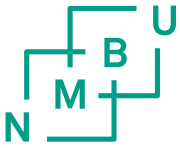
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A Quantitative Analysis of Organochlorine Pesticides in Svalbard Reindeer *(Rangifer tarandus platyrhynchus)*

Kirsten Husby Melien

Picture: Kirsten Husby Melien

Acknowledgments

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Kirsten Husby Melien

Ås, December 2014

Sammendrag

Denne studien omhandler kvantitativ analyse av utvalgte organoklor-pesticider som befinner seg i muskelvev, lever, og fettvev hos Svalbardrein (*Rangifer tarandus platyrhynchus*) på Svalbard (61 022 km^2, 74-81°N, 15-35°Ø), Norge. Studien inneholder også en kort sammenlikning med Svalbardrype (*Lagopus muta hyperborea*) og kortnebbgås (*Anser brachyrhynchus*). Prøvene ble innhentet av lokale jegere. Utvinnings- og opprensingsprosedyre bestod av ekstraksjon på kald kolonne, syrebehandling og silica gel, før analyse ved bruk av gasskromatografi-massespektrometri. HCB ble kvantifisert i de fleste prøvene, hvor majoriteten av resultatene inneholdt lavere verdier enn rapportert i tilsvarende studier. Verdiene viste 0,1-1,3 ng/g våtvekt i muskelvev, 1,7-7,0 ng/g våtvekt i fettvev og ett resultat viste 10,2 ng/g våtvekt i lever i Svalbardrein. I kun tre prøver ble *p,p'*-DDE kvantifisert, og ble funnet i enda lavere konsentrasjoner. Verdien viste 0,1-1,8 ng/g våtvekt. Andre utvalgte pesticider var under deteksjonsgrensen. Resultatene i denne studien er noe usikre siden linearitetsområdet av interstandard og kvantifiseringsstandard viste for lave verdier, og gjennvinningsstandarden presterte ikke optimalt.

Abstract

This study is a quantitative analysis targeting levels of selected organochlorine pesticides (OCP) located in muscle, liver, and fat of Svalbard reindeer (*Rangifer tarandus platyrhynchus*) in Svalbard (61 022 km^2, 74-81°N, 15-35°E), Norway. A brief comparison to Svalbard rock ptarmigan (*Lagopus muta hyperborea*) and pink-footed goose (*Anser brachyrhynchus*) is included. Samples were collected by local hunters. Extraction and clean-up procedures included cold column extraction, acid treatment, and silica gel, before gas chromatographic separation and mass selective detection analysis. HCB was quantified in most samples with the majority of the results at lower values than reported in comparing studies; ranging 0.1-1.3 ng/g ww for muscle, 1.7-7,0 ng/g ww for fat, and one result of 10.2 ng/g ww for liver in Svalbard reindeer. At even lower values, *p,p'*-DDE was quantified in three samples only, ranging 0.1-1.8 ng/g ww. Other target pesticides were <LOQ. Results from this study have a degree of uncertainty as the linear range of the internal standard and the quantification standard contained too low concentrations and the recovery standard did not demonstrate optimal performance.

Abbreviations

AEPS	Arctic Environmental Protection Strategy
AMAP	Arctic Monitoring and Assessment Programme
CAFF	The Conservation of Arctic Flora and Fauna program
CAS	Chemical Abstract Service
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
EI	Electron Ionization
GC	Gas Chromatography
GPC	Gel Permeation Chromatography
He	Helium
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
H_2SO_4	Sulfuric acid
ID	Inner diameter
LOD	Limit of Detection
LOQ	Limit of Quantification
LRTAP	Convention on Long-range Transboundary Air Pollution
m/z	Mass-to-charge ratio
MRL	Maximum Residue Level
MS	Mass Spectrometry
N	Total number of
N_2	Nitrogen
n/a	Not available
NaOH	Sodium hydroxide
Na_2SO_4	Sodium sulfate
NMBU	The Norwegian University of Life Sciences
OCN	Octachloronaphtalene
OCP	Organochlorine Pesticides
o,p'	*ortho*,-, *para'*-substitution
PAME	Protection of the Arctic Marine Environment working group
p,p'	*para*,-, *para'*-substitution
POPs	Persistent Organic Pollutants

R^2	Regression coefficient
RRF	Relative Response Factor
RT	Retention Time
SB	Spiked Blank
SDU	Sustainable Development and Utilization
SIM	Single Ion Monitoring
S/N	Signal-to-noise
SOP	Standard Operating Procedure
UNECE	United Nations Economic Commission for Europe
UNIS	The University Centre in Svalbard
v/v	Volume/Volume
ww	Wet Weight

Key words

POPs, OCPs, Svalbard reindeer, Svalbard rock ptarmigan, pink-footed goose, muscle, liver, fat, Arctic, Svalbard, Longyearbyen

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1. Introduction

1.1 Aim of study

This study is a quantitative analysis targeting levels of selected persistent organic pollutants (POPs) analyzed and examined in Svalbard Reindeer (*Rangifer tarandus platyrhynchus*) in Svalbard (61 022 km^2, 74-81°N, 15-35°E), Norway. There will be presented a brief comparison to Svalbard rock ptarmigan (*Lagopus muta hyperborea*) and pink-footed goose (*Anser brachyrhynchus*).

The Svalbard environmental act § 24 claims "Flora and fauna on land and in the sea shall be managed in such manner that the natural productivity, diversity, and habitats are maintained, and Svalbard's wilderness protected for future generations" (Svalbard Environmental Protection Act 2001). Persistent pollutants travel long distances to reach Svalbard do not cause an immediate effect, but are harmful over time. It is of global interest to have decreasing levels of harmful chemicals in the environment. This study will contribute with new results aiming for increased knowledge about the distribution and levels of POPs in the terrestrial environment in Svalbard.

"Monitoring the levels of legacy POPs in the Arctic is of interest because it provides information about environmental degradation and fate and the impact of policy decisions. It may also give an indication of how other factors, including climate change, may influence the levels in the environment. Moreover, information about legacy POPs is important in assessing the combined effects of different pollutants on wildlife and human health" (AMAP 2009 p. 26).

1.2 Persistent organic pollutants

The Arctic is a region with a delicate ecosystem. Small changes can have large impacts on vegetation and wild life, and as the world changes, the Arctic is in need of protection. In Finland, in 1991, a non-binding environmental protection agreement was founded among the eight Arctic nations: Canada, Greenland/Denmark, Finland, Iceland, Norway, Russia, Sweden, and the United States called the Arctic Environmental Protection Strategy (AEPS). The AEPS had three primary focus areas; (1) past Arctic Ocean dumping of radioactive and other hazardous materials as potential threats to human health and the environment, (2) bilateral and multi-lateral assistance to clean-up and manage present and future problems, and (3) scientific findings of abnormally high levels of POPs and heavy metals in Arctic indigenous people and their food sources likely to come from industrial nations in the

northern hemisphere (Emergency Prevention Prepareness and Response s.a.). Five programs were then established for the AEPS; (1) the Arctic Monitoring and Assessment Program (AMAP), (2) the Conservation of Arctic Flora and Fauna program (CAFF), (3) Protection of the Arctic Marine Environment working group (PAME), and (5) Sustainable Development and Utilization (SDU).

AMAP was established in 1991 to monitor identified pollution risks and their impacts on Arctic ecosystems (AMAP 2009). Information obtained from AMAP is useful in documenting trends and in showing whether persistent substances are accumulating in the Arctic, assisting further evaluation and development of the protocols on POPs to the United Nations Economic Commission for Europe (UNECE), Convention on Long-range Transboundary Air Pollution (LRTAP Convention), and the Stockholm Convention on Persistent Organic Pollutants (AMAP 2009).

The Stockholm Convention is a global treaty entered into force in 2004 aiming to reduce or eliminate intentional or unintentional production of POPs and target listing of new POPs merging to protect human health and the environment. Given by the Stockholm Convention, POPs are defined as (1) carbon-based organic chemical substances with physical and chemical properties that enable them to remain intact for a very long time, (2) widely distributed throughout the environment, (3) accumulate in fatty tissue of living organisms, and (4) are toxic to humans and wildlife when released in nature (Stockholm Convention s.a.-c).

1.3 Long Range Transport

The Arctic receives contaminants from sources far outside the Arctic region (AMAP 2009). POPs emerge in the Arctic by a combination of increasing urbanization in high latitude areas and atmospheric transport from agricultural areas from lower latitudes (Hoferkamp et al. 2010). The most rapid route of transport for POPs to the Arctic is via the atmosphere (AMAP 2014), where they act as mere passengers in the atmospheric winds (Levy II 1990). Weather systems transport airborne contaminants, while contaminants that partly dissolve in water follow a much slower route following ocean currents (AMAP 2009). The remoteness of the Arctic makes it largely free from direct inputs of industrial and agricultural chemicals (Hoferkamp et al. 2010).

In the Arctic, POPs have been detected in samples of air, water and ice, soil and sediment, and biota (AMAP 1997). In recent years, several of the POP compounds have shown a slight decrease in air levels; however, there are no new data available for terrestrial components

(AMAP 2014). An overview of legacy POPs temporal trend provided by Rigét et al. (2010) also show a general decreasing trend of POPs in biota

AMAP (2009 p. 5) states that "the only long-term solution to the high levels of POP in the Arctic is to reduce the emission of POPs into the environment".

1.4 Target organisms

This study targets animals in the second trophic level, terrestrial herbivores. The main focus is Svalbard reindeer. A few specimen of Svalbard rock-ptarmigan and pink-footed goose were included for comparison. Samples from all animals in this study were provided by local hunters in Svalbard, from areas near Longyearbyen (78°13'N, 15°38'E). Samples of Svalbard reindeer were collected through the project "Hunting for POPs in School" (Carlsson & Kallenborn 2012).

Svalbard reindeer, Svalbard rock-ptarmigan, and pink-footed goose are all part of human diet in Svalbard, where the Governor of Svalbard allocates annual quotas to local hunters (The Governor of Svalbard s.a.).

Previous studies of POPs in terrestrial herbivores in the Arctic have shown general low values of pollutants (e.g. Hassan et al. 2013; Pollock et al. 2009; Vorkamp et al. 2004). Concentrations of POPs increase with trophic status (biomagnification), so predators will in general contain higher concentrations of pesticides than prey, and from this experience greater harm from the toxins (Gill & Garg 2014; Zitko 2003). This is why top predators in both from the marine and the terrestrial food web such as various species of seals and whales, polar bears (*Ursus maritimus*), and humans have been more frequently studied than terrestrial herbivores.

In terrestrial ecosystems, contaminants are selectively taken up by microorganisms and plants from water, sediments, and soil (AMAP 1997). Food supplies for terrestrial herbivores in Svalbard are limited, as only about 13% of the land area is covered by vegetation, and the rest by 27% barren rock, and approximately by 60% glaciers (Hisdal 1985). For reasons just mentioned, biota in lower trophic levels is expected to contain low level of POPs. Pink-footed geese, however, are migrating during winter, and AMAP (1997) states that some species contain large metal and POP burdens from overwintering at lower latitudes and deliver these to the Arctic on their return in the summer.

1.4.1 Svalbard reindeer

Hunting season takes place August 15th to September 20th for Svalbard reindeer; varying from 117 to 235 animals trapped annually from 1983 to 2012 (Pedersen & Bårdsen 2014). Representation of the hunting areas for Svalbard reindeer given by the Governor of Svalbard is presented in figure 1.1. In this study, samples were collected from five of the six hunting areas presented, all listed in appendix 1.

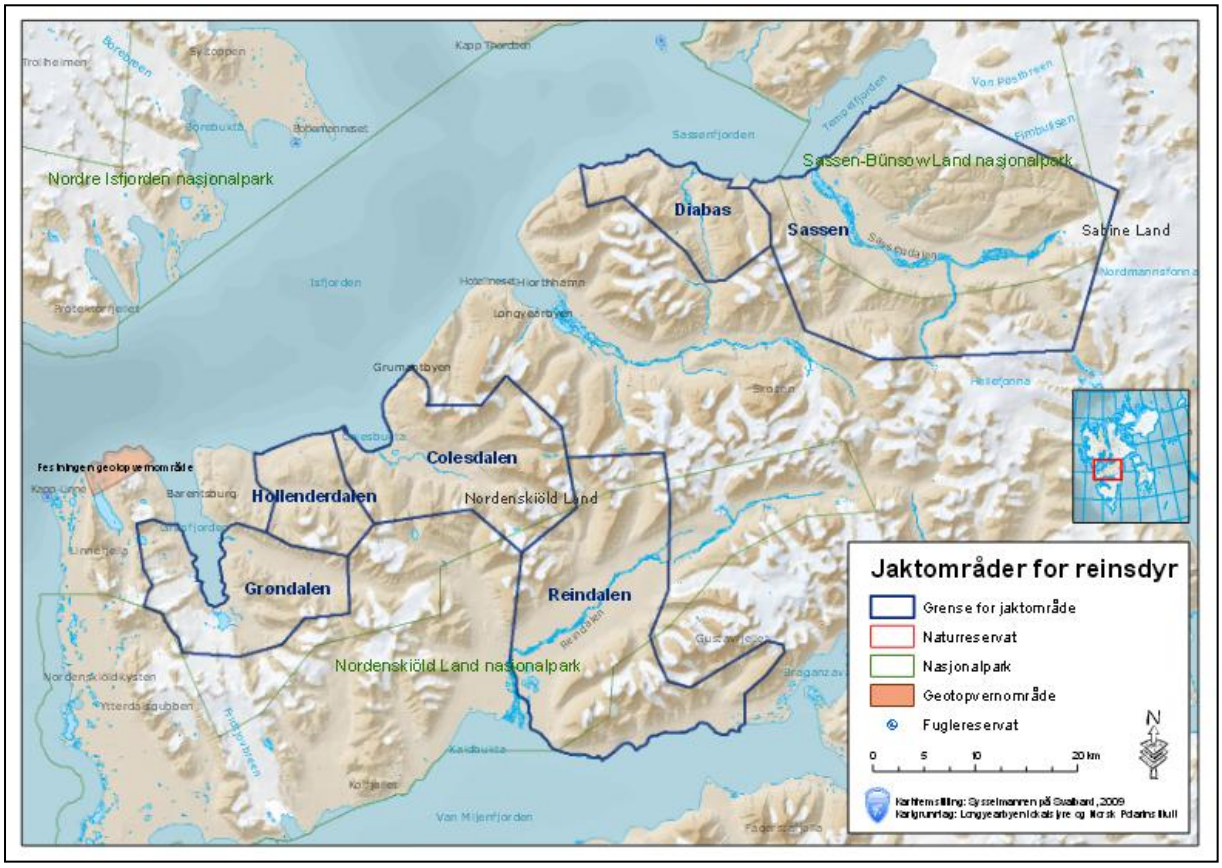


Figure 1.1 Presentation of hunting areas for reindeer (blue color), given by the Governor of Svalbard

Svalbard reindeer is an endemic species and can only be found here. There is no accurate count of the population size on the whole of Svalbard; however, the population size is under close monitoring in Adventdalen, Reindalen, and the Brøgger peninsula (MOSJ s.a.-b). Numbers from 2013 indicate about 130 Svalbard reindeer in Adventdalen, 800 in Reindalen, and 1200 at the Brøgger peninsula (MOSJ s.a.-b). Due to these close monitorings in bounded areas, as well as additional observations, it is reason to believe there are over ten thousand Svalbard reindeer in total (cf. Punsvik 2009).

