UNEP Chemicals
POPs Training Project

Practical Training Course
at the Environmental Management Bureau of the Department of Environment and Natural Resources,
Quezon City, Philppines.
August 13 – August 17, 2018

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Background

The Global Environment Facility (GEF) has granted to the United Nations Environment Program (UNEP) a project on capacity building for POP analyses for the Global Monitoring Plan. This 4-year project addresses country needs for laboratory analysis of POPs, pursuant to the Stockholm Convention, and conditions necessary to conduct such analysis in a sustainable manner. The project focuses on the analysis of all POPs of the Stockholm Convention. This project will assist laboratories in countries that are Party to the Stockholm Convention on the ground with training and inter laboratory assessment studies to prove their expertise in an international context or to improve the existing performance.

The UNEP is currently executing projects to enhance POPs Analysis Capacity. According to article 16 of the Stockholm Convention on POPs, the Conference of the Parties has agreed on a Global Monitoring Plan (GMP) consisting of regional reports. This project will also build-up a network of POPs laboratories at a global level.

Introduction

Training of laboratory personnel for the analysis of POPs at the Stockholm Convention list is offered to the Environmental laboratory Services Section of the Environmental Management Bureau in the Philippines. Two trainers from the Vrije Universteit (VU) Amsterdam, the Netherlands will give a seven-day course to demonstrate analytical methods for POPs. The course will include lectures with theory (ca. 30%) and practical ('hands-on') work (ca. 70%) in the laboratory. The theoretical part (2 days) already took place in December 2017 by Prof. Dr. Jacob de Boer.

The matrices selected by UNEP for its Global Monitoring Program (GMP) are human milk, water and air. Provided there is an interest, the participants may also get an introduction to POPs analysis in other matrices such as fish and sediment. This project will include analyses of POPs passively sampled from air by the participating laboratory.

In addition to the on-site training, a 'mirror' study will be carried out after the course. The results of the analysis carried out by the participating laboratory and that of the analysis done by VU in the same samples will be compared and used for further evaluation of laboratory performance on air samples.

Aim of the Training

The aim of the training is to build up capacity in the participating laboratory for the analysis of the organochlorinated pesticides listed in the Stockholm convention POP list and poly chlorinated biphenyls (PCBs) in environmental matrices.

Attention will be paid to sampling, sample handling, sample storage, extraction, cleanup of samples, gas chromatography, reporting and various aspects of quality assurance and quality control (QA/QC) such as method validation, blanks, calibration, internal standards, reference materials, limit of detection, limit of quantification, etc.

Table 1 Overview of the compounds of interest*

Compound	Acronym
Aldrin	Aldrin
Chlordane	cis-Chlordane trans-Chlordane cis-Nonachlor trans-Nonachlor Oxychlordane
Dichlorodiphenyltrichloroethane	2,4'-DDT 4,4'-DDT 2,4'-DDE 4,4'-DDE 2,4'-DDD 4,4'-DDD
Dieldrin	Dieldrin
Endrin	Endrin
Heptachlor	Heptachlor cis-Heptachlorepoxide trans-Heptachlorepoxide
Hexachlorobenzene	HCB
Mirex	Mirex
Hexachlorocyclohexane	α-HCH β-HCH γ-HCH
Endosulfan	α-Endosulfan
Polychlorinated biphenyls	PCB28 PCB52 PCB101 PCB138 PCB153 PCB180

*New POPs will be discussed during the theoretical part of the training.

Proposed Training Schedule

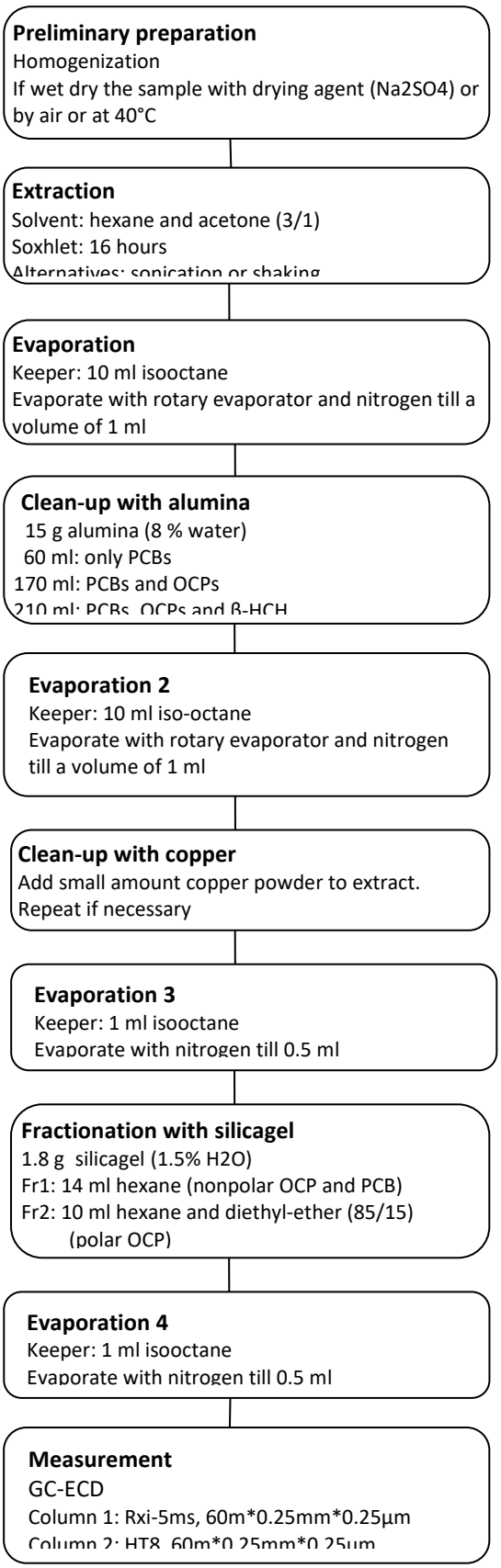
Day 1-2: (took place in December 2017)

