC/Alox cleanup.

Compound		11/2386 GPC new A		11/2386 GPC new B		11/2387 GPC new A		11/2387 GPC new B		11/2388 GPC new A		11/2388 GPC new B		11/2389 GPC new A		11/2389 GPC new B	
		Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery	Consentration	Recovery
Structure	IUPAC-no	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%
PeCB		0,67	24	0,68	30	0,47	40	0,5	38	0,49	38	0,49	39	0,59	37	0,61	33
HCB		9,46	48	9,76	42	9,38	47	10,1	49	8,48	53	8,44	52	5,71	50	5,84	46
2,2',5-TriCB	18	0,25		0,27		2,13		2,35		2,12		2,12		0,33		0,34	
2,4,4'-TriCB	28	1,96	71	2,04	60	11,1	74	12,3	74	11,1	73	11,1	74	0,84	72	0,91	65
2,4',5-TriCB	31	0,57		0,59		4,09		4,53		3,92		3,93		0,64		0,68	
2',3,4-TriCB	33	0,12		0,13		0,6		0,67		0,72		0,72		0,2		0,22	
3,4,4'-TriCB	37	0,02		0,02		0,19		0,23		0,17		0,17		0,02		0,02	
Sum-TriCB		3,85		4,05		25,8		28,6		25,7		25,7		2,75		2,96	
2,2',4,4'-TetCB	47	2,39		2,5		15,2		16,5		13,3		13,4		0,65		0,72	
2,2',5,5'-TetCB	52	4,62	71	4,77	61	26,5	72	29,2	73	20,7	71	20,6	70	2,48	68	2,64	62
2,3',4,4'-TetCB	66	5,62		5,84		36		40,8		30,3		30,7		1,36		1,61	
2,4,4',5-TetCB	74	3,58		3,64		19,7		22,2		15,9		16,2		0,81		0,96	
Sum-TetCB		18,5		19,1		114		127		94,6		95,6		6,49		7,21	
2,2',4,4',5-PenCB	99	12,30		12,7		54,8		61,8		46,5		46,8		2,73		2,97	
2,2',4,5,5'-PenCB	101	13,8	80	14,3	68	64,6	80	72,6	82	48,2	78	48,3	78	4,64	75	4,97	71
2,3,3',4,4'-PenCB	105	7,46	86	7,63	75	29,6	89	33	92	24,1	84	24,1	85	1,16	80	1,22	78
2,3,4,4',5-PenCB	114	0,6	78	0,63	68	2,41	64	2,65	67	1,91	76	1,9	78	0,11	74	0,12	70
2,3',4,4',5-PenCB	118	22,60	88	23,1	76	76,8	85	87,4	86	67	84	65,9	86	3,55	83	3,81	79
2'3,3',4,5-PenCB	122	0,02		0,02		0,12		0,13		0,11		0,12		0,01		0,01	
2',3,4,4',5-PenCB	123	0,34	85	0,35	76	1,32	90	1,49	92	1,19	86	1,19	87	0,05	84	0,05	81
Sum-PenCB		57,2		58,8		230		259		189		188		12,2		13,2	
2,2',3,3',4,4'-HexCB	128	5,89		6		23,7		25,6		18,9		18,8		1		1,05	
2,2',3,4,4',5'-HexCB	138	40,2	86	41	74	77,6	61	183	61	121	82	118	84	5,7	84	5,97	80
2,2',3,4,5,5'-HexCB	141	2,42		2,45		11,8		13,2		8,89		8,85		0,66		0,7	
2,2',3,4',5',6-HexCB	149	6,81		6,9		30,2		34,4		18,7		18,4		4,04		4,16	
2,2',4,4',5,5'-HexCB	153	71,80	82	73,3	71	219	83	245	85	192	79	189	80	8,8	79	9,46	75
2,3,3',4,4',5-HexCB	156	3,4	91	3,5	79	10,1	92	10,9	96	8,27	89	8,18	90	0,36	84	0,38	82
2,3,3',4,4',5'-HexCB	157	0,79	87	0,82	76	2,11	88	2,29	92	1,75	85	1,74	87	0,08	83	0,09	81
2,3',4,4',5,5'-HexCB	167	2,24	90	2,28	79	5,86	89	6,52	92	5,1	85	5,04	87	0,22	83	0,23	81
Sum-HexCB		134,00		136		381		521		375		368		20,9		22	
2,2',3,3',4,4',5-HepCB	170	6,34		6,4		22,5		24,6		19,7		19,2		0,77		0,81	
2,2',3,4,4',5,5'-HepCB	180	19,20	83	19,5	73	67,4	86	72,7	89	56,6	83	56,2	83	2,31	78	2,4	76
2,2',3,4,4',5',6-HepCB	183	3,84		3,9		17,2		19		14,8		14,9		0,52		0,55	
2,2',3,4',5,5',6-HepCB	187	7,67		7,87		29,8		32,7		19,6		19,6		2,36		2,37	
2,3,3',4,4',5,5'-HepCB	189	0,41	91	0,41	79	1,06	95	1,15	98	0,9	95	0,9	92	0,03	83	0,03	82
Sum-HepCB		37,40		38,1		138		150		112		111		5,99		6,16	
2,2',3,3',4,4',5,5'-OctCB	194	1,99		2,09		7,4		7,84		6,63		6,54		0,22		0,24	
2,2',3,3',4,4',5,5',6-NonCB	206	1,12		1,14		3,56		3,87		3,33		3,33		0,11		0,12	
DecaCB	209	1,43	85	1,47	73	1,27	88	1,38	89	1,24	85	1,24	84	0,07	77	0,07	76
Sum 7 PCB		174,00		178		543		702		517		509		28,3		30,2	
Sum PCB		255		261		900		1099		807		800		48,7		51,9	

Appendix 8.