The Svalbard reindeer's summer and winter diet consists of any plants available to them (Bjørkvoll et al. 2009). They are not subject to predation, except for rear killings by polar bear

(Brage Bremset Hansen 2011). Svalbard reindeer has on average about 17 % body fat (Schytte Blix 2005), but adds on up to 30 % before winter (Fuglei & Aanes s.a.). Towards the end of winter, females and calves will have lost about 44 % of their maximum autumn total body weight, while males will have lost about 55 % (Reimers 1984). In body fat alone, calves will have lost 97 % and adults 90 % (Reimers 1984).

1.4.2 Svalbard rock ptarmigan

Hunting season takes place September 10th to December 23rd; varying from about 500 to just over 2000 animals trapped annually from 1997 to 2013 (MOSJ s.a.-a). The samples from Svalbard rock-ptarmigan provided for this study were collected in Bolterdalen (78°08'N, 14°59'E) presented in figure 1.2.

Figure 1.2 Sample collection site for Svalbard rock ptarmigan

Svalbard rock ptarmigan resides in the archipelago, and is the only land-inhabiting bird which resides throughout the year in Svalbard (Fuglei & Pedersen s.a.). The population size is estimated 2.4 males per km^2 (Fuglei & Pedersen 2008). They are herbivores and their diet consists of various crops depending on the season; i.e. *Saxifraga oppositifolia* and *Saxifraga cespitosa* during winter, *Salix Polaris* during spring, and *Polygonum viviparum* during summer and fall (Pedersen et al. 2005). It has been observed that Svalbard rock ptarmigan also co-feed with Svalbard reindeer in the feeding craters excavated by reindeer in search of food during the winter (Pedersen et al. 2006).

The body weight of Svalbard rock ptarmigan shifts during the year. Their body weight increase due to fat accumulation from September-October until they peak in November-December when they may exceed 30 % body fat (Pedersen et al. 2005). Even though the birds

double their food intake from February to March, they are almost fat free in April (Pedersen et al. 2005).

1.4.3 Pink-footed goose from Svalbard

Hunting season takes place August 20th to October 31st. Recently, about 2000 pink-footed geese were trapped in the Norwegian mainland, about 6000 in Denmark, and a few hundred in Svalbard (Madsen & Tombre s.a.). There was no information provided where the pink-footed goose samples were collected for this study other than "near Longyearbyen".

There are two populations of pink-footed goose; one in Iceland/East Greenland wintering in the British Isles and one in Svalbard staging in Norway and wintering in Denmark, the Netherlands, and Belgium (Madsen & Williams 2012). The population size of pink-footed goose in Svalbard is about 81,600 birds (Madsen et al. 2013). The average exchange of individuals between the two populations is calculated 0.7 % per year (Madsen et al. 1999).

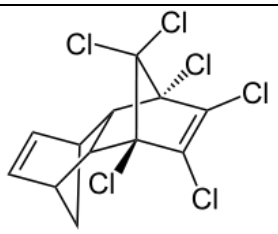
When in Svalbard, pink-footed geese feed on a wide range of different plant species utilizing snow-free areas of the tundra (Speed et al. 2014). But these birds do not reside in Svalbard all year; the birds depart from Svalbard around mid-September and return around mid-May (Madsen & Williams 2012). When pink-footed geese are in North-West Europe, the preferred spring habitat is in newly sown fields where they feed on grain (Madsen 1985). Flocks commute between open water roosts and farmland feeding at dusk and dawn (Crossley & Couzens 2014). As they are herbivores, they will also settle on grasslands as a feeding source, as they need grain as a source for carbohydrates and grass as a source for proteins (Ødegaard 2013).

Pink-footed geese does not experience significant weight shift throughout the year like Svalbard rock ptarmigan does as they are leaving winter for warmer climate, however, they do put on weight before spring migration (Aagaard 2014).

1.5 Compounds in the study and their effects on biota

POPs are categorized as pesticides, industrial chemicals, or by-products (Stockholm Convention s.a.-a). This study targets selected compounds of organochlorine pesticides (OCPs) presented in table 1.1.

Table 1.1 Presentation of selected OCPs in this study. Information includes common name, Chemical Abstract Service (CAS) registry number, molecular formula, and molecular structure of each compound respectively.

Aldrin	
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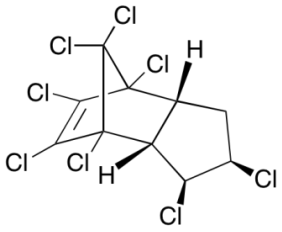
***cis*-Chlordane**	
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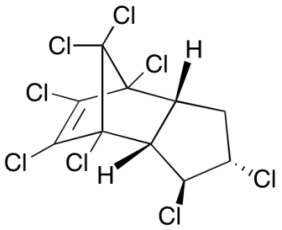
***trans*-Chlordane**	
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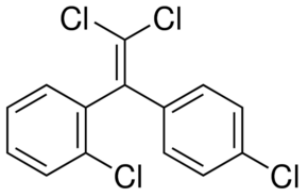
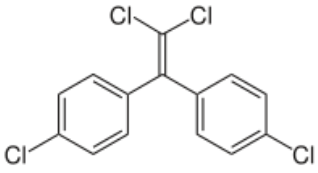
$C_{10}H_6Cl_8$	
***ortho,para'*-Dichlorodiphenyldichloroethylene (*o,p'*-DDE)**	
3424-82-6	
$C_{14}H_8Cl_4$	
***para,para'*-Dichlorodiphenyldichloroethylene (*p,p'*-DDE)**	
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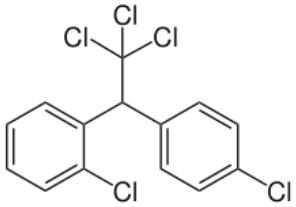
***ortho,para'*-Dichlorodiphenyltrichloroethane (*o,p'*-DDT)**	
789-02-6	
$C_{14}H_9Cl_{15}$	

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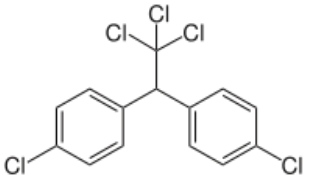
***para,para'*-Dichlorodiphenyltrichloroethane (*p,p'*-DDT)**	
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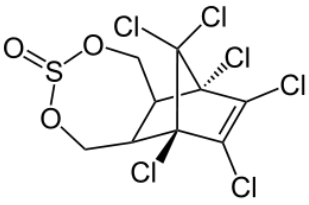
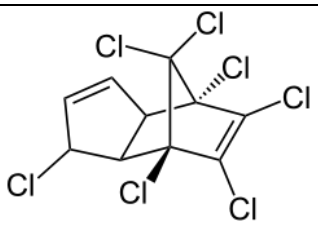
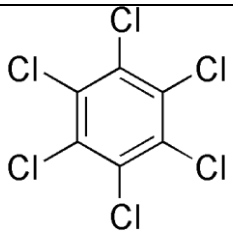
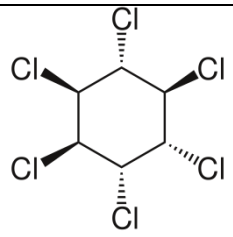
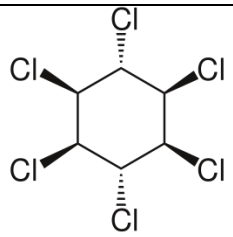
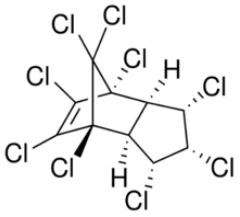
***alpha*-Endosulfan (α-Endosulfan)**	
959-98-8	
$C_9H_6Cl_6O_3S$	
Heptachlor	
76-44-8	
$C_{10}H_5Cl_7$	
Hexachlorobenzene (HCB)	
118-74-1	
C_6Cl_6	
***alpha*-Hexachlorocyclohexane (α-HCH)**	
319-84-6	
$C_6H_6Cl_6$	
***gamma*-Hexachlorocyclohexane (γ-HCH) (Lindane)**	
58-89-9	
$C_6H_6Cl_6$	
***cis*-Nonachlor**	
5103-73-1	
$C_{10}H_5Cl_9$	

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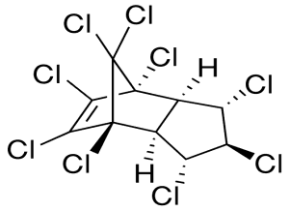
***trans*-Nonachlor**	
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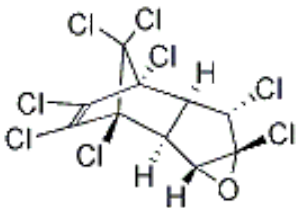
$C_{10}H_5Cl_9$	
Oxychlordane	
27304-13-8	
$C_{10}H_4Cl_8O$	

OCPs started being commercially used in the 1940s. “Although, pesticides were used initially to benefit human life through increase in agricultural productivity and by controlling infectious disease, their adverse effects have overweighed the benefits associated with their use” (Gill & Garg 2014 p. 210). Now, Aldrin, chlordane, DDT, heptachlor, and HCB are listed among the 12 initial POPs by the Stockholm Convention, while α-endosulfan, α-HCH, and Lindane are listed among the new nine POPs. All compounds are on the elimination list banned from production, with a few exceptions; DDT is acceptable for disease vector control (mainly malaria-carrying mosquitoes) (Stockholm Convention s.a.-a), HCB is unintentionally released as a by-product of manufacturing certain industrial chemicals (Stockholm Convention s.a.-a), and γ-HCH is allowed as pharmaceutical second line treatment for head lice and scabies (Stockholm Convention s.a.-b). DDE is a toxic and persistent breakdown product of DDT, as nonachlor and oxychlordane are of chlordane.

Common properties of OCPs are low solubility in water and high solubility in lipids (Zitko 2003). OCPs have been used to control e.g. insects, termites, rodents, weeds, and fungi in agriculture. As mentioned previously, POPs can have a toxic effect on terrestrial biota. The general toxic effects of OCPs include a number of chronic health issues as a result of a prolonged presence of pesticides in biota (Zitko 2003). Long-term impacts include e.g. reproductive harm, harm of the immune system and nervous system, endocrine disruption, and cancer (AMAP 2009; Hotchkiss et al. 2008; WHO s.a.) For birds, pesticides have the potential to alter feeding behavior, reproduction, failure to regulate body temperature, and eggshell thinning (Gill & Garg 2014). As described in 1.2, POPs accumulate in fatty tissue of living organisms.

Usually persistency of pesticides is presented as soil half-life measured in days. Presented by Vouge et al. (1994), soil half-life of OCPs in this study range from 50 days (endosulfan) to 2000 days (DDT). As pesticides and other POPs reach the Arctic through LRT, the half-life is generally monitored in air and no longer soil. OCPs in Arctic air have a general half-life between 3-16 years (AMAP 2009). Half lives are however difficult to define in environmental context and can only be used as an indication of persistence (Zitko 2003).

1.6 Method selection

The method was chosen and validated based on physical-chemical properties of the target chemicals, expenses, availability, level of sophistication, robustness/ reliability, quality (recovery, uncontrolled loss, repeatability etc.), sensitivity and selectivity, comparability, and time consumption.

Choice of sample clean-up and analysis was based on validated methods and Standard Operating Procedure (SOP) (Andreassen 2009; Carlsson & Halse 2012). Clean-up was necessary in order to remove target compounds from a biological matrix to eliminate interferences during analysis. Analysis consisted of gas chromatographic separation and mass selective detection. All sampling occurred in field in Svalbard and laboratory work and analysis was performed at the University Centre in Svalbard (UNIS).

2. Materials and methods

2.1 Sample collection and preparation

Samples of liver, muscle, and fat from Svalbard reindeer, Svalbard rock ptarmigan, and pink-footed goose were collected by local hunters in areas close to Longyearbyen during fall 2010.

Each sample was taken on site, bagged separately in plastic or aluminum foil, and stored at -20 °C at UNIS prior to analysis. There were no field blank provided for any of the samples.

In total, there were samples taken from eight Svalbard reindeers, two Svalbard rock ptarmigans, and two pink-footed geese, listed in appendix 1. From each animal, there was one replica of muscle, liver, and fat provided. From the Svalbard reindeer, all muscle samples were cut from the cheek of the animal. Fat samples from Svalbard reindeer, and muscle and fat samples from Svalbard rock ptarmigan and pink-footed goose were chosen by the hunters, and additional information was not available.

All apparatus and reagents used for sample preparation, as well as cleaning procedures and pre-treatment procedures and are listed in appendix 2.

2.2 Homogenization

Sample preparation was performed at UNIS during March 2012. All samples were thawed in room temperature and then homogenized in a Wilfa kitchen hand blender. Approximately 5 g tissue material was used from muscle and liver and 3 g from fat; one replica from each. In order to retract all moisture from the samples, solid sodium sulfate (Na_2SO_4) (pre-treated at 450 °C for 6 hours) was weight in and added to the blender just before the samples. The liver samples contained most moisture, so about 70-85 g Na_2SO_4 was added to each sample. About the tenfold of the sample amount of Na_2SO_4 was used for each muscle sample (50-60 g), and each fat sample (30 g).

The lid of the blender did not close properly, so parafilm was used to secure it. The blades of the blender did not reach completely down to the bottom, nor to the sides, so it had to be shaken manually in all directions (excluding up-side-down) while homogenizing the sample with the Na_2SO_4.

The homogenate was weighed in, packed in aluminum foil, and left over night in the freezer at –20 °C. To check whether the samples still contained any water, the weight should not differ more than 5 %.

2.3 Cold column extraction

A cold column extraction by gravitation chromatography was performed to retract all lipophilic compounds from the samples. The homogenate was transferred to a glass column (length 80 cm, inner diameter (ID) 15 mm, Merck, Darmstadt, Germany) and 25 µl of 0.2 ng/µl ^{13}C *p,p'*-DDE internal standard was added directly on column for all samples. 50 ml 1:1 volume/volume (v/v) cyclohexane/acetone was added and the columns were kept closed for 60 minutes before the cyclohexane was allowed to run through without drying the column. This step was repeated three times. The samples were collected in TurboVap®-glasses and two drops of 2,2,4-Trimethylpentane (isooctane) were added as a keeper. The volume was reduced to 1 mL by TurboVap® at 38 °C, medium fan speed. The samples were transferred into 4 mL vials, filled up with cyclohexane to about 2 mL, and numbered as listed in appendix 1. 200 µl from each sample of fat and 400 µl of each sample of liver and muscle were removed in order to calculate extracted organic matter (EOM). EOM was then estimated by

letting all new vials dry over night at room temperature, and compare weight of vial before and after.

2.4 Acid treatment

To remove lipids and organic matrix from the sample, acid treatment is an effective clean-up method as it can remove over 90 % of lipids in a sample (Zhao et al. 2005). Due to the initial volume in the vials, each sample was divided into two10 mL vials, and sulfuric acid (H_2SO_4) was added to each vial with approximately equal volumes sample/acid. The acidified extracts were shaken by hand and left over night to let the two phases separate. The top layer was transferred into a new vial, and the process was repeated until both phases were clear colored; five repetitions for the fat samples, and four repetitions for the rest. After this process, each divided sample was combined in a single new vial. End volume was reduced under a gentle stream of nitrogen gas (N_2) by Reacti-Vap^{TM} Evaporator without any added heat from about 5 mL to 2 mL. 5 drops of isooctane were added as a keeper.

2.5 Silica gel clean-up

Silica gel gravitation chromatography was chosen to clear sample of hydrophilic matter. For the silica gel, the same columns were used as for the cold column extraction. 2 g of activated silica (pre-treated at 450 °C for 6 hours) were added to each column and a layer of approximately 0.5 cm Na_2SO_4 was added on top of the silica. The columns were conditioned with 10 mL cyclohexane. Each sample was added, and 12 mL of a 3:1 (v/v) cyclohexane/DCM mixture was used for the column extraction. The samples were collected in TurboVap® glasses and the volume was reduced to 0.5 mL. Two drops of isooctane were used as a keeper during volume reduction. Each sample was transferred to 4 mL vials.

