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á Akureyri

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Occurrence of different persistent organic pollutants in Atlantic cod (*Gadus morhua L.*) in Icelandic waters

Vordís Baldursdóttir

Supervisors:
Kristín Ólafsdóttir, Ph.D., Department of Pharmacology and Toxicology, University of Iceland
Rannveig Björnsdóttir, Ph.D., University of Akureyri / Matís ohf.
Helga Gunnlaugsdóttir, Ph.D., Matís ohf.
Hrönn Jörundsdóttir, Ph.D., Matís ohf.

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Declaration

I hereby declare that I am the only author of this work and it is the product of my own research.

_____

Vordís Baldursdóttir

We, the undersigned, hereby confirm that this work satisfies examination requirements for M.Sc. degree in Natural Resource Sciences from the School of Business and Science, faculty of Natural Resource Sciences at the University of Akureyri.

_____

Kristín Ólafsdóttir, Ph.D., University of Iceland

_____

Rannveig Björnsdóttir, Ph.D., University of Akureyri and Matís ohf.

Abstract

Persistent organic pollutants (POPs) can have various harmful effects on the health of living organisms. They can be carcinogenic, affect the hormonal balance, the nervous system and the reproductive system. POPs have very long half lives in the environment and in living organisms, they are very lipophilic and accumulate in the food chain. Therefore, it is necessary to monitor these substances in nature.

The main objective of this project was to analyse the levels of some POPs in the Icelandic cod (*Gadus morhua*) and evaluate the effect that different factors such as; sex, age, sexual maturation, location of catch, season, have on these levels. In addition, to assess whether the analytical method that has been developed in our laboratory gives comparable results to other researchers.

Eleven POPs were analysed in the muscles from 64 individual cods and 38 livers from these same individuals. The amount of POPs detected in the cod was far below the previously set maximum levels in food Iceland. The levels observed in the liver were about 300 times higher than in the muscle, reason for this is that these substances are lipophilic and the muscle contains very little fat. The analytical method was found to be comparable to methods used by other researchers, and appears to be adequate for the same substances in chicken.

There is a need for further studies regarding the amount of POPs in other marine species as well as other organisms that feed on fish based products such as fish meal and are used for human consumption.

Keywords: POPs, PCBs, pesticides, Atlantic cod (*Gadus morhua*), *traceability, PSE (pressurised solvent extraction)*

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Útdráttur

Rannsóknir hafa sýnt að þrávirk lífræn efni geta haft margvísleg skaðleg áhrif á heilsu lífvera. Þau geta m.a. verið krabbameinsvaldandi, haft áhrif á hormónabúskap, taugakerfi og æxlunarfæri. Þrávirk lífræn efni brotna hægt niður í umhverfinu og í lífverum, þau eru afar fituleysin og magnast að styrk upp fæðukeðjuna og því er nauðsynlegt að fylgjast vel með efnunum í náttúrunni.

Helsta markmið verkefnisins var að meta magn og breytileika þrávirkra lífrænna efna í íslenska þorskinum (*Gadus morhua*) og hvort þættir eins og kyn, aldur, kynþroski, fiskimið, árstími, hefðu áhrif á magn efnanna. Einnig hvort þær mæliaðferðir sem þróaðar hafa verið á tækjabúnað Matís ohf. á Akureyri til mælinga á þrávirkum lífrænum efnum í fiskafurðum séu sambærilegar við þær aðferðir sem beitt er annarsstaðar.

Mæld voru nokkur þrávirk lífræn efni í holdi 64 þorska og lifrum 38 þeirra. Lítið magn þrávirkra lífrænna efna greindist í þorskinum og langt undir þeim mörkum sem leyfð eru. Magnið sem mældist í lifrunum var u.þ.b. 300 sinnum meira en í holdinu, þar sem efnin fylgja fitunni en þorskvöðvinn er afar fitulítill. Sú mæliaðferð sem notast var við stenst fullkomlega samanburð við þær aðferðir sem verið er að nota annarsstaðar, og virðist nýtanleg til að greina sömu efni í kjúklingi.