- Introduction (trainer, participants)
- Theory, power point presentations
- Background information:
    - Safety
    - Sample forms / LIMS
    - Blanks / Reference Material / Control charts / Validations
    - Testing silica / evaluation
    - Standards / Recoveries / Calibration curves / Weigh lists
    - LOD / LOQ
    - Testing and calibrating GC-µECD

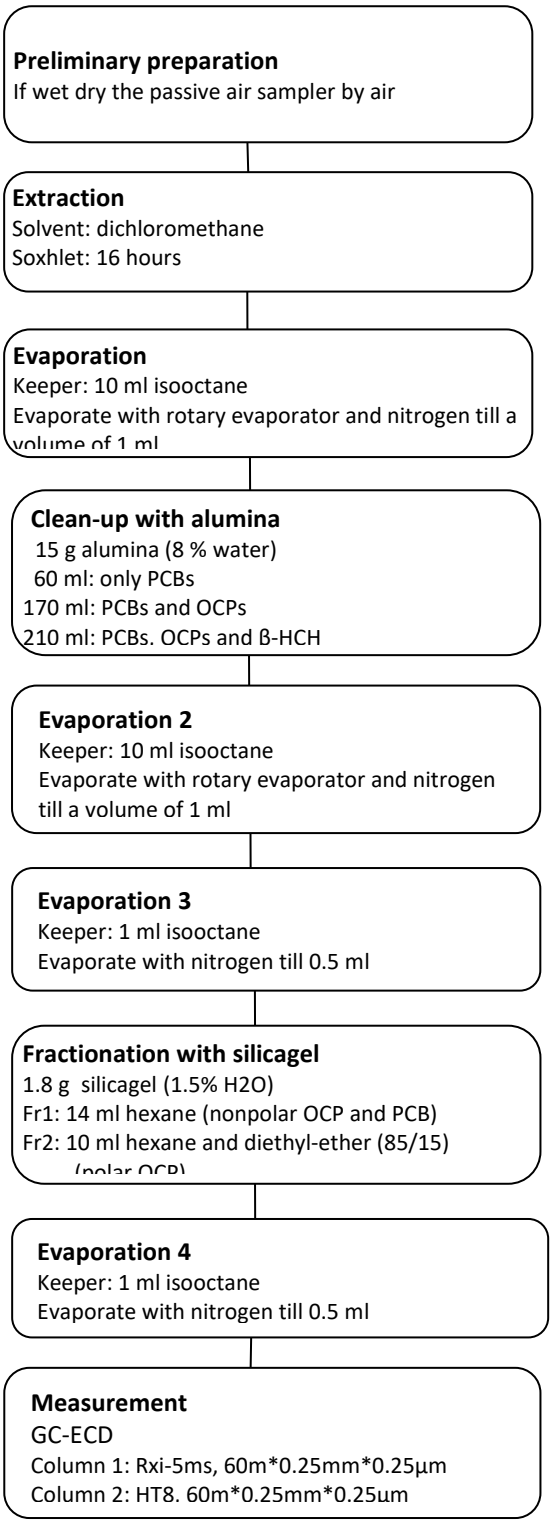
Day 3-7:

- Extraction, clean up and analysis of PUFs and other matrices
- Interpretation of chromatograms
- Integration, calculation and reporting of concentrations
- PUF sampling
- Clean up options for sediment and fish (i.e. removing sulphur and fat)
- Drying methods (i.e. freeze drying)
- Fat determinations (Bligh & Dyer or extractable fat)
- Dry weight determinations (sediments or biota)