In order to remove all DCM from the samples, the volume in each vial was reduced to approximately 200-300 µl three times under a gentle stream of N_2 by Reacti-Vap^{TM} Evaporator without any added heat. Two drops of isooctane were added as a keeper. After final volume reduction, each 4 ml vial were filled half full with cyclohexane.

2.6 Transport

The samples were transported in a sealed box to the Norwegian University of Life Sciences (NMBU) for quantitative analysis. Each sample was weighed in before and after transportation, to see if any volume changes had occurred. Prior to analysis, the volume of each sample was again reduced under a gentle stream of N_2 gas using a Reacti-Vap^{TM} Evaporator without any added heat. Two drops of isooctane were used as a keeper and the

volume of the samples was reduced in their vials and transferred to pointy vials for analysis. Due to malfunction of the GC-MS at NMBU, all samples had to be re-packed and air mailed back to UNIS for analysis in November 2012. Before transportation, the final volume was approximately 150 mL for each sample and the caps were screwed on tight and secured with parafilm.

At UNIS the volumes of the samples were again reduced with N_2 gas in the vials to about 25 µl, and 25 µl octachloronaphtanene (OCN) with a 200 pg/µl concentration was added as recovery standard before running the samples at the GC-MS.

2.7 Quality assurance and quality control

As quality assurance and quality control, there were provided blank samples, spiked blank samples, and linearity tests for internal standard and quantitative standard.

2.7.1 Method Blank samples

Three method blank samples were provided labeled Blank 1, Blank 2, and Blank 3. These samples were made from pre-cleaned Na_2SO_4, and followed the same preparation procedure in the laboratory as all biota samples, starting from cold column extraction. 25 µl of 0.2 ng/µl ^{13}C *p,p'*-DDE internal standard was added directly on column to each blank sample.

2.7.2 Spiked Blank samples

There were provided two spiked blank (SB) samples labeled SB1 and SB2. These samples were also made from pre-cleaned Na_2SO_4, and followed the same preparation procedure in the laboratory as all biota samples, starting from cold column extraction. 25 µl of 0.2 ng/µl ^{13}C *p,p'*-DDE internal standard and 50 µl quantitative standard of ^{12}C OCP components of 0.2 ng/µl (OCP mix of aldrin, *p,p*-DDE, *p,p*-DDT, α-HCH, and trans-nonachlor) from stock solution listed in appendix 3 were added directly on column for both spiked blank samples.

2.7.3 Recovery standard

As a recovery standard, 25 µl OCN with concentration of 200 pg/µl was added to each sample just before analysis.

2.7.4 Linearity test

There were made two linearity tests, one for internal standard and one for quantitative standard. There was also made a linearity comparison of recovery standard OCN and internal standard ^{13}C *p,p'*-DDE.

Based on SOP and expected low contamination, it was desired to create a linearity test ranging from 25 pg/µl to 200 pg/µl. Since the OCN mix had concentrations from 308 pg/µl to 2154 pg/µl, there were made two linearity tests, one for the higher concentrations and one for the lower concentrations. The complete list of linearity tests with concentrations is listed in appendix 3.

2.8 Analysis

The Gas chromatographer (GC) (TRACE™ Ultra GC, Thermo Fisher Scientific, Waltham, MA, USA) was equipped with a 5 m pre-column (0.53 mm ID; Agilent Technologies) and a 30 m DB5-MS+DG column (0.25 mm ID and 0.10 µm film thickness; Agilent Technologies).

Helium (He) (6.0 quality, Hydrogas, Porsgrunn, Norway) was used as carrier gas at a flow rate of 1.5 mL/min.

The Mass spectrometer (MS) (PolarisQ ion trap, Thermo Fisher Scientific, Waltham, MA, USA) was operated in electronic ionization (EI) mode with single ion monitoring (SIM). Ionization energy was 70 eV. The transfer line temperature was held at 275 °C and the source temperature was set to 200 °C. Mass range 141.25 to 409.75.
The injection was performed on-column with an injection volume of 1 µl for the linearity test and 2 µl for the sample run. The transfer line temperature was 275 °C and the source temperature was 200 °C.

Temperature program was set to start at 70 °C hold 3 min, 15 °C/min up to 180 °C, hold 0 min, and 5 °C/min up to 280 °C, hold 7 min.

For quantification work, Xcalibur™ (version 2.0.7, Thermo Scientific, Waltham, MA, USA) was used as software program.

3 Results

3.1 Retention time, signal to noise, and masses

In order to know if the target OCP compounds in fact are the correct compounds, and not e.g, impurities or noise, there is a need to set identification criteria. This is to improve the accuracy of the method.

The following validation criteria were chosen:

(1) The retention time (RT) must be ± 0.1 min compared to standard run

(2) Signal to noise (S/N) >3 = analyte is above limit of detection (LOD)
S/N > 10 = analyte is above limit of quantification (LOQ)

(3) Quantifying and reference ions are present and the ion ratio deviates <20 % compared to standard

Standard run with retention time and masses used for quantification ions and reference ions is presented in table 3.1. Retention times of linearity test and biota sample run are provided in appendix 4 and S/N of linearity test and biota sample run are provided in appendix 5.

Table 3.1 Presentation of standard run with retention time and masses. n/a = retention time could not be determined due to low sensitivity.

Compound	Retention time [min]	Quantifying ion [*m/z*]	Reference ion [*m/z*]	Reference ion 2 [*m/z*]
α-HCH	14.57	181	219	
γ-HCH	15.46	181	219	
HCB	14.66	284	286	
heptachlor	17.54	272	274	
aldrin	18.66	263	293	265
oxychlordane	19.87	387	185	
trans-chlordane	20.65	373	239	
o,p'-DDE	20.72	246	318	
cis-chlordane	21.07	373	239	
α-endosulfan	21.11	241	239	
p,p'-DDE	21.82	246	318	
13C-*p,p'*-DDE	21.81	258	330	
trans-nonachlor	21.22	409	407	
cis-nonachlor	23.23	409	407	
o,p'-DDT	23.34	235	237	
p,p'-DDT	24.58	235	237	
OCN	n/a	404	332	

Based on table 3.1, OCN did not show any sharp peak in the chromatogram enabling to tell correct retention time. It does, however, show a consistent retention time throughout the linearity test and biota sample run presented in appendix 4.

The biota sample run (appendix 4) showed all RTs slowly undergo a consistent delay during analysis. However, heptachlor detected in SB1 had over three minutes delay which was not validated as it was much higher than the rest and was removed. For the linearity test, HCB did not have a valid retention time for the lower amounts, so they were removed from the linearity test. The values of S/N were calculated by Xcalibur™. For blank sample 1, ion ratio for heptachlor deviated >20 % and were removed.

3.2 Linear regression

A linear correlation of recovery standard and internal standard is presented in figure 3.1. A perfect linearity gives $R^2 = 1$, and valid linearity criteria was set to 10 % deviation.

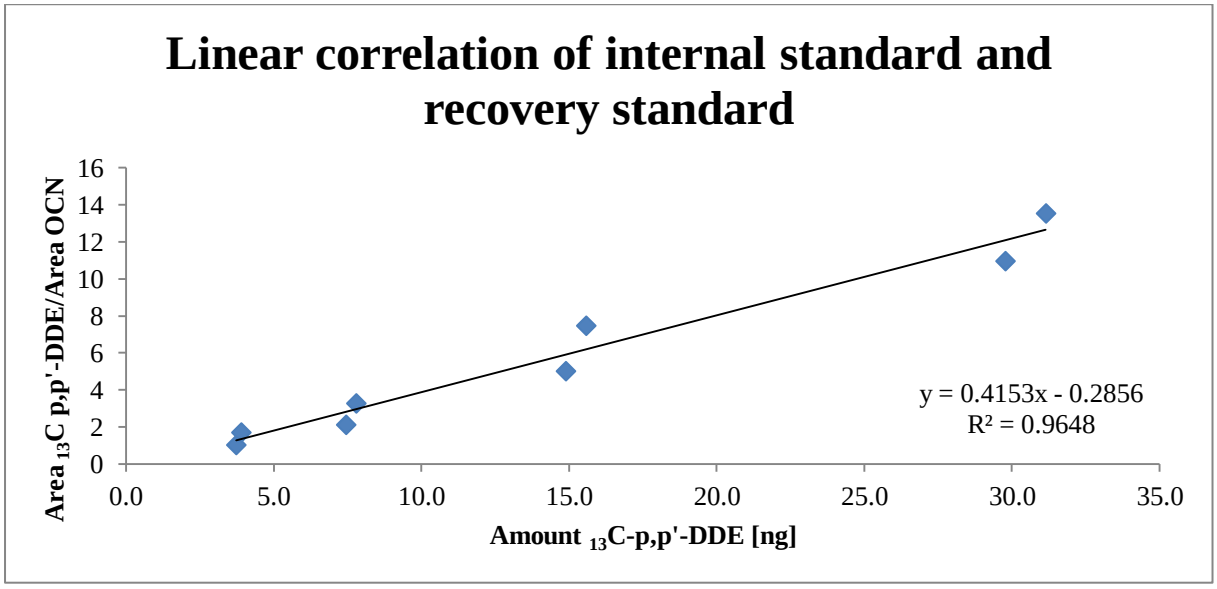


Figure 3.1 Linear correlation between internal standard ^{13}C p,p'-DDE and recovery standard OCN

The linearity test for each individual compound in the OCP mix is listed in appendix 6. *Trans*-Nonachlor was not within 10 % deviation with a R^2 of 0.8841. All other compounds showed accepted linearity.

The sequence for the linearity test run on the GC-MS is presented in appendix 7.

3.3 Relative response factor

To quantify OCP present, a relative response factor (RRF) was calculated; RFF_i for ^{13}C *p,p'*-DDE / ^{12}C components and RFF_g for recovery standard OCN / ^{13}C *p,p'*-DDE. RRF_i was calculated by using formula 3.1 and RRF_g was calculated using formula 3.2.

Formula 3.1 $$RRFi = \frac{Amount\ 13C\ x\ Area\ 12C}{Amount\ 12Cx\ Area\ 13\ C}$$

Formula 3.2 $$RRFg = \frac{Amount\ recovery\ standard\ x\ Area\ 13C}{Amount 13C\ x\ Area\ recovery\ standard}$$

Since the linearity test for each compound was made from four to eight measurements, there was made an average RRF_i and RRF_g for each component presented in table 3.2. For a complete overview of all RRF_i and RRF_g, see appendix 8.

Table 3.2 Average RRF_i and RRF_g from linearity test.

Compound	RRF_i average	RRF_i standard deviation
aldrin	0.06	0.01
cis-chlordane	0.09	0.01
trans-chlordane	0.11	0.00
o,p'-DDE	0.18	0.03
p,p'-DDE	0.16	0.02
o,p'-DDT	0.09	0.02
p,p'-DDT	0.08	0.01
α-endosulfan	0.03	0.01
HCB	0.16	0.02
α-HCH	0.05	0.01
γ-HCH	0.06	0.01
heptachlor	0.04	0.00
cis-nonachlor	0.03	0.00
trans-nonachlor	0.003	0.00
oxychlordane	0.02	0.01
	RRF_g average	RRF_g standard deviation
^{13}C *p,p'*-DDE and OCN	7.63	1.38

3.4 Results of biota samples

Based on RRF_i presented in table 3.2, calculations were made to quantify each sample by finding amount of target component in a sample (M_i).

Calculating M_i [ng] was made by using formula 3.3.

Formula 3.3

$$Mi = \frac{Amount\ 13Ci\ x\ Area\ 12Ci}{RRFi\ x\ Area\ 13Ci}$$

The amount M_i (ng) was the total amount of compound found in the total sample of liver, muscle, or fat. Therefore, the compounds will be presented as M_i [ng/g] wet weight (ww). Wet weight was found by dividing the total amount M_i [ng] by weight of samples presented in appendix 9. Also included in appendix 9 is the amount of extracted organic matter (EOM) in each sample.

Sample 1 Svalbard reindeer liver, sample 5 Svalbard reindeer fat, and sample 20 pink-footed goose liver did not give any results. No recovery standard, internal standard, or OCP compounds were possible to detect in the chromatogram, and were removed from the presented data.

Results from the quantitative analysis determining amount of OCPs in the samples (HCB and p,p'-DDE) are presented in table 3.3. Other OCPs in study (aldrin, *cis*-chlordane, *trans*-chlordane, *o,p'*-DDE, *o,p'*-DDT, *p,p'*-DDT, α-endosulfan, heptachlor, HCB, α-HCH, γ-HCH, *cis*-nonachlor, *trans*-nonachlor, and oxychlordane) where not detected in samples (>LOQ). The sequence for the sample run on the GC-MS is presented in appendix 6.

Table 3.3 Amount OCP [ng/g ww] detected in samples. Results marked with [-] means amount OCP were >LOQ.

Sample	Animal	Tissue	HCB [ng/g ww]	*p,p'*-DDE [ng/g ww]
2	Svalbard reindeer	liver	-	-
3	Svalbard reindeer	liver	10.2	1.8
4	Svalbard reindeer	liver	-	-
6	Svalbard reindeer	muscle	-	-
7	Svalbard reindeer	muscle	0.3	-
8	Svalbard reindeer	muscle	0.3	-

Table continues on next page

9	Svalbard reindeer	muscle	1.3	-
10	Svalbard reindeer	muscle	-	-
11	Svalbard reindeer	muscle	0.1	-
12	Svalbard reindeer	fat	3.5	-
13	Svalbard reindeer	fat	3.9	-
14	Svalbard reindeer	fat	2.7	-
15	Svalbard reindeer	fat	7.0	-
16	Svalbard reindeer	fat	-	-
17	Svalbard reindeer	fat	1.7	-
18	Svalbard reindeer	fat	2.2	0.1
19	Pink-footed goose	liver	-	-
21	Svalbard rock ptarmigan	muscle	0.3	-
22	Svalbard rock ptarmigan	fat	2.0	0.2

Based on the results from the samples given in table 3.3 there are too few variables to continue statistical analysis.

3.5 Method validation

3.5.1 Blank samples

Results from OCP found in blank samples are presented in figure 3.2. Ideally, there should not be any OCP present in these samples. The results are presented as total amount ng present in sample.

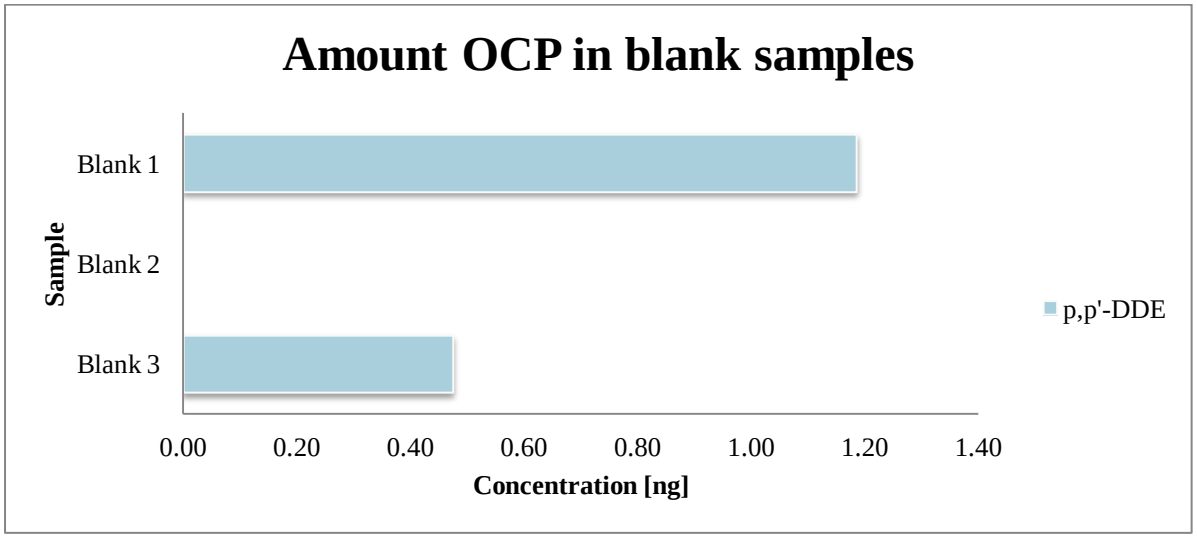


Figure 3.2 Amount OCP [ng] detected in blank samples

3.5.2 Spiked blank samples

Results from OCP found in spiked blank samples are presented in figure 3.3. These samples were spiked with 10 ng of each OCP compound. The results are presented as total amount ng present in sample.