Full þörf er á frekari rannsóknum hvað varðar magn efnanna í öðrum tegundum sjávarlífvera sem og í lífverum sem fóðraðar eru á fiskafurðum og nýttar eru til manneldis.

Lykilorð: Þrávirk lífræn efni, PCB, varnarefni, þorskur, rekjanleiki, PSE (pressurised solvent extraction)

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List of abbreviations

AMAP	Arctic monitoring and assessment programme
ADI	Acceptable daily intake
ASE	Accelerated solvent extraction
CB	Chlorinated biphenyls
CRM	Certificate reference material
DDD	Dichlorodiphenyltrichloroethane
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
ECD	Electron capture detector
EPA	U .S Environmental Protection Agency
GC	Gas chromatography
Geom	Geometric mean
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HCl	Hydrogen chloride
He	Helium
IARC	International agency for research on cancer
IUPAC	International union of pure and applied chemistry
OSPAR	Oslo and Paris agreement
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzo-furans
PFOS	Perfluorooctane sulfonic acid
POPs	Persistent organic pollutants
PSE	Pressurized solvent extraction
PTDI	Provisional tolerable daily intake
Quasimeme	Quality assurance of information for marine environmental monitoring in Europe
TDI	Tolerable daily intake
Tox26	Toxaphene 26
Tox50	Toxaphene 50
UNEP	United Nations Environment Programme
WHO	World Health Organization

Units of weight

mg = milligrams (1 mg = 0.001 g)
µg = micrograms (1 µg = 0.001 mg)
ng = nanograms (1 ng = 0.001 µg)

1 Introduction

The present work was a part of a larger project, "Factors influencing the quality and value of the Icelandic cod; a value chain perspective" [Grandskoðum þann gula frá miðum í maga], that was funded by the AVS R&D Fund of Ministry of Fisheries in Iceland and Matis ltd. The overall aim of the project is to collect information on factors that may influence the quality and safety of cod caught in Icelandic waters. The analysis included various quality parameters of fillets through processing, liver weight and the chemical composition of the muscle and liver, including nutrients and undesirable substances. All these factors will be traced to fishing grounds, fishing season, age, sex, and sexual maturation among other factors, with the intention to maximize the value of the catch.

The aim of the work presented here was to analyse selected persistent organic pollutants (POPs) in samples collected from the muscle and liver of the fish.

1.1 Persistent organic pollutants (POPs)

Persistent organic pollutants (POPs) are compounds that can affect the environment and organisms for a long time. Many of them have long half-lives in the environment, some for many years and even up to decades. These compounds are lipophilic as well as very stable and therefore they magnify easily up the food chain (Borja *et al.*, 2005; Lohmann *et al.*, 2007). Most of the POPs have the possibility to undergo long range transport and therefore they can be found a long way from where they were used. Most of the compounds can also be found at higher concentrations in the northern region than in the region they were mostly used. The way the compounds travel has been described as global distillation (Fig. 1) or by the grasshopper effect (Wania *et al.*, 1998; Wania & Mackay, 1993). There are factors that affect how the POPs can be transferred to and within the northern regions such as climate change, geographical location and physical properties of the compounds. The compounds can be carried to the north with the atmosphere, sea currents, ice drift and rivers (Barrie *et al.*, 1992; Burkow & Kallenborn, 2000).

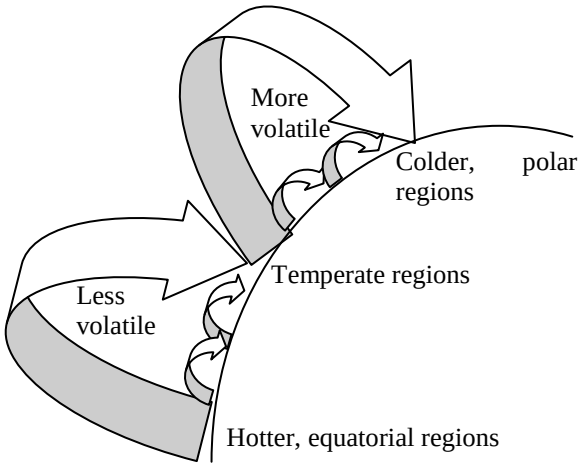

Figure 1. *The grasshopper effect or global distillation*

Most of these substances have either been banned or restricted for years or even decades, but because of their persistence remains of them can still be found widely in the environment. They are sometimes referred to as the "legacy" POPs because existent contamination is mostly due to remains from former emission and use of the chemicals (AMAP, 2009b; Riget *et al.*, 2010).