Table. The calculated concentrations and recovery's of HCHs, DDTs and chlorinated pesticides from the samples that went through cold column extractions and GPC/Alox cleanup.

Compound	11/2386 GPC new A		11/2386 GPC new B		11/2387 GPC new A		11/2387 GPC new B		11/2388 GPC new A		11/2388 GPC new B		11/2389 GPC new A		11/2389 GPC new B	
	Consentration	Recovery	Consentration	Recovery	Consentra	Recovery	Consentra	Recovery	Consentra	Recovery	Consentra	Recovery	Consentra	Recovery	Consentra	Recovery
Structure	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%	ng/g	%
a-HCH	0,63	57	0,67	42	0,44	49	0,4	52	0,43	60	0,47	60	0,81	54	0,81	54
b-HCH	0,45	96	0,36	62	0,59	63	0,57	64	0,66	69	0,69	67	0,42	68	0,47	59
y-HCH	0,34	69	0,43	50	0,4	55	0,4	58	0,5	64	0,46	65	0,33	59	0,37	55
d-HCH	<0,03	62	0,05	43	0,04	48	0,04	60	0,05	49	0,07	45	0,56	3	0,6	2
o,p'-DDE	0,27		0,26		0,11		0,13		0,11		0,1		0,43		0,43	
p,p'-DDE	139	131	138	101	249	83	288	82	217	74	216	74	20,7	68	21,1	66
o,p'-DDD	1,15		1,26		1,81		2,11		1,8		1,76		0,91		0,95	
p,p'-DDD	24,9		23,9		58,5		70,2		53,6		51,6		7,4		8,28	
o,p'-DDT	1,27		1,42		0,11		0,18		0,31		0,25		1,05		0,96	
p,p'-DDT	12,5	164	11,9	99	3,76	79	4,57	74	4,55	62	4,24	64	3,44	54	3,39	49
Sum DDT	179		177		313		365		278		274		33,9		35,1	
Dieldrin	15,5	105	16,4	89	11,6	121	12,9	117	12,6	93	11,9	100	8,54	89	8,9	82
Aldrin	0,01	76	0,01	67	0,01	75	0,01	79	0,01	78	0,01	78	0,01	83	0,01	78
Isodrin	0,02	74	0,03	64	0,03	74	0,02	79	0,02	76	0,02	77	0,01	76	0,01	73
Endrin	0,25	135	0,68	94	0,26	89	0,39	86	0,36	89	0,26	101	0,15	87	0,1	84
eptachlor-exo-epoxid	0,97	101	2,06	75	0,74	93	0,51	96	0,63	88	0,74	93	1,45	79	1,3	83
ptachlor-endo-epoxi	0,34		0,73		0,27		0,18		0,22		0,26		0,2		0,08	
trans-Chlordane	0,69	99	0,68	76	0,54	91	0,63	97	0,54	84	0,52	86	0,52	79	0,54	79
cis-Chlordane	6,38	86	5,97	74	4,33	88	4,78	88	3,53	83	3,61	83	3,59	78	3,63	77
Oxy-chlordane	4,94	96	5,23	80	3	93	3,02	92	2,73	85	2,34	87	1,05	79	1,03	82
Chlordene	0,1		0,15		0,08		0,11		0,11		0,08		0,06		0,04	
Heptachlor	0,02	90	0,04	67	0,02	81	0,02	84	0,03	81	0,02	84	0,01	68	0,01	69
trans-Nonachlor	16,4	101	16,4	83	12,6	86	14,2	86	9,75	81	9,97	79	4,79	77	4,96	74
cis-Nonachlor	7,72	93	7,87	76	6,44	0	0,01	0	3,9	85	3,86	85	2,24	83	2,23	80
Tox-26	7,49		6,96		2,73		2,95		1,92		2,26		3,77		4,1	
Tox-32	6,13		3,16		2282		0,01		2,49		2,55		1,35		1,74	
Tox-40+Tox-41	5,26		4,62		24,2		0,01		1,76		1,88		4,53		5,04	
Tox-42a	0,24		0,27		3348		0,01		0,07		0,02		1,33		1,51	
Tox-44	16,7		15,3		83,9		0,01		2,63		2,94		12,1		13,9	
Tox-50	12,4		11,7		11,2		0,01		2,38		2,6		6,17		6,62	
Tox-62	1,18		1,79		922		0,01		1,07		0,84		1,93		2,53	
Endosulfan-I	0,04	102	0,05	83	0,02	92	0,04	96	0,03	85	0,04	89	0,13	72	0,13	76
Endosulfan-II	0,14	7	1,25	2	0,12	23	0,06	41	0,27	4	0,48	2	7,74	0	0,81	0
Endosulfan-sulphate	40,9	0	3,59	0	0,01	0	3,06	0	2,52	0	6,02	0	0,01	0	1,92	0
HCB	9,28	46	9,3	44	8,42	54	9,12	58	7,88	57	7,85	85	5,24	58	5,38	54
Trifluralin	0,08	88	0,12	61	0,06	74	0,07	85	0,08	78	0,06	78	0,31	62	0,28	67
Mirex	1,45	143	1,46	103	0,96	105	1,07	99	0,89	85	0,72	103	0,21	83	0,21	89