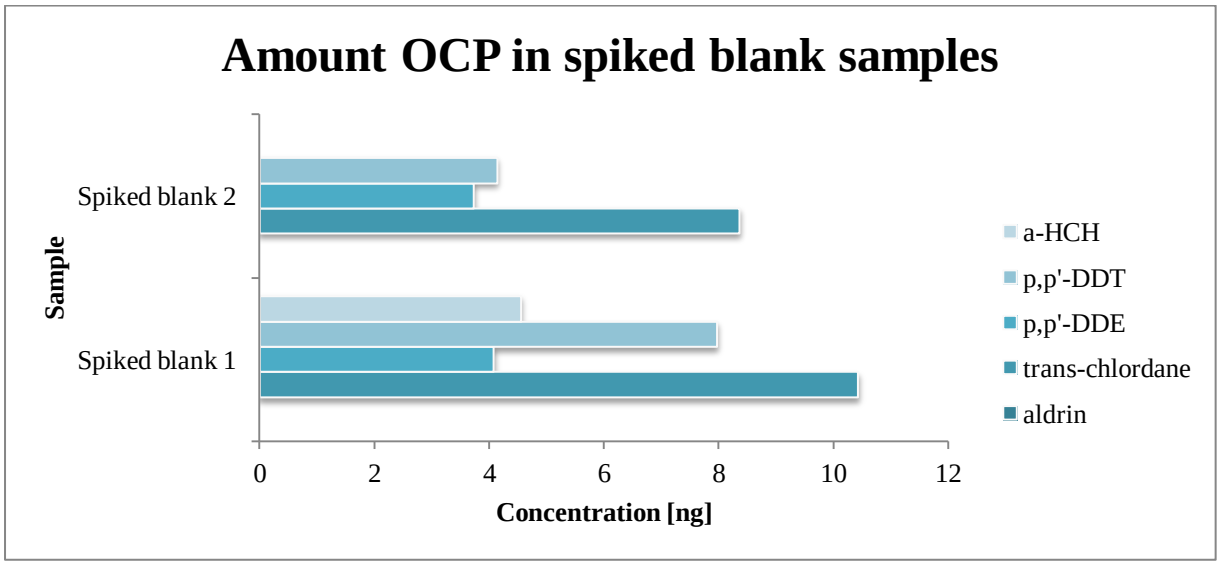


Figure 3.3 Amount OCP [ng] detected in spiked blank samples

3.6 Recovery

Based on RRF_g presented in table 3.2, calculations were made to find the recovery [%] based on amount OCN added to each sample just before analysis.

Calculating recovery [%] was made by using formula 3.4. To validate the results, the recovery should be 40 – 120 %.

Formula 3.4

$$\text{Recovery \%} = \frac{Amount\ recovery\ standard\ x\ Area\ 13Ci\ x\ 100}{RRFg\ x\ Amount\ 13Ci\ x\ Area\ recovery\ standard}$$

3.6.1 Recovery for blank samples

The recovery [%] of each biota sample is presented in table 3.4. Blank 1 and 2 showed valid recovery, while Blank 3 is just above 120 %.

Table 3.4 Recovery [%] of blank samples

Sample	Recovery [%]
Blank 1	58
Blank 2	74
Blank 3	126

3.6.2 Recovery for spiked blank samples

The recovery [%] of spiked blank sample is presented in table 3.5. Recoveries are not within the accepted area.

Table 3.5 Recovery [%] of spiked blank samples. Results marked with n/a means areas of OCN in the chromatogram could not be set and recovery could not be calculated.

Sample	Recovery [%]
Spiked blank 1	19
Spiked blank 2	n/a

Since there was a known added amount of OCP mix (10 ng) added to each spiked blank sample, an expected amount of recovery would be 4-12 ng. Calculated recovery [%] for each single compound was made from comparing known added amount of OCP with calculated amount [M_i] after analysis, presented in table 3.6.

Table 3.6 Recovery [%] of compounds detected in spiked blank sample. Results marked with – means they were not found and thereby <LOQ.

Compound	Spiked blank 1 Recovery [%]	Spiked blank 2 Recovery [%]
aldrin	-	-
trans-chlordane	104	84
p,p'-DDE	41	37
p,p'-DDT	80	41
α-HCH	46	-

3.6.3 Recovery for biota samples

The recovery [%] of each biota sample is presented in table 3.7.

Table 3.7 Recovery [%] of biota samples. Results marked with n/a means areas of OCN in the chromatogram could not be set and recovery could not be calculated.

Sample	Animal	Tissue	Recovery [%]
2	Svalbard reindeer	liver	n/a
3	Svalbard reindeer	liver	n/a
4	Svalbard reindeer	liver	n/a
6	Svalbard reindeer	muscle	29
7	Svalbard reindeer	muscle	n/a
8	Svalbard reindeer	muscle	204
9	Svalbard reindeer	muscle	131
10	Svalbard reindeer	muscle	n/a
11	Svalbard reindeer	muscle	82
12	Svalbard reindeer	fat	44
13	Svalbard reindeer	fat	50
14	Svalbard reindeer	fat	102
15	Svalbard reindeer	fat	14
16	Svalbard reindeer	fat	n/a
17	Svalbard reindeer	fat	123
18	Svalbard reindeer	fat	130
19	Pink-footed goose	liver	n/a
21	Svalbard rock ptarmigan	muscle	n/a
22	Svalbard rock ptarmigan	fat	109

4 Discussion

When working in the Arctic, the choice of method is limited to what equipment and chemicals are available. So it was important to always have a second option if anything would not go as planned. Time consumption in the laboratory was performed just as scheduled, while analysis did take much longer than expected due to malfunction of equipment.

4.1 Quality assurance and quality control

Since this is an ultra trace analysis, working in pg and ng range, a systematic error can easily occur. Small errors can have a big impact on the results.

4.1.1 Random errors

Cross contamination risk

There was a possibility for cross contamination due to how each sample was packed after sampling. Even though samples were individually wrapped in plastic foil or aluminum foil, they were not safely packed for thawing. When thawing the three zip-lock bags containing (1) all muscle samples from Svalbard reindeer, (2) all liver samples from Svalbard reindeer, and (3) samples from pink-footed goose there were leaking fluids from the samples sieving out of each individual wrapping. The fluids were blending in the zip-lock bags, possibly leading to small amounts of cross contamination. There were no such problems with the three zip-lock bags containing (1) all fat samples from Svalbard reindeer, (2) sample 11 and 12 (see appendix 1) from Svalbard reindeer, and (3) all samples from Svalbard rock-ptarmigan. The solution to this problem would have been to wrap each sample in aluminum foil and then in a small zip-lock bag before storing samples together in a bigger zip-lock bag.

Another source of possible minor cross contamination could have occurred when using the Wilfa blender for homogenization of each sample. The plastic of the Wilfa hand blender bowl had previously been melted by mistake with acetone, so the walls were not smooth. This made the bowl harder to clean between each new sample. A new blender would have been preferred, but working in the Arctic, there was no time to wait for a new one to arrive.

Other contamination risks

There was no detailed information provided on how each sample was precisely sampled in the field and brought back to storage at UNIS. Each sample was most likely cut with the local hunter's own knife.

Control parameters

Since all samples only had one replica due to time restrictions, it is not possible to know for certain whether a random error may have occurred to one more of the samples. The method could benefit from one or a few samples running three parallels as a random check.

4.1.2 Systematic errors

Acid treatment

Acid treatment is not the best choice for cleaning up OCPs as some compounds are not resistant to sulfuric acid (Martins et al. 2013). Intentionally, all the samples were supposed to be run through a Gel Permeation Chromatography (GPC) system, but there was a malfunction and no time to wait for it to be fixed. In GPC, a sample is separated based on hydrodynamic

volume where the mobile face function as an eluent through the stationary phase with pore size capable of discriminating analytes (Miller 2005). As this method is more gentle to the compounds, and does not create any chemical reactions or added heat, it would have been preferred for clean-up.

Since the samples were intended for the GPC, they had a volume of 2 ml. This volume was divided into 2 x 10 ml vials for acid clean-up. Instead of suddenly having to use twice as many vials, it would have been much easier to reduce the volume of each sample by ReactiVap to about 1 ml prior to acid treatment.

Preferably, the samples should have been transferred to glass tubes and shaken by e.g. a vortex mixer in order to blend all the acid well with each sample. Because the lab did not have a vortex mixer, all vials were shaken by hand. Improving the method, the samples could also have been centrifuged to separate the layers even better. When doing final analysis on the GC-MS the volume of each sample was reduced so much that some samples showed signs of impurities still in the samples, which was partly the reason the GC clogged up.

Clean-up with silica

It was debated whether the silica used should have been activated, or deactivated with 5 % water. Activated silica was chosen, and the samples should have been filtered in order to remove any particles following the samples out of the column. When doing final analysis on the GC-MS the volume of each sample was reduced so much that some samples showed signs of particle residue, which was the partly the reason the GC clogged up.

4.1.3 Linearity test

The linearity proved to be within the accepted area of 10 % for all compounds without *trans*-nonachlor.

Linearity range internal standard

For internal standard ^{13}C *p,p'*-DDE there was a desire to have a linearity test up to at least 200 pg/µl, but there was no standard available with high enough concentration. Therefore, the linear range became too low at 102 pg/µl. This means, when 5 ng internal standard was added to each sample and in the end all samples had a final volume of about 50 µl, the end concentration of ^{13}C *p,p'*-DDE would be about 100 pg/ µl. Preferably, the linearity test should at least reach 600 pg/µl seeing the areas present from the sample run.

Linearity proved to be too low, as almost all blank samples, spiked blank samples, and biota samples detected ^{13}C *p,p'*-DDE well above the linear range. Since this is a validated method, the linearity is assumed to continue, however, all samples quantified with internal standard outside linear area could not be validated in this study.

Linearity range quantification standard

There was a challenge working with a quantification standard with such a variation of concentrations, ranging from 308 pg/µl to 2154 pg/µl in stock solution. Even though the linearity test was desired to be in the area of 25 – 200 pg/µl, some compounds only ended up at about 100 pg/µl as highest concentration.

HCB was one of the compounds with desired range up to 199 pg/µl. However, half of the samples (7 of 14) where HCB was detected, the area was still above linear range. Trans-chlordane was above linear area for SB1 and heptachlor above linear area for blank 1.

Results of α-HCH, *o,p'*-DDE, *p,p'*-DDE, *cis*-nonachlor, *o,p'*-DDT, and *p,p'*-DDT were all within linear area. There were no detected γ-HCH, aldrin, oxychlordane, *cis*-chlordane, or α-endosulfan in any sample, so whether the linearity tests were of valid range is not possible to tell.

There were no issues in the lower area, so perhaps a linearity test up to 400 pg/µl would have been sufficient.

4.1.4 Validation of retention time, signal to noise, and masses

As sample clean-up was not sufficient, the GC clogged up during sample run which could explain why there was a continuous delay in RT during the sample run, but no changes during the linearity test run. OCN proved difficult to locate, and due to poor peakshape it was also difficult to determine correct RT. The recovery standard will be discussed further in 4.1.7. The linearity test showed valid RT except for lower concentrations for heptachlor (appendix 4). Even though the RT was delayed, the delay was fairly similar in all compounds.

S/N was <LOQ for the all biota samples detected by RT except for internal standard in sample 2, 16, and 19. No OCP compounds were detected in sample 2 and 19, but in sample 16 there was HCB >LOQ. The amount [ng] could not be calculated when absence of internal standard.

Some of the quantifying ions and reference ions had close masses, such as e.g. HCB searching for *m/z* 284 and 286 (table 3.1). Preferably, reference ion should not be so close in masses, but due to the mass range set, other masses possible to use were outside mass range area.

4.1.5 Blank samples

The blank samples were primarily used to check for any contaminations. They should only contain internal standard and recovery standard after analysis. Any results of OCP found in the blank samples would be an indication of cross contamination during sample preparation or carry-over contamination during analysis.

Results show that blank 2 were clean from any OCP. Blank 1 and 3 contained small amounts of *p,p'*-DDE. Looking at the sequence run in appendix 7, the impurities could not come from any carry-over. Blank 3 did turn milky white for no apparent reason after the final acid treatment, so there might have been a random error.

Recovery of blank 1 and blank 2 were within accepted area of 40 – 120 %, and blank 3 was just above with 126 %.

Improving the method, it would have been desired to have field blank samples to follow sample collection. Also, it would have been preferred a transportation blank sample following samples by air plane from and back to UNIS. Including such supplementary blank samples would act as an additional method quality assurance parameter, intended to pick up traces of random errors possibly occurring during field work and transport.

4.1.6 Spiked blank samples

Spiked blank samples were used to indicate how well the method would work. They should have contained all components, but did not. Out of the two spiked blanks following the method, neither showed all components. Aldrin was missing in both samples. Most likely it was lost during clean-up as the compound is acid-sensitive (cf. Martins et al. 2013).

Sample SB1 contained more of the added amount [ng] quantification standard than SB2. The reason being could be because SB2 was accidently added to the same vial as what should have been sample SB3. The solution was divided back in two separate vials. Between acid treatment and silica gel clean-up, the SB2 sample turned yellow in color and had slightly evaporated after resting over night in a closed vial. Evaporation could be reason for α-HCH missing.

The recovery for each sample was not sufficient with 19 % for SB 1 and no OCN with adequate results in the chromatogram to make a calculation for SB 2. Looking at the recovery of each single compound, the recovery is within the accepted limit of 40-120 %, with the only exception of *p,p'*-DDE which was just below with 37 %. This indicated the recovery standard was causing low recovery, not the method itself.

Improving the method, there should preferably have been tree parallel spiked blank samples. In order to see if the cyclohexane/DCM solvent used on the silica column runs carried through all OCPs, another spiked blank sample should have been made only to run through the silica column and then analyzed. The analysis would show if any compounds were left in the silica column or if all compounds would eluate as intended.

4.1.7 Internal standard

Calculating the amount of sample [ng] present in the biota samples was made from RRF_i, which was calculated from performance of the internal standard ^{13}C *p,p'*-DDE. The highest calculated RRF_i standard deviation for any compound was 0.03 (table 3.1) which indicates the method is working to desired accuracy. The R^2 from the linear correlation between internal standard and recovery standard OCN presented figure 3.1 was accepted within 10 % deviation.