The POPs are often placed in three categories; pesticides, industrial chemicals and by-products, some of them can be categorized both as industrial chemicals and by-products such as Polychlorinated biphenyls (PCBs) and Hexachlorobenzene (HCB) (Stockholm Convention on Persistent Organic Pollutants).

Analysis of POPs in different types of food from Sweden, fish, meat, dairy, egg, fat and pastry showed much higher concentration in fish than in other products. Not only in the fish from the Baltic Sea, which is the most polluted fish caught in Sweden but in all fish analysed in the study (Darnerud *et al.*, 2006). Research on different kinds of food in northwest Russia shows that concentrations of POPs in fish is higher than in most other types of food, it is mostly in chicken eggs and fatty foods like butter and pork fat where the substances are found at higher concentrations than fish (Polder *et al.*, 2010).

Bioaccumulation and biomagnification is sometimes considered to be the same term but they are not. Some chemicals bioaccumulate but do not biomagnify. Bioaccumulation describes how chemicals build up in the organism; it could be based on age but also differ between species. However, biomagnification means that the concentration of these

substances increases from one trophic level to another. The uptake of POPs in the marine environment can be either through the skin, the respiratory system or by dietary absorption (Mackay & Fraser, 2000). The food chain is generally longer and more complicated in the aquatic ecosystem than the terrestrial food chain and therefore we can expect higher concentrations of POPs in marine animals than in terrestrial animals. Bioaccumulation seems to vary between species indicating that some of them have greater ability to excrete some POPs than other species (Olafsdottir *et al.*, 2001).

1.1.1 Stockholm Convention on Persistent organic pollutants

May 2001 in Stockholm, representatives of 90 countries signed a global agreement called: The Stockholm Convention on Persistent Organic Pollutants. Iceland ratified the Stockholm Convention in May 2002, first of the Nordic countries (Ministry for the Environment, 2004). The Convention itself was not fully ratified until May 2004 when the condition of fifty national confirmations of the ratification was fulfilled (Stockholm Convention on Persistent Organic Pollutants).

The purpose of the Convention is to protect human health and the environment from persistent organic chemicals. The agreement addresses how the member states should take measures pertaining to various issues related to persistent organic chemicals. According to the convention, measures should be taken to reduce or restrict the use of the chemicals and eliminate release of their products either intentionally or not. In addition, in which way the countries could reduce emissions of waste that may contain persistent organic pollutants and how their destruction should be organized. The member states need to make action plans on how they intend to honour the agreement and collect information concerning pollutant levels and in which way they intend to inform the public about the convention, the potential risks posed by the chemicals, the sources and replacements. The member states also commit themselves to promote research and monitoring on the use and unintentional production of these substances (Stockholm Convention on Persistent Organic Pollutants).

Initially twelve substances or groups of substances were included, all of which have known negative effects on both the ecosystem and humans:

- ● Aldrin
- ● Chlordane
- ● Dichlorodiphenyltrichloroethane (DDT)
- ● Dieldrin
- ● Endrin
- ● Heptachlor
- ●■♦ Hexachlorobenzene (HCB)
- ● Mirex
- ■ Polychlorinated dibenzo-p-dioxins (PCDD)
- ■ Polychlorinated dibenzo-furans (PCDF)
- ■♦ Polychlorinated biphenyl (PCBs)
- ● Toxaphene

In May 2009, it was agreed to add nine new substances or groups of substances as persistent organic pollutants under control of the Stockholm convention:

- ●■ α -hexachlorocyclohexane (α-HCH)
- ●■ β-hexachlorocyclohexane (β-HCH)
- ● Chlordecone
- ♦ Hexabromobiphenyl
- ♦ Hexabromodiphenylether (hexa-PBDE) and heptabromodiphenylether (hepta-PBDE)
- ● Lindane (γ-HCH)
- ● ♦ Pentachlorobenzene
- ♦ Perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride
- ♦ Tetrabromodiphenylether (tetra-PBDE) and pentabromodiphenylether (penta-PBDE)

● Pesticide ■ By-product ♦Industrial chemical

(Stockholm Convention on Persistent Organic Pollutants).