Synopsis of the analytical protocol for PCB/OCP analysis in soil



Synopsis of the analytical protocol for PCB/OCP analysis in passive air samplers


Preliminary preparation
If wet dry the passive air sampler by air
Extraction
Solvent: dichloromethane
Soxhlet: 16 hours
Evaporation
Keeper: 10 ml isooctane
Evaporate with rotary evaporator and nitrogen till a volume of 1 ml
Clean-up with alumina
15 g alumina (8 % water)
60 ml: only PCBs
170 ml: PCBs and OCPs
210 ml: PCBs. OCPs and ß-HCH
Evaporation 2
Keeper: 10 ml isooctane
Evaporate with rotary evaporator and nitrogen till a volume of 1 ml
Evaporation 3
Keeper: 1 ml isooctane
Evaporate with nitrogen till 0.5 ml
Fractionation with silicagel
1.8 g silicagel (1.5% H2O)
Fr1: 14 ml hexane (nonpolar OCP and PCB)
Fr2: 10 ml hexane and diethyl-ether (85/15)
(polar OCP)
Evaporation 4
Keeper: 1 ml isooctane
Evaporate with nitrogen till 0.5 ml
Measurement
GC-ECD
Column 1: Rxi-5ms, 60m*0.25mm*0.25µm
Column 2: HT8. 60m*0.25mm*0.25um

Materials needed for the analysis during the training course

UNEP will provide a number of essential consumables, such as GC columns, glassware and other tools. The participating laboratory should ensure that other materials are available. A beamer/projector for power point presentations should also be provided to the trainer for his presentations. The consumables and chemicals needed are:

Glassware etc:

- Common laboratory glassware: several volumetric flasks/ Erlenmeyer's
- Pasteur's pipettes
- Mortar + pestel (1x)
- Soxhlet setup including *glass* thimbles (6x, extractor volume 100 ml)), coolers, glass rods
- Rotary evaporator
- Glass columns for Al_2O_3 (3x, provided by E&H)
- Glass columns for Silica (3x, provided by E&H)
- Test tubes (minimal 15 ml) (12x)
- GC-vials
- Tweezers
- Aluminum foil

Chemicals/Solvents etc:

- Na_2SO_4 (± 250 g)
- Silica (50 g)
- Al_2O_3 (200 g)
- Pentane, picograde or equal quality
- Hexane picograde or equal quality
- Dichloromethane picograde or equal quality
- Iso-octane picograde or equal quality
- Di-ethylether picograde or equal quality
- Methanol picograde or equal quality
- Copper powder
- Hydrochloric Acid
- PCB 112
- Glass pearls
- Glass wool

Equipment etc:

- Pipetman (10 – 100 µl) including tips
- Pipetman (100 – 1000 µl) including tips
- Heating for Soxhlets (75 °C)
- Coolers voor Soxhlet
- Stands/supports, clamps, to support columns
- Evaporation apparatus for solutions in test tubes using N_2 and a water bath (30 °C)
- Balance
- GC-ECD or GC-MS, including autosampler
- Column / liners / syringe / Helium (or hydrogen) as carrier gas

- Computer for the evaluation/calculation of the samples

Detailed Analytical Protocol

Preparation of drying agent

Prepare Na_2SO_4 (approx. 3 g Na_2SO_4 per ±1g sample) by 'baking out' for 16 hours at 400 °C to remove contaminants.

Drying and activating column materials

Dry SiO_2 overnight at 200 °C to make it 100% active (remove all water).

Deactivate aluminum oxide (8%, w/w)

Add 16 g of HPLC grade water to 184 g of Al_2O_3 in a 250 ml Erlenmeyer and shake, by hand, until all lumps are gone. Put the Erlenmeyer on a shaking table for an hour. After deactivation, leave this overnight to condition.

Activation of copper powder

Add 10 ml of 10% HCL to 0,5 – 1 g of copper powder and shake this for a few minutes. Then centrifuge for 1 minute, 300 rpm, to separate the powder from the liquid. Discard the liquid and add an amount of clean methanol. Shake again and centrifuge again. Repeat the latter step another 2 times. Dry the copper powder with a gentle stream of nitrogen. When dry, it is ready to be used, but keep it well under some hexane before use because the air will oxidize the Cu very rapidly.

Deactivate silica (40% H_2SO_4 w/w)

Add 80 g of concentrated H_2SO_4 to 120 g of silica in a 250 ml Erlenmeyer and shake, by hand, until all lumps are gone. Put the Erlenmeyer on a shaking table for an hour. After deactivation, leave this overnight to condition.