4.1.8 Recovery standard

Recovery standard was added right before analysis, so the peaks should be easily detected in the chromatograms. The peakshapes were generally very poor; where other components had a clean peak, OCN did not at all. Even in standard run, it was difficult to get the proper retention time and area of the peak. A standard run of only 200 pg/µl stock solution OCN is given in figure 4.1

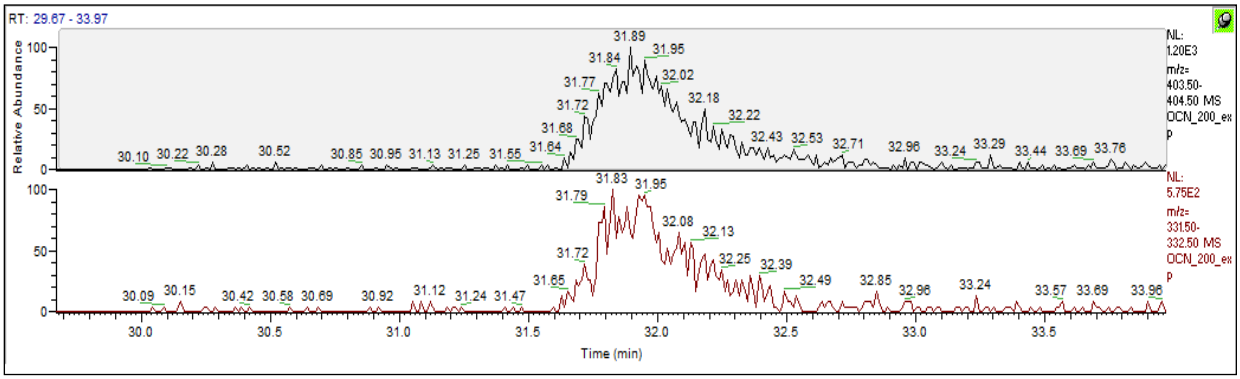


Figure 4.1 Standard run of 200 pg/µl OCN, m/z *404 and 332.*

This is an indication that there might have been an issue with the OCN standard, with such low sensitivity. As the recovery [%] of all compounds was based on this recovery standard, there is reason to assume the OCN was cause of poor recovery, not the method itself. Referring to the results of recovery of the spiked blank samples in 3.6.2, the recovery [%] was valid for all samples except for *p,p'*-DDE which was just below the valid value. This does not match the poor recovery [%] calculated to SB1 at 19 % and SB2 where area of OCN in the chromatogram was too poor to be set. This is the same reason why the recovery could not be calculated for many of the biota samples, as there was peak of OCN to draw an area from. And for the samples where recovery [%] was calculated, the area of the recovery standard was set to best ability, meaning the peaks were not optimal.

For the linearity test, 20 ng recovery standard was added to each vial before analysis, which was five times the amount added to each biota sample, blank sample, and spiked blank sample. Even though the OCN did preformed better at this concentration, the RRF_g calculated from the linearity test did give average 7.63 ± 1.38, meaning the standard deviation is high since the desired standard deviation should be as close to zero as possible.

Improving the method, the OCN should have been exchanged for a new solution of OCN, preferably at a higher concentration, or a different recovery standard compound.

4.2 Summary of quantification method

Working with GC-MS was the preferred method, and with better clean-up the method should have worked out fine for quantification, preferably change acid clean-up to e.g. GPC. The linearity tests all showed acceptable linearity for all compounds except for trans-nonachlor, but most compounds found were outside linear range meaning the linear range should have reached higher concentrations.

Two of the three method validation blank samples showed low levels of p,p'-DDE. The spiked blank samples contained the added compounds except for aldrin in both samples and α-HCH in SB2. The recovery [%] was within the accepted area of 40-120 % for all compounds detected, except for p,p'-DDE in SB2 at 37 %. The recovery [%] was not good at 19 % for SB2 and not possible to calculate for SB2 based on internal standard and recovery standard. Also, not being able to draw an area of OCN from the chromatogram of multiple samples, it appears the recovery standard caused poor recovery and not the method. Preferably, the OCN should have been changed for a new recovery standard.

4.3 Sample quantification and comparison

4.3.1 Quantified results for selected biota

As samples were being analyzed, the clean-up procedure had not been fully successful as the GC-MS clogged up. There were multiple samples that never made the analysis, but due to time restrictions, there was no time to do additional clean-up and a sample run. Based on the results given, HCB and *p,p'*-DDE was the only OCP contaminants found >LOQ to be quantified in biota samples.

In general, HCB results were slightly higher in fat samples compared to muscle samples. This complement research mentioned in the introduction; expecting higher results of OCP in fatty tissues as the compounds are lipophilic.

Looking at the results from the biota sample run, the highest concentration of any compound was HCB found in Svalbard reindeer liver sample number 3, presented in table 3.3. Notably, this particular Svalbard reindeer was the same reindeer that gave muscle liver sample number 3 and fat sample number 16 (appendix 1). Unfortunately, the fat sample could not be quantified as internal standard was <LOQ and the reason why the muscle sample does not show equally high results, could be because the sample nearly dried out during volume reduction in the laboratory. So if this particular reindeer, it's uncertain if the HCB value in the liver is as high or if there has occurred a systematic or random error. For all three samples the recovery was below 40 %, though this could be caused by the recovery standard as mentioned in 4.1.7.

Svalbard rock-ptarmigan was numbered sample 21 for muscle and 22 for fat. Both samples showed results of HCB, and a small amount of *p,p'*-DDE was detected in the fat. Compared to the Svalbard reindeer samples, the results are about the same and showing same components of OCP.

Pink-footed goose did not show any results of OCP above LOQ, and sample 20 was removed as described previously due to lack of internal standard and recovery standard. Sample 19 did not show any visible peak for OCN in the chromatogram, and no pesticides were detected. As such, this analysis did not generate adequate results from pink-footed geese to compare to Svalbard reindeer based on this analysis.

4.3.2 Accepted limit for pesticides in food

The European Union (EU) has set a maximum residue level (MRL) of any pesticide residue legally tolerated in/on food and feed. MRL is given in mg/kg, appearing to be wet weight (Bitterhof s.a.). The MRL of pesticides in this study are calculated to ng/g table 4.1.

Table 4.1 MRL of pesticides in/on food or feed under "Products of animal origin-terrestrial animals" *(EU Pesticides database s.a.).*

Compound	MRL [ng/g]
Aldrin	200
Chlordane [1)]	50
DDT [2)]	1000
Endosulfan [3)]	50
HCB	200
α-HCH	200
γ-HCH	20
heptachlor	200

[1)] *Sum of* cis-*chlordane and* trans-*chlordane*

[2)] *Sum of* o,p'-*DDT,* p,p'-*DDT,* p,p'-*DDE, and* p,p'-*DDD*

[3)] *Sum of* α-*HCH,* β-*HCH, and endosulfan-sulfate*

Comparing MRL to the results of HCB and *p,p'*-DDE (table 3.3) from Svalbard reindeer and Svalbard tock ptarmigan, all samples are well under the limit as the highest result of HCB was 45.9 ng/g ww and *p,p'*-DDE was 1.8 ng/g ww. The database does not contain any information about *cis*-nonachlor, *trans*-nonachlor, and oxychlordane.

4.3.3 Comparison to other studies

There have been selective quantitative analysis studies of terrestrial herbivores in the Arctic, but no recent temporal trends or spatial trends. According to Rigét et al. (2010) the only available time-series from terrestrial ecosystem is reindeer from northern Sweden, though this study were of industrial chemicals and not pesticides.

Analysis of selected OCPs in this study showed only HCB and *p,p'*-DDE detected above LOQ. A comparison to similar selected studies found of terrestrial biota in the Arctic is listed in table 4.2.

Table 4.2 Levels of OCPs [ng/g ww] median (range) detected in selected studies from arctic regions such as Greenland (Vorkamp et al. 2004), Canada (Pollock et al. 2009), and Norway (Hassan et al. 2013) for comparison. N = total number of species

Species	Location	Year	Tissue	N	HCB	N	*p,p'*-DDE
Cribou (*Rangifer tarandus*)	Isortoq, Greenland	2000	Liver	5	6.2 (3.9-7.4)	0	-
			Muscle	4	8.7 (6.8-9.5)	0	-
			Blubber	5	7.3 (3.7-9.3)	0	-
	George River herd, Labrador, Canada	2001	Perirenal fat	27	24.2 (11.8-36.2)	0	-
Reindeer (*Rangifer tarandus tarandus L.*)	Northern Norway	2004-2005	Liver	27	2.56 (<0.11-4.55)	23	(0.11-0.13)
			Meat	25	0.62 (0.31-1.14)	23	(0.10-0.89)
			Tallow	23	37.83 (16.59-53.25)	30	(0.49-3.01)
Ptarmigan (*Lagupus mutus*)	Nuuk, Greenland	1999	Liver	5	2.9 (2.1-5.1)	0	-
			Muscle	5	3.6 (3.5-6.8)	0	-

Species for comparison listed in table 4.2 are not the same species analyzed in this study; however this was the closest available recent studies of arctic terrestrial herbivores found. For all studies presented, HCB appears to give predominant results for most terrestrial studies of OCP compounds. In general, the amount of HCH measured in this study is lower than the comparing studies, except for the reindeer liver concentration. Discussed in 4.2.1; liver sample 3 was showing a significant higher result than the rest of the Svalbard reindeer samples. As sample 3 was the only liver sample giving any quantitative result in this study, and the result came from the Svalbard reindeer showing concentrations much higher than the rest, it would have been preferable with more results before a valid comparison could be made.

5 Conclusion

Since presence of OCPs is a long-term issue for humans, wildlife and environment in the Arctic, it is important to do continuous monitoring of wildlife to keep track of increasing or decreasing levels of legacy POPs as well as detection of new merging compounds.

The results of this study showed in general low levels of detected HCB and *p,p'*-DDE in selected Svalbard reindeer and Svalbard rock ptarmigan, although based on validation of all results conclusions are to be made with caution. This study targeted a small geographic area around Longyearbyen in Svalbard, and all samples were from the same year. Even though research such as this cannot alone show temporal and spatial trends, it can be compared to other studies for getting a broader knowledge of OCPs in Arctic terrestrial herbivores.

6 Literature

Aagaard A. (ed.). (2014). Pesticide Risk Assessment for Birds and Mammals: Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009. Version 1.1. 138 pp.

AMAP. (1997). Arctic pollution issues: a state of the Arctic environment report. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xii+188 pp.

AMAP. (2009). Arctic Pollution 2009. Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. xi+83 pp.

AMAP. (2014). Trends in Stockholm Convention Persistent Organic Pollutants (POPs) in Arctic Air, Human media and Biota. AMAP Technical Report to the Stockholm Convention No. 7 (2014). Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. 54 pp.

Andreassen, I. (2009). *New Emerging Persistant Organic Pollutants in Selected Arctic Biota: Accumulation, Distribution and Transformation in Arctic Food Webs*: Norwegian University of Science and Technology, Biology. 90 pp.

Bitterhof, A. (s.a.). *Legal aspects of relevant EU legislation related to pesticides*: European Commission DG Health and Consumers, Unit E.3. Available at: http://www.bfr.bund.de/cm/343/legal-aspects-of-relevant-eu-legislation-relatet-to-pesticides.pdf (accessed: 2014/12/14).

Bjørkvoll, E., Pedersen, B., Hytteborn, H., Jónsdóttir, I. S. & Langvatn, R. (2009). Sasonal and Interannual Dietary Variation during winter in Female Svalbard Reindeer (Rangifer tarandus platyrhynchus). *Arctic, Antarctic, and Alpine Research*, 41 (1): 88-96.

Brage Bremset Hansen, R. A., Ivar Herfindal, Jack Kohler, and Bernt-Erik Sæther. (2011). Climate, icing, and wild artic reindeer: past relationships and future prospects. *Ecology*, 92 (10): 1917-1923.

Carlsson, P. & Halse, A. K. (2012). *Standard Operating Procedure AT-324-1. Version 1.2*. The University Centre in Svalbard (UNIS). 15 pp. Unpublished manuscript.

Carlsson, P. & Kallenborn, R. (2012). POPjakt i skolen: Sluttrapport til Svalbard miljøvernfond, november 2010-desember 2012. Available at: http://www.sysselmannen.no/Documents/Svalbard_Miljovernfond_dok/Prosjekter/Rapporter/popjakt_rapport_dec12.pdf?epslanguage=no.

Crossley, R. & Couzens, D. (2014). *The Crossley ID guide: Britain and Ireland*, vol. 1: Prinveton University Press. 304 pp.

Emergency Prevention Prepareness and Response. (s.a.). *Minististerial Direction: Background*. Available at: http://www.arctic-council.org/eppr/reports/ministerial-direction/ (accessed: 2014/11/15).

EU Pesticides database. (s.a.). *Pesticides Residues*. Available at: http://ec.europa.eu/sanco_pesticides/public/?event=substance.selection (accessed: 2014/12/11).

Fuglei, E. & Pedersen, Å. Ø. (2008). Bestandsovervåkning av Svalbardrype (Lagopus muta hyperborea): Registrering av territoriell stegg våren 2008.

Fuglei, E. & Aanes, R. (s.a.). *Survival strategies*: Svalbard museum. Available at: http://www.svalbardmuseum.no/eindex.php?id=12&kategori=3 (accessed: 2014/10/10).

Fuglei, E. & Pedersen, Å. Ø. (s.a.). *Svalbard rock ptarmigan (Lagopus muta hyperborea)*. Available at: http://www.npolar.no/en/species/svalbard-rock-ptarmigan.html (accessed: 2014/09/01).

Gill, H. K. & Garg, H. (2014). Pesticides: Environmental Impacts and Management Strategies. In Larramendy, M. L. & Soloneski, S. (eds) *Pesticides - Toxic Aspects*, p. 238: InTech.

Hassan, A. A., Rylander, C., Brustad, M. & Sandanger, T. M. (2013). Persistent organic pollutants in meat, liver, tallow and bone marrow from semi-domesticated reindeer (Rangifer tarandus tarandus L.) in Northern Norway. *Acta Veterinaria Scandinavica*, 55 (1): 57-57.

Hisdal, V. (1985). *Geography of Svalbard*, vol. nr. 2 (1985). Oslo: Norsk polarinstitutt. 75 s. : ill. pp.

Hoferkamp, L., Hermanson, M. H. & Muir, D. C. G. (2010). Current use pesticides in Arctic media; 2000–2007. *Science of the Total Environment*, 408 (15): 2985-2994.

Hotchkiss, A. K., Rider, C. V., Blystone, C. R., Wilson, V. S., Hartig, P. C., Ankley, G. T., Foster, P. M., Gray, C. L. & Gray, L. E. (2008). Fifteen Years after "Wingspread"—Environmental Endocrine Disrupters and Human and Wildlife Health: Where We are Today and Where We Need to Go. *Toxicological Sciences*, 105 (2): 235-259.

Levy II, H. (1990). Regional and Global Transport and Distribution of Trace Species Released at the Earth's Surface. In Kurtz, D. A. (ed.) *Long Range Transport of Pesticides*, p. 480. USA: Lewis Publishers.

Madsen, J. (1985). Relations between Change in Spring Habitat Selection and Daily Energetics of Pink-Footed Geese Anser brachyrhynchus. *Ornis Scandinavica*, 16 (3): 222-228.

Madsen, J., Cracknell, G. & Fox, T. (1999). *Goose populations of the western Palearctic: a review of status and distribution*. Rønde, Denmark: National Environmental Research Institute. 343 s. : ill. pp.

Madsen, J. & Williams, J. H. (2012). International Species Management Plan for the Svalbard Population of the Pink-footed Goose Anser brachyrhynchus. *AEWA Technical Series No. 48*.

Madsen, J., Cottaar, F., Nicolaisen, P. I., Tombre, I., Verscheure, C. & Kuijken, E. (2013). Svalbard pink-footed goose: Population Status Report 2012-2013. *Technical Report from DCE – Danish Centre for Environment and Energy No. 29*. Aarhus University.

Madsen, J. & Tombre, I. M. (s.a.). Internasjonal forvaltningsplan for kortnebbgås. Available at: http://gasejakt.no/doc/Madsen%20&%20Tombre_%20Internasjonal%20forvaltningsplan%20for%20kortnebbgas.pdf (accessed: 2014/11/18).

Martins, J. G., Amaya Chávez, A., Waliszewski, S. M., Colín Cruz, A. & García Fabila, M. M. (2013). Extraction and clean-up methods for organochlorine pesticides determination in milk. *Chemosphere*, 92 (3): 233-246.

Miller, J. M. (2005). *Chromatography: Concepts & Contrasts*. 2nd ed. NJ, USA: John Wiley & Sons. 490 pp.

MOSJ. (s.a.-a). *Bag of Svalbard Ptarmigan (Lagopus muta hyperborea)*. In Nilsen, S. Ø. (ed.): Miljøovervåkning Svalbard og Jan Mayen (MOSJ). Available at: http://mosj.npolar.no/en/influence/hunting/indicators/svalbard-rock-ptarmigan-hunting.html (accessed: 2014/11/18).

MOSJ. (s.a.-b). *Svalbardrein (rangifer tarandus platyrhynchus)*. In Nilsen, S. Ø. (ed.): Miljøovervåking Svalbard og Jan Mayen (MOSJ). Available at: http://mosj.npolar.no/no/fauna/terrestrial/indicators/reindeer.html (accessed: 2014/10/08).

Pedersen, Å. Ø., Overrein, Ø., Unander, S. & Fuglei, E. (2005). Svalbard Rock Ptarmigan (Lagopus mutus hyperboreus): a status report. *Rapportserie Norsk Polarinstitutt No. 125*. Tromsø: Norsk Polarinstitutt. 21 s. : ill. pp.

Pedersen, Å. Ø., Lier, M., Routti, H. A. I., Christiansen, H. H. & Fuglei, E. (2006). Co-feeding between Svalbard Rock Ptarmigan (Lagopus muta hyperborea) and Svalbard Reindeer (Rangifer tarandus platyrhynchus). *Arctic*, 59 (1): 61-64.

Pedersen, Å. Ø. & Bårdsen, B.-J. (2014). *Reinsdyrkjever og jaktsatistikk*: Norsk Polarinstitutt. Available at: http://www.npolar.no/no/kronikker/2014/2014-08-04-jegerdata.html (accessed: 2014/11/17).

Pollock, B., Penashue, B., McBurney, S., Vanleeuwen, J., Daoust, P.-Y., Burgess, N. M. & Tasker, A. R. (2009). Liver Parasites and Body Condition in Relation to Environmental Contaminants in Caribou (Rangifer tarandus) from Labrador, Canada. *Arctic*, 62 (1).

Punsvik, T. (2009). Plan for forvaltning av svalbardrein: en beskrivelse av miljømål og status for reinen på Svalbard, og en veileder for forvaltningen og forskningen. *Sysselmannen på Svalbard* (Report 1/2009): 47.

Reimers, E. (1984). *Body composition and population regulation of Svalbard reindeer*. S.16-21 fig. pp.

Rigét, F., Bignert, A., Braune, B., Stow, J. & Wilson, S. (2010). Temporal trends of legacy POPs in Arctic biota, an update. *Science of the Total Environment*, 408 (15): 2874-2884.

Schytte Blix, A. (2005). *Arctic animals and their adaptations to life on the edge*. Trondheim: Tapir Academic Press. 296 pp.

Speed, J. D. M., Anderson, H. B., Madsen, J., Pedersen, Å. Ø., Tombre, I. & Wal, R. v. d. (2014). Effects of foraging by pink-footed geese on tundra vegetation in Svalbard: an assessment of extent and a proposal for a monitoring program. *Report to Svalbard envronmental protection fund*. Aarhus University.

Stockholm Convention. (s.a.-a). *The 12 initial POPs under the Stockholm Convention*. Available at: http://chm.pops.int/TheConvention/ThePOPs/The12InitialPOPs/tabid/296/Default.aspx (accessed: 2014/09/26).

Stockholm Convention. (s.a.-b). *The new POPs under the Stockholm Convention*. Available at: http://chm.pops.int/TheConvention/ThePOPs/TheNewPOPs/tabid/2511/Default.aspx (accessed: 2014/09/26).

Stockholm Convention. (s.a.-c). *Overview*. Available at: http://chm.pops.int/TheConvention/Overview/tabid/3351/Default.aspx (accessed: 2014/09/24).

Svalbard Environmental Protection Act. (2001). *Svalbard Environmental Protection Act (Svalbardmiljøloven) of 1. July 2002 No. 24*. Available at: https://lovdata.no/dokument/NL/lov/2001-06-15-79?q=svalbardmilj%C3%B8loven (accessed: 2014/11/15).

The Governor of Svalbard. (s.a.). *Jakt, fangst og fiske - jaktbare arter*: The Governor of Svalbard. Available at: http://www.sysselmannen.no/Fastboende/Jakt-fangst-og-fiske/ (accessed: 2014/11/18).

Vorkamp, K., Riget, F., Glasius, M., Pécseli, M., Lebeuf, M. & Muir, D. (2004). Chlorobenzenes, chlorinated pesticides, coplanar chlorobiphenyls and other organochlorine compounds in Greenland biota. *Science of the Total Environment*, 331 (1–3): 157-175.

Vouge, P. A., Kerle, E. A. & Jenkins, J. J. (1994). *OSU Extension Pesticide Properties Database*: National Pecticide Information Center. Available at: http://npic.orst.edu/ingred/ppdmove.htm (accessed: 2014/12/11).

WHO. (s.a.). *Persistent Organic Pollutants (POPs)*: World Health Organization (WHO). Available at: http://www.who.int/trade/glossary/story072/en/.

Zhao, R., Chu, S., Zhao, R., Xu, X. & Liu, X. (2005). Ultrasonic extraction followed by sulfuric acid silica gel cleanup for the determination of α-hexachlorocyclohexane enantiomers in biota samples. *Analytical and Bioanalytical Chemistry*, 381 (6): 1248-1252.

Zitko, V. (2003). Chlorinated Pesticides: Aldrin, DDT, Endrin, Dieldrin, Mirex. In Fiedler, H. (ed.) The Handbook of Environmental Chemistry, vol. 3O *Persistent Organic Pollutants*, pp. 47-90: Springer Berlin Heidelberg.

Ødegaard, P.-I. (2013). *Food preferences by spring migrating Pink-footed geese (Anser brachyryhnchus) in Central Norway*: Hedmark University College, Faculty of Forestry and Wildlife Management.

Appendix 1 Samples in study and numbering of each sample

A presentation of how all samples were numbered, and information about each sample regarding species, tissue sample, sex, age, sample collection date, and sample location. n/a = information not available.

Sample number	Tissue	Animal	Sex	Approximate age (years)	Date of sample collection	Location
Hunting for POPs in school: Reindeer 2010						
1	liver	Svalbard reindeer	female	n/a	2010-08-25	Colesdalen
2	liver	Svalbard reindeer	female	juvenile	2010-09	Gangdalen (Reindalen)
3	liver	Svalbard reindeer	n/a	0.5	2010-08-19	Plateau above Rusanovodden (Colesdalen)
4	liver	Svalbard reindeer	male	1.5	2010-08-31	Fardalen (Colesdalen)
5	fat	Svalbard reindeer	male	calf	2010-09	Hollenderdalen
6	muscle	Svalbard reindeer	female	juvenile	2010-09	Gangdalen (Reindalen)
7	muscle	Svalbard reindeer	female	adult	2010-08	Reindalen
8	muscle	Svalbard reindeer	female	juvenile	2010-09	Gangdalen (Reindalen)
9	muscle	Svalbard reindeer	n/a	0.5	2010-08-19	Plateau above Rusanovodden (Colesdalen)
10	muscle	Svalbard reindeer	female	n/a	2010-08-25	Colesdalen
11	muscle	Svalbard reindeer	male	5	2010-09	Diabas
12	fat	Svalbard reindeer	male	5	2010-09	Diabas
13	fat	Svalbard reindeer	female	n/a	2010-08-25	Colesdalen
14	fat	Svalbard reindeer	male	2.5	2010-08-15	Leiladalen (Colesdalen)

15	fat	Svalbard reindeer	male	1.5	2010-08-31	Fardalen (Colesdalen)
16	fat	Svalbard reindeer	n/a	0.5	2010-08-19	Plateau above Rusanovodden (Colesdalen)
17	fat	Svalbard reindeer	male	n/a	2010-08	Diabas
18	fat	Svalbard reindeer	female	juvenile	2010-09	Gangdalen
Local hunter: Pink-footed goose 2010						
19	liver	Pink-footed goose	n/a	n/a	2010-08	n/a
20	liver	Pink-footed goose	n/a	n/a	2010-08	n/a
Local hunter: Svalbard rock ptarmigan 2010						
21	muscle	Svalbard rock ptarmigan	n/a	n/a	2010-09-18	Bolterdalen
22	fat	Svalbard rock ptarmigan	n/a	n/a	2010-09-18	Bolterdalen

Appendix 2 Apparatus and reagents

General equipment

- Glassware: Vials in various sizes, single use pipettes, single use micropipettes (accuracy ≤ ± 0.25 %, precision ≤ ± 0.5 %, Blaubrand® intraMARK) , Erlenmeyer bottles, beakers, TurboVap glasses®, and columns (length 80 cm, ID 15 mm, Merck, Darmstadt, Germany)
- Parafilm "M" laboratory film (Pechiney Plastic Packaging, Chicago, IL, USA)
- Surgical blades for the scalpel (Feather surgical blades no. 32, single use stainless steel, Japan)
- Wilfa hand blender (Wilfa 700W, Skytta, Norway)

Reagents

The Na_2SO_4 and Silica were kept in Erlenmeyer glassware in an exicator. All vials used were glass vials with screw caps.

Table 1 Chemicals and absorbents used for sample preparation, all of trace analysis grade

Chemical	Supplier
Acetone	Merck, Darmstadt, Germany
Cyclohexane	Merck, Darmstadt, Germany
Dichloromethane	Merck, Darmstadt, Germany
Helium (He), 6.0 quality	Hydrogas, Porskgrunn, Norway
Isooctane	Merck, Darmstadt, Germany
Methanol (MeOH)	Merck, Darmstadt, Germany
Nitrogen (N2) gas, 5.5 quality	AGA, Oslo, Norway
Octachloronaphtalene (OCN)	Merck, Darmstadt, Germany
Pesticides standards, non-labeled and 13C labeled	Cambridge Isotope Laboratory (CIL), Andover, MA, USA
Silica, mesh size 70-230 µm, pre-treated at 450 °C for 6 hours	Sigma-Aldrich, Oslo, Norway
Sodium hydroxide (NaOH)	Sigma-Aldrich, Oslo, Norway
Sodium sulfate (Na2SO4), pre-treated at 450 °C for 6 hours	Sigma-Aldrich, Oslo, Norway
Sulfuric acid (H2SO4), 98% purity	Merck, Darmstadt, Germany

Apparatus

– Gas chromatographer (GC) (TRACE™ Ultra GC, Thermo Fisher Scientific, Waltham, MA, USA)
- Mass spectrometer (MS) (PolarisQ ion trap, Thermo Fisher Scientific, Waltham, MA, USA)
- Reacti-Vap™ Evaporator (Pierce, Rockford, IL, USA)
- TurboVap® (Zymark TurboVap® 500, Caliper Life Science, Hopkinton, MA, USA)
- Ultrasonic bath (Elma Transsonic T700, Singen, Germany)
- Xcalibur™ software (version 2.0.7, Thermo Scientific, Waltham, MA, USA)

Cleaning procedure

All glassware used was burned at 450 °C for 6 hours and cleaned in the following order; once with methanol, once with acetone, and once with cyclohexane. All openings were covered with aluminum foil.

Glass columns were soaked overnight (for a minimum of 6 hours) in a 0.1 M NaOH bath, and then cleaned once with methanol, acetone, and cyclohexane. Top and bottom was wrapped in aluminum foil. The stopcocks for the columns were cleaned in the laboratory dish washer before cleaned in methanol and left for 15 minutes in an ultrasonic bath.

A Wilfa hand blender was cleaned twice with distilled water (MilliQ gradient Millipore, filter 0.22 pm Millipak 20 Millipore) and once with methanol.

Surgical blades for the scalpel were cleaned once with acetone and once with cyclohexane.

TurboVap® stationary parts were cleaned once with acetone and once with cyclohexane

The needles to the Reacti-Vap™ Evaporator were cleaned in methanol (MeOH) in an ultrasonic bath for 15 min.

Appendix 3 Concentrations for linearity test

The table shows a representation of concentrations in stock solution OCP and the amount [ng] in solution to make the linearity tests. H200-H25 represents the higher concentrations in stock solution, and L200-L25 represents the lower concentrations, keeping the compounds as close as possible to 200 pg/µl – 25 pg/µl linear range for each compound.

Compound	Stock solution [ng/µl]	H200 [ng]	H100 [ng]	H50 [ng]	H25 [ng]	L200 [ng]	L100 [ng]	L50 [ng]	L25 [ng]
OCN		20	20	20	20	20	20	20	20
^{13}C *p,p'*-DDE	1.888 [1)]	31.1	15.6	7.8	3.9	29.8	14.9	7.4	3.7
aldrin	0.431	40.3	20.1	10.1	5.0	7.3	3.7	1.8	0.9
cis-chlordane	0.954	89.2	44.6	22.3	11.1	16.2	8.1	4.0	2.0
trans-chlordane	0.646	60.4	30.2	15.1	7.5	11.0	5.5	2.7	1.4
o,p'-DDE	0.308	28.8	14.4	7.2	3.6	5.2	2.6	1.3	0.7
p,p'-DDE	0.308	28.8	14.4	7.2	3.6	5.2	2.6	1.3	0.7
o,p'-DDT	0.369	34.5	17.3	8.6	4.3	6.3	3.1	1.6	0.8
p,p'-DDT	0.369	34.5	17.3	8.6	4.3	6.3	3.1	1.6	0.8
α-endosulfan	0.769	71.9	36.0	18.0	9.0	13.1	6.5	3.3	1.6
HCB	2.154	201.3	100.7	50.3	25.2	36.6	18.3	9.1	4.6
α-HCH	0.308	28.8	14.4	7.2	3.6	5.2	2.6	1.3	0.7
γ-HCH	0.462	43.1	21.6	10.8	5.4	7.8	3.9	2.0	1.0
heptachlor	0.769	71.9	36.0	18.0	9.0	13.1	6.5	3.3	1.6
cis-nonachlor	1.754	163.9	82.0	41.0	20.5	29.8	14.9	7.4	3.7
trans-nonachlor	0.615	57.5	28.8	14.4	7.2	10.4	5.2	2.6	1.3
oxychlordane	0.308	28.8	14.4	7.2	3.6	5.2	2.6	1.3	0.7

1) In stock solution, ^{13}C-DDE and OCP mix were in separate vials.

Appendix 4 Retention time

4.1 Retention time linearity test

All data in table is retention time from linearity test run presented in minutes. Numbers marked in red did not meet validation criteria and were removed from linearity test. n/a = compounds not detected in chromatogram.