Deactivate silica (1.5%, w/w)

Add 1.5 g of HPLC grade water to 98.5 g of silica in a 250 ml Erlenmeyer and shake, by hand, until all lumps are gone. Put the Erlenmeyer on a shaking table for an hour. After deactivation, leave this overnight to condition. And always test the performance of 1,5% SiO_2 before use with real samples.

Testing 1.5% SiO_2 column material

Rinse the appropriate column for the SiO_2 with a small amount of hexane and put a small plug of silanized glass wool at the end of the column. Add 1.8 g of 1.5% SiO_2 and put ½ - 1 cm of baked-out Na_2SO_4 on top. When adding SiO_2, it is important to tick the side of the column in order to allow the SiO_2 to settle in the column. Condition the column with 4 ml of hexane. Rinse three tubes with some hexane.

Mark three tubes and weigh them. After the conditioning add 1 ml of a standard mixture on the column. After this has eluted, place tube 1 and add 14 ml of hexane; Fraction 1.
Next, place the second tube under the column and elute the column with 10 ml of hexane:diethylether (85:15 v/v); fraction 2.
After collecting the second fraction place the last tube under the column and elute with 5 ml of hexane:diethylether (85:15 v/v); Fraction 3.

Add 1 ml of iso-octane to each tube and evaporate to 1 ml under a gentle stream of nitrogen and weigh the three tubes. Homogenize the extracts and transfer them in three autosampler vials with the correct codes. The extracts are ready to be analyzed. Identification is done on the basis of retention time.

In fraction 1, we can expect PCBs and some OCPs. In fraction 2, no more than 2% of the PCBs should be present. The OCPs will be divided over the 2 fractions. In fraction 3, no more than 2% of the OCPs found in fraction 2 should be present. (This is a check of the elution volume.) See table 1 for an overview of the partition.

Some OCPs (*e.g*. HCB) will be found exclusively in the first fraction. Other OCPs (*e.g.* dieldrin) will be found exclusively in the second fraction. Other OCPs (*e.g*. pp-DDE) will be present in both the 1st and 2nd fractions and therefore must be quantified in both fractions separately and summed after quantification.

Table 2: Likely partition of the PCBs and OCPs by the two fractions

Fraction 1	Fraction 2
Pentachlorobenzene	α-HCH
Hexachlorobenzene	β-HCH
Heptachlor	γ-HCH
Aldrin	cis-Heptachlor epoixde
PCB-28	trans-Heptachlor epoixde
PCB-52	oxychlordane
PCB-101	trans chlordane
PCB-138	cis chlordane
PCB-153	α-endosulfan
PCB-180	
trans chlordane	trans chlordane
	Dieldrin
	Endrin
24-DDE	
44-DDE	44-DDE
	24-DDD
	44-DDD
24-DDT	24-DDT
44-DDT	44-DDT

When the OCPs are divided in both fractions and must therefore be quantified in both fractions separately and summed after quantification.

Now that the tests have been done, we proceed with the extraction.

Extraction

The first step of the extraction is the drying of the sample. Sediment samples can be dried by freeze or heat drying. But there is a possibility that a part of the volatile compounds (like hexachlorobenzene) will be lost. When analyzing semi-volatile compounds the best method for the drying is the use of an inert drying agent, for instance sodium sulphate. Normally for each gram of wet sample three gram of Na_2SO_4 must be added. The maximum amount of a biological sample that can be analyzed depends on the percentage of fat in the sample. The maximum allowable amount of fat that can be separated from the PCBs and OCPs with the alumina oxide column is 250 mg.

If a wet sample (*e.g*. fish tissue) is to be analyzed, place the homogenized wet sample in mortar. And add for each gram of wet sample 3 g Na_2SO_4. Grind this with the pestle to a homogeneous powder. Cover it (aluminum foil) and leave it overnight to dry further.

Transfer the dry sample to the Soxhlet thimble and add 100 µl of 100 ppb internal standard[1]. Add 175 ml of hexane:acetone (3:1, v/v) to the flask (250 ml). Boiling stones should be added for smooth boiling. Allow the extraction to continue for at least 16 hours (overnight).

Alternative method:

Transfer the dry sample to an Erlenmeyer (250 ml) and add 100 µl of 100 ppb internal standard[1]. Add 50 ml acetone (the mixture of sample and Na_2SO_4 must completely be covered with acetone) and close the Erlenmeyer. Shake or sonicate the Erlenmeyer for 8 hours, add subsequently 50 ml of hexane and shake/sonicate again for 8 hours.

Add 2 ml of isooctane as a keeper and evaporate the extract with the rotary evaporator to 3 ml. Transfer the extract to a clean tube and rinse the flask 3 times with 1 ml hexane. Evaporate under a gentle stream of nitrogen to 1 ml.

Cleanup with Al_2O_3

The cleanup of the extract starts with an alumina oxide cleanup. As mentioned earlier the maximum allowable amount of fat for this step is 250 mg.