Compounds	H200	H100	H50	H25	L200	L100	L50	L25
OCN	31.35	31.35	31.35	31.34	31.38	31.35	31.34	31.35
^{13}C *p,p'*-DDE	21.77	21.83	21.83	21.83	21.82	21.83	21.83	21.83
α-HCH	14.57	14.57	14.57	14.57	14.57	14.66	14.57	n/a
γ-HCH	15.38	15.47	15.46	15.46	15.46	15.46	15.46	15.46
HCB	14.66	14.57	14.57	14.57	14.57	(14.84)	(14.84)	(14.84)
heptachlor	17.50	17.50	17.50	17.55	n/a	n/a	n/a	n/a
aldrin	18.62	18.62	18.62	18.67	18.62	18.62	18.62	18.62
oxychlordane	19.84	19.84	19.84	19.84	19.84	19.84	19.84	n/a
trans-chlordane	20.63	20.63	20.63	20.66	20.63	20.66	20.66	20.66
cis-chlordane	21.05	21.05	21.05	21.08	21.05	21.08	21.08	21.05
o,p'-DDE	20.73	20.73	20.73	20.76	20.76	20.76	20.76	20.76
p,p'-DDE	21.82	21.83	21.83	21.83	21.82	21.83	21.83	21.88
α-endosulfan	21.08	21.08	21.08	21.08	21.08	21.08	n/a	21.08
trans-nonachlor	21.23	21.23	21.23	21.23	21.23	21.23	21.23	n/a
cis-nonachlor	23.21	23.22	23.23	23.23	23.22	23.23	23.23	23.23
o,p'-DDT	23.31	23.32	23.32	23.32	23.32	23.32	23.32	23.32
p,p'-DDT	24.53	24.54	24.59	24.54	24.54	24.54	24.54	n/a

4.2 Retention time biota sample run

All data in table is retention time from biota sample run, including method blank samples and spiked blank samples, are presented in minutes. All samples are presented in the order they were analyzed. Samples marked [-] indicates shape of peak in chromatogram had low sensitivity and retention time could not be determined accurately.

Compound	Sample 7	Sample 12	Sample 9	Sample 11	Sample 14	Sample 17	Sample 18	Sample 21	Blank 1	Blank 2	Sample 3	Sample 8	Sample 13	Blank 3	Sample 22	SB1	Sample 19	Sample 6	Sample 15	Sample 2	Sample 16	SB2	Sample 4	Sample 10
OCN	-	31.49	31.65	31.93	31.53	31.53	31.55	-	31.52	31.54	-	31.99	31.68	31.61	31.68	31.59	-	31.99	31.83	-	31.84	-	-	-
^{13}C *p,p'*-DDE	21.91	21.87	21.87	21.96	21.91	21.91	21.88	21.97	21.89	21.89	21.97	21.99	21.95	21.92	21.96	21.93	21.96	21.96	21.96	21.98	21.99	22.05	22.42	22.52
α-HCH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	14.66	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
γ-HCH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HCB	14.77	14.75	14.77	14.79	14.77	14.79	14.81	14.97	<LOD	<LOD	15.06	14.89	14.85	<LOD	14.84	<LOD	<LOD	<LOD	14.86	<LOD	14.93	<LOD	<LOD	<LOD
heptachlor	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	17.60	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
aldrin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
oxychlordane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
trans -chlordane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	20.78	<LOD	<LOD	<LOD	<LOD	<LOD	20.81	<LOD	<LOD
cis -chlordane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
o,p'-DDE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
p,p'-DDE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	21.92	<LOD	21.89	<LOD	21.93	<LOD	<LOD	21.96	21.96	21.97	<LOD	<LOD	<LOD	<LOD	<LOD	22.00	<LOD	<LOD
α-endosulfan	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
trans -nonachlor	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	21.23	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
cis -nonachlor	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
o,p'-DDT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
p,p'-DDT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	24.73	<LOD	<LOD	<LOD	<LOD	<LOD	24.72	<LOD	<LOD

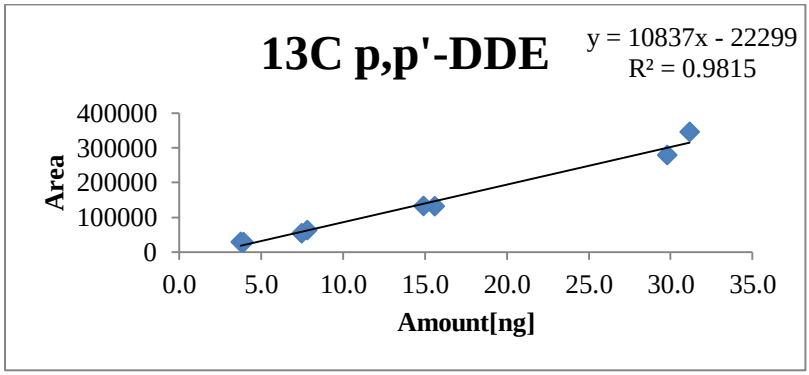
Appendix 5 Signal to noise

All data in table is signal-to-noise (S/N) values from biota sample run, including method blank samples and spiked blank samples, presented as S/N. All samples are presented in the order they were analyzed. Samples marked [-] indicates shape of peak in chromatogram had low sensitivity and area could not be determined. All values >10 meets criteria >LOQ and compounds could be used for quantitative analysis.

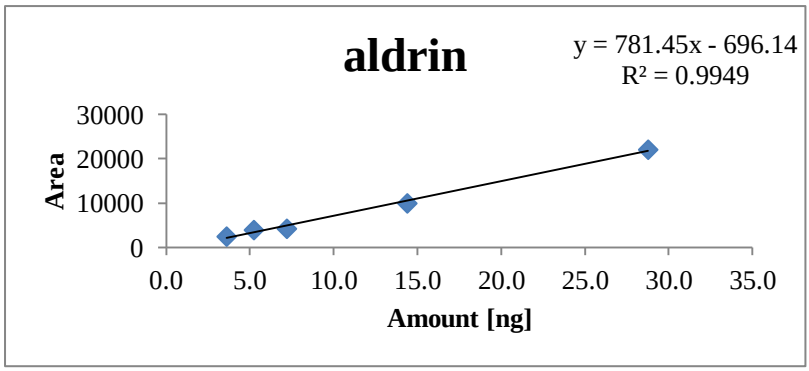
Compound	Sample 7	Sample 12	Sample 9	Sample 11	Sample 14	Sample 17	Sample 18	Sample 21	Blank 1	Blank 2	Sample 3	Sample 8	Sample 13	Blank 3	Sample 22	SB1	Sample 19	Sample 6	Sample 15	Sample 2	Sample 16	SB2	Sample 4	Sample 10
OCN	-	270	129	156	49	181	64	-	79	96	-	47	40	79	65	70	-	42	39	-	12	-	-	-
^{13}C *p,p'*-DDE	1783	990	836	612	2709	1934	1119	694	601	963	257	639	734	1992	1012	1411	>LOD	230	190	>LOD	>LOD	120	91	77
α-HCH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	20	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
γ-HCH	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
HCB	120	174	250	20	517	191	529	30	<LOD	<LOD	40	26	234	<LOD	163	<LOD	<LOD	<LOD	73	<LOD	137	<LOD	<LOD	<LOD
heptachlor	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	92	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
aldrin	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
oxychlordane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
trans -chlordane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	271	<LOD	<LOD	<LOD	<LOD	<LOD	143	<LOD	<LOD
cis -chlordane	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
o,p'-DDE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
p,p'-DDE	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	13	<LOD	38	<LOD	17	<LOD	<LOD	37	16	71	<LOD	<LOD	<LOD	<LOD	<LOD	35	<LOD	<LOD
α-endosulfan	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
trans -nonachlor	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
cis -nonachlor	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
o,p'-DDT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
p,p'-DDT	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	46	<LOD	<LOD	<LOD	<LOD	<LOD	23	<LOD	<LOD

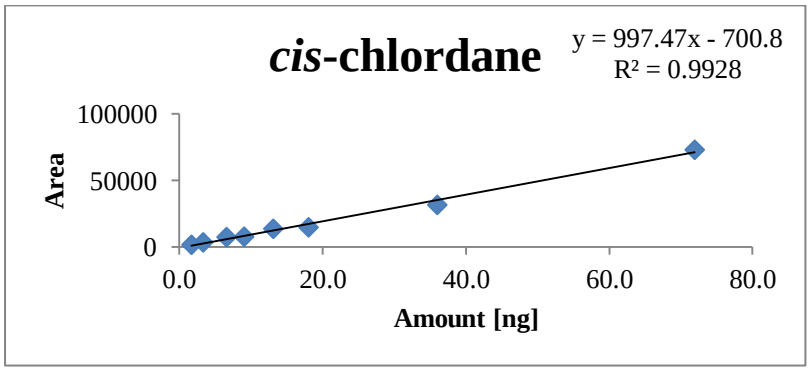
Appendix 6 Linearity test

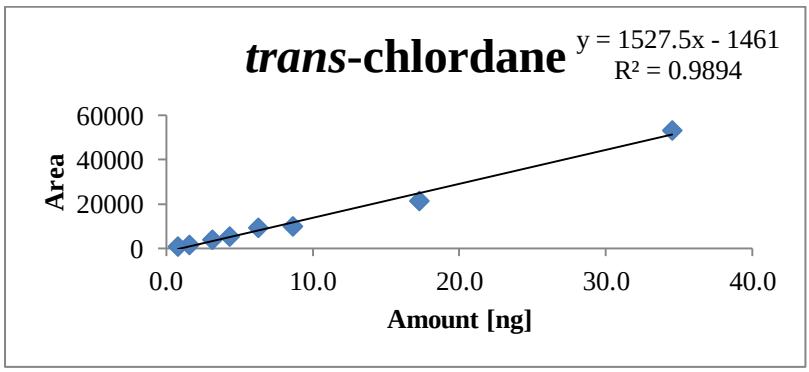
6.1 Linearity test for internal standard ^{13}C *p,p'*-DDE

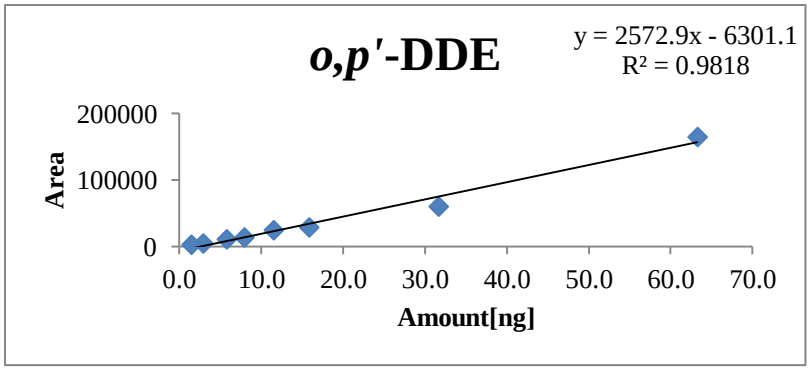


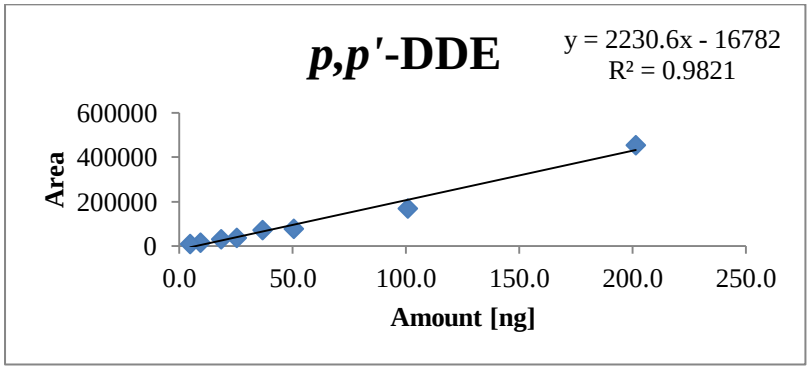
6.2 Linearity tests for quantification standard OCP mix

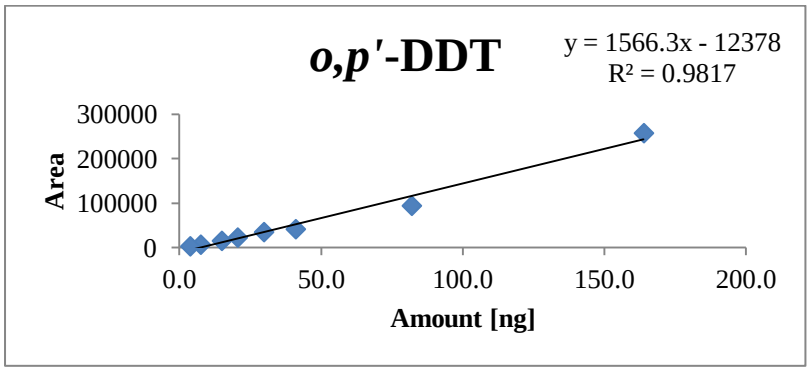


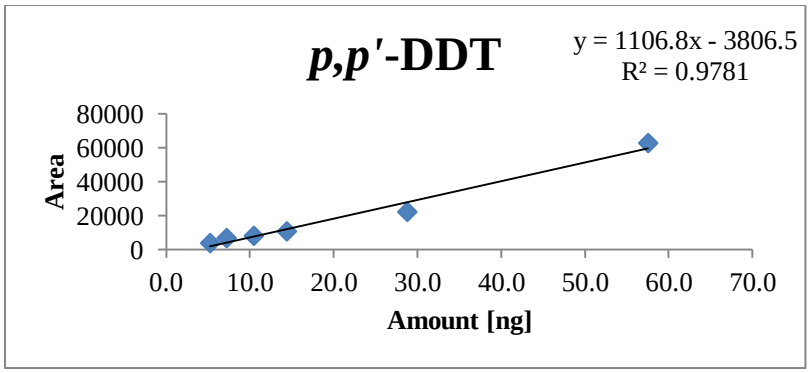


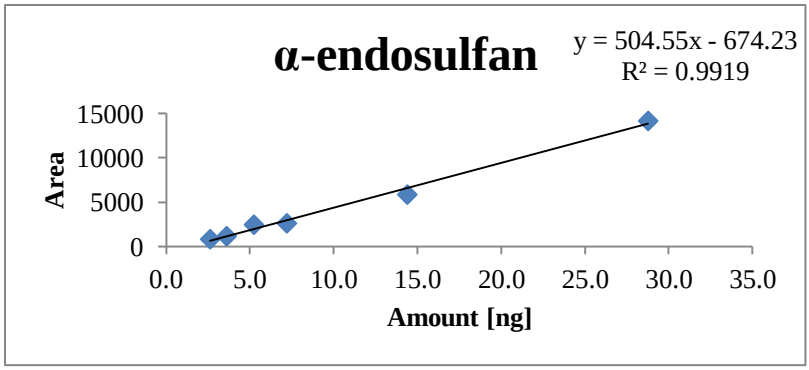


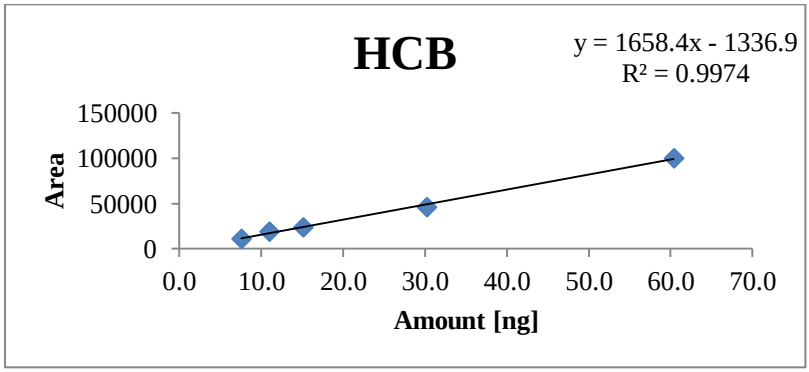

o,p'-DDE
y = 2572.9x - 6301.1
R² = 0.9818
Area
200000
100000
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Amount[ng]

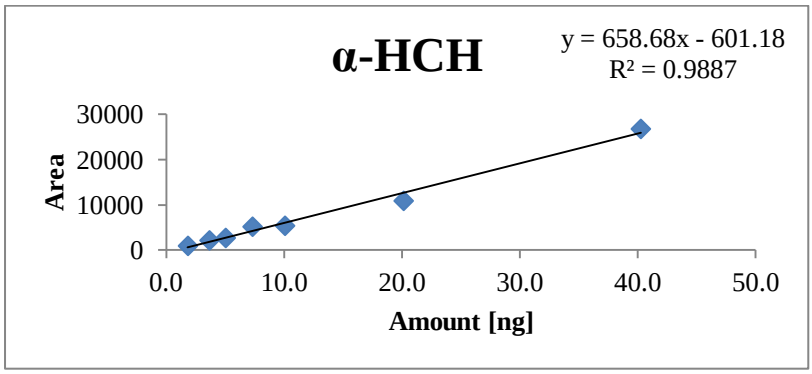

p,p'-DDE
y = 2230.6x - 16782
R² = 0.9821
Area
600000
400000
200000
0
0.0
50.0
100.0
150.0
200.0
250.0
Amount [ng]

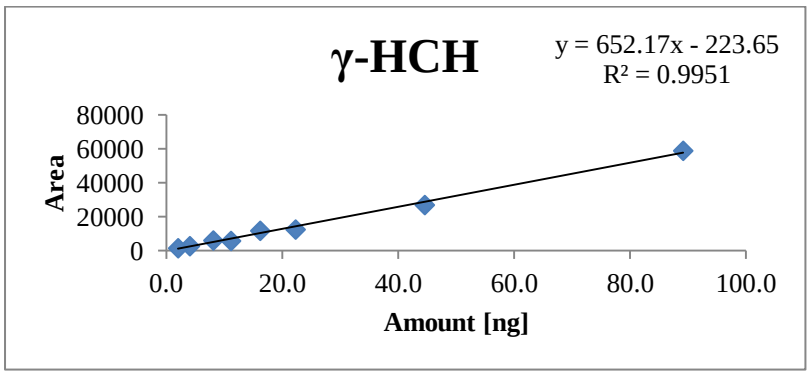

o,p'-DDT
y = 1566.3x - 12378
R² = 0.9817
Area
300000
200000
100000
0
0.0
50.0
100.0
150.0
200.0
Amount [ng]

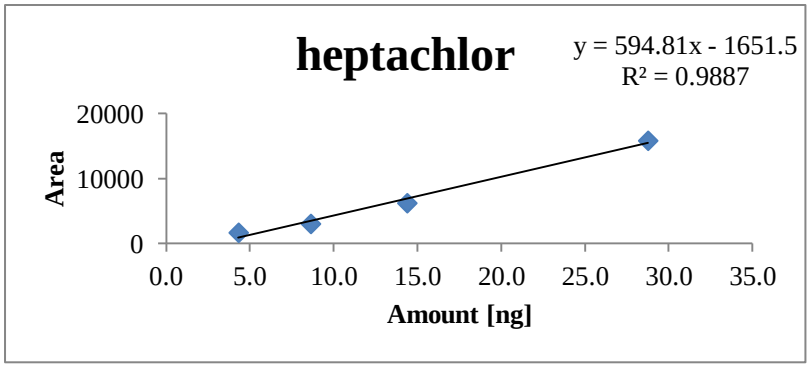

p,p'-DDT
y = 1106.8x - 3806.5
R² = 0.9781
Area
80000
60000
40000
20000
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Amount [ng]

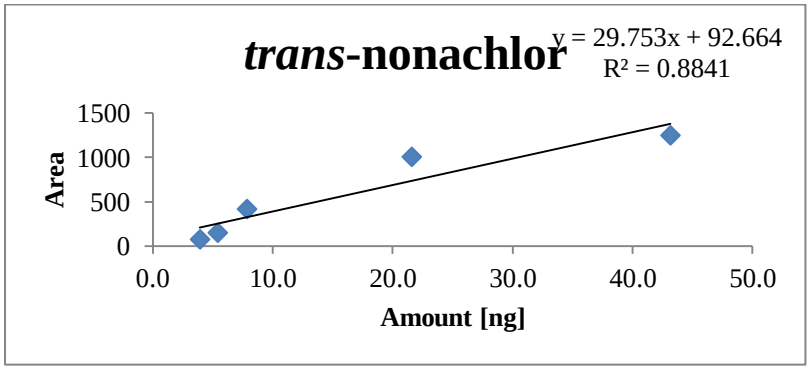

α-endosulfan
y = 504.55x - 674.23
R² = 0.9919
Area
15000
10000
5000
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
Amount [ng]

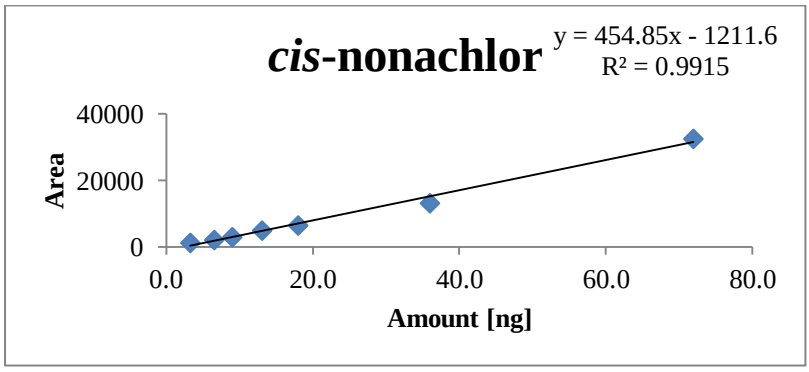

HCB
y = 1658.4x - 1336.9
R² = 0.9974
Area
150000
100000
50000
0
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
Amount [ng]

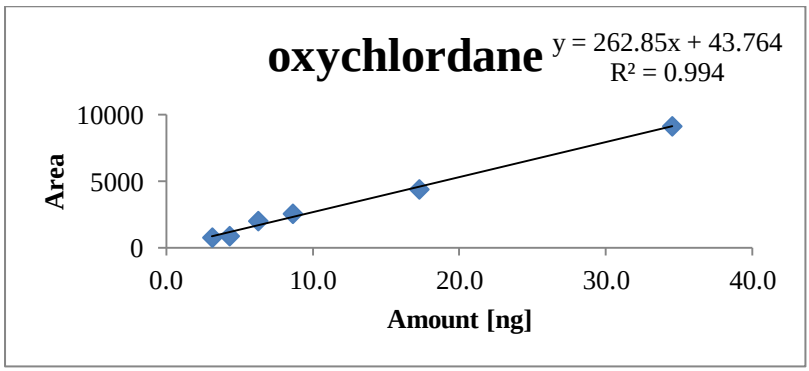

α-HCH
y = 658.68x - 601.18
R² = 0.9887
Area
30000
20000
10000
0
0.0
10.0
20.0
30.0
40.0
50.0
Amount [ng]


γ-HCH
y = 652.17x - 223.65
R² = 0.9951
Area
80000
60000
40000
20000
0
0.0
20.0
40.0
60.0
80.0
100.0
Amount [ng]


heptachlor
y = 594.81x - 1651.5
R² = 0.9887
Area
20000
10000
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
Amount [ng]


trans-nonachlor
y = 29.753x + 92.664
R² = 0.8841
Area
1500
1000
500
0
0.0
10.0
20.0
30.0
40.0
50.0
Amount [ng]


cis-nonachlor
y = 454.85x - 1211.6
R² = 0.9915
Area
40000
20000
0
0.0
20.0
40.0
60.0
80.0
Amount [ng]


oxychlordane
y = 262.85x + 43.764
R² = 0.994
Area
10000
5000
0
0.0
10.0
20.0
30.0
40.0
Amount [ng]

Appendix 7 Sample run

Linearity test run	
1 µl injection volume	
File name	**Comment**
hexane	
PCB25	
PCB50	
PCB100	
PCB200	
hexane	
pcb25_slowprog	
hexane	
pest_H_200	not all masses in the program
hexane	
pest_H_cal25	not all masses in the program
hexane	
eHCH_100	in MeOH
hexane	
hexane	
pest_H_200	not all pest found
hexane	
pest_H_50	PCB program
pest_H_50	
pest_H_50	
pest_H_50	all pest program
hexane	
pest_H_25	25 pg/ul OCN
pest_H_50	25 pg/ul OCN
pest_H_100	25 pg/ul OCN
pest_H_200	25 pg/ul OCN
hexane	
hexane	
pest_H100_tuning	1850V 0.6, 25 pg/ul OCN
pest_H100_tuning	1850V 1.0
pest_H50_tuning	1850V 1.0
pest_H50_tuning	1850V 0.6
hexane	
OCN_50	
OCN_100	
hexane	
pest_L_25	50 pg/ul OCN, 1850V, 1.0
pest_L_50	50 pg/ul OCN, 1850V, 1.0
pest_L_100	50 pg/ul OCN, 1850V, 1.0

Sample run	
2 µl injection volume	
File name	**Comment**
hexane	needle test
pest_L2_100	
pest_L2_200	
pest_H2_25	
pest_H2_50	
pest_H2_100	
pest_H2_200	
hexane	
S-07	
hexane	
S-12	
hexane	
S-9	
hexane	
S-11	
hexane	
S-14	
hexane	
S-17	
hexane	
S-23	not inj
hexane	
S-18	
hexane	
S-21	
hexane	
hexane	
hexane	
pest_H2_100	
hexane	
B-01	
B-01_2nd	
hexane	
B-02	
hexane	block
S-03	
hexane	
S-08	
hexane	

pest_L_200	50 pg/ul OCN, 1850V, 1.0	S-13	
hexane		hexane	
pest_L_25	50 pg/ul OCN, 1850V, 0.6	B-03	
pest_L_50	50 pg/ul OCN, 1850V, 0.6	hexane	
pest_L_100	50 pg/ul OCN, 1850V, 0.6	S-22	
pest_L_200	50 pg/ul OCN, 1850V, 0.6	hexane	
hexane		hexane	
hexane		pest_H2_200	
hexane		hexane	
pest_H2_25	syringe was changed	MVB-01	
pest_H2_50		hexane	
pest_H2_100		S-19	
pest_H2_200		hexane	
hexane		S-06	
pest_L2_25		hexane	
pest_L2_50		S-02	
pest_L2_100		hexane	
pest_L2_200		S-16	not inj
hexane		hexane	
hexane	syringe	S-24	not inj
hexane		hexane	
pest_H2_25	second injection	S-25	not inj
pest_H2_50	second injection	hexane	
pest_H2_100	second injection	S-16_2nd	
pest_H2_200	second injection	hexane	
hexane		S-5	
pest_L2_25	second injection	hexane	
pest_L2_50	second injection	OCN_200_pg-ul	
pest_L2_100	second injection	OCN_50	
pest_L2_200	second injection	OCN_100	
hexane		hexane	
masstest_H_200		hexane	
masstest_H_100		hexane	
hexane		OCN_new_230_pg-ul	
hexane		hexane	
PCB50	old program	burning	burnng the col
S26_KHM		OCN_100	
hexane		hexane	
S13_KHM		OCN_50	
hexane		hexane	
PCB200		hexane	
PCB50+OCN		hexane	
hexane		hexane	
PCB50	1850V 1.0	hexane	
PCB50_06	1850 0.6	hexane	
PCB50_old	1850 0.3	hexane	

hexane	
hexane	
hexane	
	hexane
	OCN_new_230_pg-ul
	OCN_200_exp
	OCN_100
	hexane
	pest_H2_25
	hexane
	32PCB_50_pg_ul
	hexane
	OCN_50
	pest_H2_100
	hexane
	hexane
	MVB-02
	hexane
	S-01
	hexane
	S-04
	hexane
	S-20
	hexane
	S-10
	hexane
	hexane
	pest_H1_new_100_pg_ul RT changed
	hexane
	pest_H2_200_badOCN
	hexane
	pest_H2_200_badOCN 2
	hexane
	hexane
	hexane
	hexane
	hexane
	pest_H2_100_badOCN
	hexane
	hexane
	hexane
	hexane
	hex_after_col_cut
	hex_cut
	hex_cut02
	hex_cut03
	hex_cut04

Appendix 8 Relative Response Factor

8.1 Results of RRF_i

Compounds	H200	H100	H50	H25	L200	L100	L50	L25
α-HCH	0.06	0.05	0.05	0.05	0.06	0.05	0.05	-
γ-HCH	0.06	0.05	0.05	0.05	0.07	0.07	0.06	0.07
HCB	0.15	0.14	0.14	0.13	0.16	0.16	0.18	0.19
heptachlor	0.05	0.04	0.04	0.04	-	-	-	-
aldrin	0.07	0.06	0.05	0.06	0.07	0.07	0.04	-
oxychlordane	0.02	0.02	0.03	0.02	0.03	0.02	-	-
trans-chlordane	0.14	0.11	0.10	0.11	0.13	0.11	0.09	0.09
cis-chlordane	0.09	0.08	0.07	0.08	0.09	0.10	0.10	0.09
o,p'-DDE	0.23	0.17	0.16	0.16	0.19	0.17	0.15	0.17
p,p'-DDE	0.20	0.15	0.14	0.13	0.18	0.15	0.14	0.17
α-endosulfan	0.04	0.04	0.03	0.03	0.04	0.03	-	-
trans-nonachlor	0.003	0.004	0.002	0.003	0.005	0.002	-	-
cis-nonachlor	0.04	0.03	0.03	0.03	0.03	0.03	0.03	-
o,p'-DDT	0.14	0.10	0.09	0.10	0.10	0.09	0.07	0.05
p,p'-DDT	0.10	0.07	0.07	0.08	0.07	0.06	-	-

8.2 Results of RRF_g

Compounds	H200	H100	H50	H25	L200	L100	L50	L25
^{13}C *p,p'*-DDE and OCN	8.70	9.60	8.43	8.77	7.37	6.75	5.70	5.70

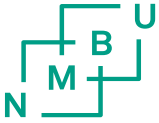
Appendix 9 Wet weight of sample and extracted organic matter

Wet weight of samples removed from biota tissue and lipid estimation by percentage extracted organic matter (EOM). ww = wet weight

Sample	Animal	Tissue	sample [g ww]	volume removed [µl]	EOM [%]
Blank 1				400	0.00
Blank 2				400	0.00
Blank 3				400	0.00
Spiked bank 1				400	0.00
Spiked blank 2				400	0.00
1	Svalbard reindeer	liver	5.10	400	7.84
2	Svalbard reindeer	liver	5.02	400	8.89
3	Svalbard reindeer	liver	5.02	400	12.22
4	Svalbard reindeer	liver	5.12	400	9.80
5	Svalbard reindeer	fat	1.02	200	32.86
6	Svalbard reindeer	muscle	5.06	400	9.17
7	Svalbard reindeer	muscle	5.25	400	18.72
8	Svalbard reindeer	muscle	5.06	400	13.52
9	Svalbard reindeer	muscle	5.16	400	55.96
10	Svalbard reindeer	muscle	5.18	400	20.98
11	Svalbard reindeer	muscle	5.12	400	8.62
12	Svalbard reindeer	fat	1.11	- [1)]	-

13	Svalbard reindeer	fat	3.06	200	65.03
14	Svalbard reindeer	fat	2.98	200	58.29
15	Svalbard reindeer	fat	3.00	200	52.22
16	Svalbard reindeer	fat	2.93	200	55.88
17	Svalbard reindeer	fat	3.20	200	43.31
18	Svalbard reindeer	fat	3.03	200	49.78
19	Pink-footed goose	liver	5.23	400	16.76
20	Pink-footed goose	liver	5.50	400	12.53
21	Svalbard rock ptarmigan	muscle	4.91	400	5.32
22	Svalbard rock ptarmigan	fat	1.00	200	36.63

[1)] No outtake was made as the sample contained too much fat as it had turned solid in the vial, separated from the solvent



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