Rinse the appropriate column with some hexane and put 15 g of Al_2O_3 on top of the frit, followed by ½ - 1 cm of Na_2SO_4. When adding Al_2O_3, it is important to tick the side of the column in order to allow the Al_2O_3 to settle in the column. Condition the column with 15 ml of pentane (or hexane if pentane is not available). Rinse a clean 250 ml round-bottom flask with some hexane.

After the conditioning of the column is finished, put the rinsed 250 ml round-bottom flask beneath the column. Transfer the sample extract to column, rinse the tube with 1 ml of pentane and transfer it to the column as soon as the sample extract has eluted. Repeat the rinse with 1 ml pentane another two times for best results. Avoid allowing the column to dry out completely during the rinsing. Hereafter elute the column with another 170 ml of pentane or hexane.

After the elution is finished add 2 ml of isooctane to the round-bottom flask and evaporate the extraction to approximately 3 ml. Transfer the extract to a clean rinsed tube and rinse the flask 3 times with 1 ml of hexane. Evaporate the extract to 1 ml with a gentle stream of nitrogen. If the extract is a soil or sediment sample the next step is a cleanup with copper powder. This cleanup step is not necessary for the other matrices.

[1] Please note that at this point a choice can be made for two systems: external standard or internal standard approach; in the following text the internal standard method is described; the external standard method would start here with a recovery standard solution of PCBs and OCPs to be taken through the entire procedure.

Cleanup with copper powder

Soil and especially sediment samples may contain sulphur. This can be removed by adding copper powder to the extract. Sulphur will be oxidized when copper is added to the extract. Before the copper powder can be used, it has to be activated.
Add the copper powder stepwise and in very small amounts to the sediment extract. When sulfur is present, the copper powder will turn black (oxidation). Repeat the copper powder addition until this reaction no longer occurs.

Fractionation

Rinse the appropriate column for the SiO_2 with a small amount of hexane and put a small plug of silanized glass wool at the end of the column. Add 1.8 g of 1.5% SiO_2 and put ½ - 1 cm of baked-out Na_2SO_4 on top. When adding SiO_2, it is important to tick the side of the column in order to allow the SiO_2 to settle in the column. Condition the column with 4 ml of hexane. Rinse two tubes with some hexane.

Mark two tubes and weigh them. After the conditioning of the column, transfer the extract on the column wait until it is eluted. Rinse the sample tube with 1 ml of hexane, place tube 1 under the column and transfer the hexane on column. Repeat this twice and add 11 ml of hexane on the column after the last rinse has eluted; Fraction 1.
Next, place the second tube under the column and elute the column with 10 ml of hexane:diethylether (85:15 v/v); fraction 2.

Add 1 ml of iso-octane to both tubes and evaporate the 2 fractions to 0.5 ml each (in case of air samples it may be necessary to evaporate until 0.1 ml) under a gentle stream of nitrogen and weigh the two tubes. Homogenize the extracts and transfer them in two autosampler vials with the correct codes. The extracts are ready to be analyzed by GC-ECD or GC-MS.

Extra clean-up with 40% H_2SO_4/silica (if necessary)

Note that not all components will survive the clean-up with 40% H_2SO_4/silica. Dieldrin, endrin, telodrin, α-endosulfan and cis-and transheptachlorepoxide will be oxidized by the 40% H_2SO_4/silica. These components should be analyzed **before** the extra clean-up.

Mark a tube and weight it. Transfer the extract from the analysis vial into the tube. Rinse the vial 3 times with 1 ml iso-octane and transfer the liquid to the tube. Evaporate to 1 ml under a gentle stream of nitrogen.

Put a small plug of silanized glass wool at the end of a pasteurspipette. Fill the pipette with 1 gram of 40% H_2SO_4/silica. Rinse the column 4 times with 1 ml hexane/dichloromethane (4:1 v/v).

Place the tube under the pipette and transfer the extract on top of the column. Rinse the original tube 2 times with 0.5 ml hexane/dichloromethane (4:1 v/v) and transfer the liquid to the column. Elute with 2 ml hexane/dichlomethane (4:1 v/v).

Evaporate the eluate to 0.5 ml (in case of air samples it may be necessary to evaporate until 0.1 ml) under a gentle stream of nitrogen and weigh the tube. Homogenize the extract and transfer it in a autosampler vial with the correct code. The extracts are ready to be analyzed by GC-ECD or GC-MS.

Preparation of the Calibration Curve

The calibration curve will be prepared from the stock solution containing the PCB/OCPs. The curve will consist of 6 levels which are obtained by different dilutions from the stock solution. Serial dilution should be avoided. These solutions will be prepared directly into autosampler vials. The concentrations are calculated by exactly weighing all stock solution additions and iso-octane.

Performing a test injection

Make sure that the correct method is loaded in *Chemstation* (or other integration/calculation program) and perform a test injection with the level 1 standard (highest concentration). Compare the response of all compounds to the response of the same level injected in the multi-level calibration of the last sequence. If both are comparable you can start the new sequence. Otherwise, try to determine what is causing the problem. Resolve this and perform a new test injection. If the deviation is more than 5%, you should prepare a new calibration curve.

Injections

If all samples are ready to be analyzed a sequence can be made. The order in which the samples are measured is as follows: First a solvent blank run (iso-octane) followed by the most diluted standard from the calibration curve and the mid-calibration curve standard. Then the blank sample(s), the reference sample(s), the real samples and the other calibration curve standards are injected at random. The last injection should be the mid-calibration curve standard again.

GC conditions

The procedure is designed for measuring the extracts on a GC equipped with an ECD detector. The settings below are optimized for an Agilent 6890 GC with a split/splitless injector.

With **helium** as carrier gas:

Oven program:

Initial temperature:	90 °C	Final temp 2:	265 °C
Initial time:	3 min	Final time 2:	5 min
Ramp 1:	30 °C/min	Ramp 3:	3 °C/min
Final temp 1:	200 °C	Final temp 3:	275 °C
Final time 1:	15 min	Final time 3:	15 min
Ramp 2:	5 °C/min	Total run time:	60 min

Injector settings:

Mode:	Pulsed splitless	Pulse time:	1.5 min
Initial temp:	250 °C	Purge flow:	55.4 ml/min
Pressure:	23.43 psi	Purge time:	1.4 min
Pulse pressure:	66 psi	Total flow:	63.5 ml/min

With **hydrogen** as carrier gas:

Oven program:

Initial temperature:	90 °C	Final temp 2:	265 °C
Initial time:	3 min	Final time 2:	4 min
Ramp 1:	38 °C/min	Ramp 3:	3.85 °C/min
Final temp 1:	200 °C	Final temp 3:	275 °C
Final time 1:	12 min	Final time 3:	15 min
Ramp 2:	6.4 °C/min	Total run time:	50 min

Injector settings:

Mode:	Pulsed splitless	Pulse time:	1.5 min
Initial temp:	250 °C	Purge flow:	55.4 ml/min
Pressure:	13,12 psi	Purge time:	1.4 min
Pulse pressure:	40 psi	Total flow:	63.5 ml/min

With **nitrogen** as carrier gas:

Oven program:

Initial temperature:	90 °C	Final temp 2:	265 °C
Initial time:	4 min	Final time 2:	6.6 min
Ramp 1:	23 °C/min	Ramp 3:	2.26 °C/min
Final temp 1:	200 °C	Final temp 3:	275 °C
Final time 1:	20 min	Final time 3:	20 min
Ramp 2:	3.8 °C/min	Total run time:	77 min

Injector settings:

Mode:	Pulsed splitless
Initial temp:	250 °C
Pressure:	15.51 psi
Pulse pressure:	70 psi
Pulse time:	1.5 min
Purge flow:	55.4 ml/min

Purge time: 1.4 min
Total flow: 63.5 ml/min

Column:
SGE Rxi5MS (60 m x 0.25 mm x 0.25 µm)

Detector settings:
ECD detector at 300 °C using the "constant makeup flow" mode (30 ml/min of N_2; this may differ per type of ECD).

Expected retention time of the POPs in the solutions EC-5495 and ES-5476 on the Rxi5MS column

	RT	Remarks
Pentachlorobenzene	12.6	
alpha-BHC	15.8	
Hexachlorobenzene	16.3	
beta-BHC	16.9	
gamma-BHC (Lindane)	17.4	
delta-BHC		Can't be analyzed with applied method
PCB28	20.3	
Heptachlor	21.2	
PCB52	22	
Aldrin	25.8	
cis-Heptachlor Epoxide	24.9	
Oxychlordane	25	
trans-Heptachlor Epoxide	25.2	
trans-Chlordane (gamma)	26.1	
2,4'-DDE	25.5	
PCB101	26	
Endosulfan I	26.7	
cis-Chlordane (alpha)	26.8	
trans-Nonachlor	27	
4,4'-DDE	27.6	
Dieldrin	27.9	
2,4'-DDD	28	
Endrin	28.9	
Endosulfan II	29.3	Can't be analyzed with applied method
4,4'-DDD	29.5	
2,4'-DDT	29.7	
cis-Nonachlor	29.9	
PCB153	30.2	
Kepone (Chlordecone)	30.5	Can't be analyzed with applied method
Endosulfan sulfate	31.3	Can't be analyzed with applied method
4,4'-DDT	31.4	
PCB138	31.7	
PCB180	35.5	

Mirex	38	

Integration and Interpretation of Chromatograms and Calculations

Before the measurements of the samples can be evaluated and calculated, first the QA and QC has to be done. Check first if the system has been stable over the entire set of analysis by doing an overlay of the middle point of the calibration curve and all the re-injections of this point during the sequence. All chromatograms should have the same responses for all peaks within a margin of 5%.
Construct a calibration curve for all compounds with Chemstation and check the linearity of the system and compare the value of the calibration curve with the values from earlier measured calibration curves for all compounds.
Calculate the recoveries of the internal standard - PCB-112 and Isodrin of the QC samples.
Calculate the blanks and check if the blanks succeed to the requirements.
Calculate for the reference sample(s) the concentrations in the sample and check if the outcome fits in the Shewart (QC) chart of the reference material.

If all the QA/QC calculations satisfy to the requirements all the measured samples can be calculated.
First calculate the recoveries of the internal standard and if it meets the requirements the individual OCPs and PCBs can be evaluated and calculated.

The detection limits must be calculated by reviewing the noise in the chromatograms next to the place where the compound elutes. The detection limit (LOD) is set as three times the height of the noise divided by the response of this compound in the lowest calibration point multiplied by the concentration of this point (in ng injected) and corrected for the recovery. All compounds found with concentrations below this limit are reported as "< detection limit". The limit of quantification (LOQ) is calculated in the same way, using ten times the height of the noise.

Re-injections

If some of the compounds in the samples are too high to fit in the calibration curve, they can be re-injected after being diluted.

Reporting

Finally all data should be reported and checked by a second technician, before it's being reported to the customer.

About the Dept. of Environment and Health, Vrije Universiteit Amsterdam

The VU's Department of Environment & Health conducts academic research and training to strengthen our understanding of the impacts of environmental contaminants on human health and the environment. Multiple disciplines such as analytical chemistry, (eco)toxicology, risk assessment, epidemiology and health sciences are brought together to both educate students and conduct world class research. The systems studied by this unique group span from the molecular level to populations, ecosystems and society.

Graduates of E&H academic programs are able to bring their fundamental understanding of the chemical contamination, toxicological effects and health aspects needed to find solutions to global health issues. E&H's researchers make use of state-of-the-art technical equipment and methodologies and their large international network to provide knowledge of the impacts of emerging contaminants to science, industry, governments and society. E&H's expertise and research output make an important contribution to the knowledge needed for informed environmental governance of the most pressing environmental pollution issues.

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ANNEX 1

PFOS analysis:

Water:

Working solutions:

- 0.1% NH_4OH in methanol: add 0.4 ml NH_4OH solution (25%) in 100 ml methanol
- 25mM NH_4Ac pH4 buffer: dissolve 190 mg NH_4Ac in 100 ml MilliQ water, adjust to pH 4 with acetic acid
- THF:MeOH (3:1 v/v)

Pretreatment:
Weigh sample: 10 to 50 ml in polypropylene tube
Add 50 µl PFOS internal standard
Centrifuge: 5 minutes max speed
Tranfer the supernatant into a new polypropylene tube.

Cleanup:
Conditioning of the SPE cartridge (Oasis WAX 6cc 150 mg 30 µm):

- 4 ml 0.1% NH_4OH in methanol
- 4 ml methanol
- 4 ml MilliQ water

Extraction:

- Load the sample on the cartridge with a flow of 1 ml/min (if necessary, apply some pressure)

Washing of the cartridge:

- 4 ml 25mM NH_4Ac pH4 buffer
- 8 ml THF-MeOH
- Apply vacuum to dry the cartridge

Elution:

- 4 ml 0.1% NH4OH in methanol
  The eluate is collected in a 15 ml polypropylene tube

Further treatment:

- Evaporate the solvent with a gentle flow of nitrogen on a waterbath at 50 °C
- Add 50 µl of PFOS injection standard to the residue in the tube
- vortex the tube
- Add 50 µl MilliQ water
- vortex the tube
- Transfer the sample in a polypropylene autosampler vial

Soil:

Pretreatment:
Weigh 5 g soil sample
Add 50 µl PFOS internal standard
Add 2 ml 0.2M NaOH and mix carefully
Wait 30 minutes
Add 20 ml methanol
Shake for 30 minutes @ 300 spm
Centrifuge 10 minutes max speed
Transfer the methanol in a 50 ml polypropylene tube
Repeat the extraction with 10 ml methanol
Combine both methanol fractions
Evaporate until approx 5 ml with N_2 on a waterbath at 50 °C
Add 0.2 ml 2M HCl
Add 30 ml NH_4Ac pH4 buffer and mix
Check the pH: should be < pH5!

Cleanup:
Conditioning of the SPE cartridge (Oasis WAX 6cc 150 mg 30 µm):

- 4 ml 0.1% NH_4OH in methanol
- 4 ml methanol
- 4 ml MilliQ water

Extraction:

- Load the sample on the cartridge with a flow of 1 ml/min (if necessary, apply some pressure)

Washing of the cartridge:

- 4 ml 25mM NH_4Ac pH4 buffer
- 8 ml THF-MeOH
- Apply vacuum to dry the cartridge

Elution:

- 4 ml 0.1% NH4OH in methanol
  The eluate is collected in a 15 ml polypropylene tube

Further treatment:

- Evaporate the solvent with a gentle flow of nitrogen on a waterbath at 50 °C
- Add 50 µl of PFOS injection standard to the residue in the tube
- vortex the tube
- Add 50 µl MilliQ water
- vortex the tube
- Transfer the sample in a polypropylene autosampler vial

Biota:

Pretreatment:
Weigh: 1 g of sample in a 15 ml polypropylene tube
Add 50 µl PFOS internal standard
Add 3g Na_2SO_4
Mix the sample with a spatula
Leave overnight at room temperature

Extraction:
Add 10 ml methanol
Shake 30 minutes @ 300 spm
Centrifuge 10 minutes @ max speed
Transfer the methanol in new polypropylene tube
Repeat extraction with 5 ml methanol
Combine both methanol fractions
Evaporate methanol until approx 5 ml with N_2 on a waterbath at 50°C

Cleanup:
Conditioning of the SPE cartridge (Oasis WAX 6cc 150 mg 30 µm):

- 4 ml 0.1% NH_4OH in methanol
- 4 ml methanol
- 4 ml MilliQ water

Extraction:

- Load the sample on the cartridge with a flow of 1 ml/min (if necessary, apply some pressure)

Washing of the cartridge:

- 4 ml 25mM NH_4Ac pH4 buffer
- 8 ml THF-MeOH
- Apply vacuum to dry the cartridge

Elution:

- 4 ml 0.1% NH4OH in methanol
  The eluate is collected in a 15 ml polypropylene tube

Further treatment:

- Evaporate the solvent with a gentle flow of nitrogen on a waterbath at 50 °C
- Add 50 µl of PFOS injection standard to the residue in the tube
- vortex the tube
- Add 50 µl MilliQ water
- vortex the tube
- Transfer the sample in a polypropylene autosampler vial

LC-MS analysis:

HPLC Gradient:

Time (minutes)	5 mM NH_4CHO_2 (%)	Methanol (%)	Flow (ml/min)
0	65	35	0.3
2	65	35	0.3
16	5	95	0.3
22	5	95	0.3
22.5	65	35	0.3
27	65	35	0.3

QQQ settings:

Compound name	Precursor Ion	Product Ion	Polarity
13C8 PFOS	507	99	negative
13C8 PFOS	507	80	negative
13C4 PFOS	503	99	negative
13C4 PFOS	503	80	negative
PFOS	499	130	negative
PFOS	499	99	negative
PFOS	499	80	negative

ANNEX 2

HBCD analysis:

The extraction is done similar to the extraction of the PCB/OCPs on page 12.

Pretreatment:
Prepare silica with 40% sulphuric acid. Shake until no lumbs are present and leave overnight.
Prepare a hexane:dichloromethane mixture of 7:3 v/v

Cleanup part 1:
Prepare a glass column with 15 g of the sulphuric acid silica and add about 1 cm of Na_2SO_4 on top

Pre-rinse the column with 25 ml of hexane
Add the extract to the column and rinse the vial 3 times with 1 ml of hexane:DCM (7:3 v/v)
Elute the column with 147 ml of hexane:DCM (7:3 v/v)
Add 1 ml of iso-octane and evaporate until approx. 1 ml

Cleanup part 2:
SPE cartridge: Discovery DSC-NH_2 500mg (52637)

Condition the cartridge with 4 ml hexane
Transfer the sample (1 ml) to the cartridge
Wash the cartridge with 4 ml hexane (if you collect all 5 ml this contains all BDEs)
Place a clean tube under the cartridge and elute the HBCDs from the column with 10 ml of acetone.

Evaporate the sample with a gentle flow of nitrogen on a waterbath at 30 °C.
Reconstitute the sample in 200 µl MeOH:MilliQ water (3:1 v/v).

LC-MS analysis:

HPLC Gradient:

Time (minutes)	**ACN:MeOH (75:25 v/v) (%)**	**2 mM NH_4Ac (%)**	**Flow (ml/min)**
0	85	15	0.3
8	85	15	0.3

QQQ settings:

Compound name	**Precursor Ion**	**Product Ion**	**Polarity**
13C12 HBCD	652.7	81	negative
13C12 HBCD	652.7	79	negative
HBCD	640.7	81	negative
HBCD	640.7	79	negative