1.1.2 PCBs

PCBs are industrial compounds that are man-made and were first produced in 1929 (Hutzinger *et al.,* 1974). As reviewed by Borja *et al*. 2005, there are no known natural sources of PCBs.

1.1.2.1 Structure and formation

The general chemical formula of PCBs is $C_{12}H_{10-n}Cl_n$ with n= 1-10 and the basic structure are two benzene rings that are linked together on the first carbon atom (Erickson, 1997). When the biphenyl molecules react with Cl_2 the hydrogen atoms are replaced by chlorine atoms and thus forming the PCBs (Baird & Cann, 2005). The chlorine atoms can bind to any carbon atom with different degree of chlorination and therefore there are 209 theoretical congeners of PCBs (Erickson, 1997), 130 of which are likely to occur in commercial products (UNEP, 1999). The toxicity of the PCB congeners differs, depending on the number and position of the chlorine atoms (Baird & Cann, 2005).

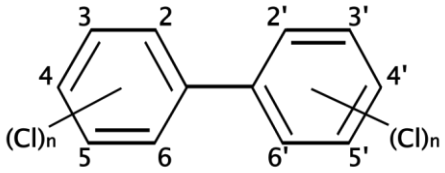

Figure 2. *General structure of PCBs*
Source: www.wikipedia.org

Ballschmiter and Zell presented a numbering system that was in accordance with the International Union of Pure and Applied Chemistry (IUPAC**)** rules where each congener got its own number from 1-209 (Ballschmiter & Zell, 1980). These numbers are usually used as abbreviations for the PCBs (Erickson, 1997), and this method is applied in this thesis.

The presence of PCBs in the biosphere is commonly analysed by quantifying the PCB7, which are seven different PCBs, the so-called marker PCBs or Dutch 7. These seven PCBs are CB-28 CB-52 CB-101, CB-118, CB-138, CB-153 and CB-180. These PCBs have been selected as indicators for the presence of PCBs in the samples because of their abundance and/or toxicity, but to analyse the overall amount of PCB compounds in the sample would require the quantification of 30-50 different congeners (Muir & Sverko, 2006).

The half-lives of PCBs are estimated to be from a few hours up to decades depending on the congener in question and there is a large difference between air, water, soil/sediment

and biota. For CB-28 the half-life is 3 days to about 3 years, but for CB-180 it is over a year in air and about 30-40 years in soil and sediment (Sinkkonen & Paasivirta, 2000).

1.1.2.2 Origin and use

As previously stated, the PCBs have mainly been produced intentionally by humans but the substances can also be formed unintentionally during combustion of waste and as a by-product in the production of various chemicals that contain chlorine (Erickson, 1997).

PCBs or mixtures containing PCBs were produced under several different trade names depending on manufacturer such as Aroclor (USA), Chlorofen (Poland), Clophen (Germany), Kanechlor (Japan), Phenoclor, Pyraléne (France) (EPA, 2009; Ishikawa *et al.*, 2007). PCBs were widely used because of their stability and were either used alone or mixed with oils, as insulators in transformers and capacitors. PCBs were also used for other purposes such as softeners in various oil products, as additives in all kinds of plastics even packaging materials for food, glue, printing ink, paint, waxes and copy paper (Borja *et al.*, 2005; Breivik *et al.*, 2004). PCBs still exist in some old electrical devices but due to the Stockholm Convention, countries have until 2028 to eliminate those equipments (AMAP, 2009b; Stockholm Convention on Persistent Organic Pollutants). That extensive use of PCBs and their ability for long range transport has resulted in their presence almost anywhere on earth, regardless of where they were used (Riget *et al.*, 2010; Wania & Mackay, 1993).

The extent of the PCBs problem became apparent when Sören Jensen, a Danish scientist, found unknown peaks in the chromatograms when analysing pesticides (p,p´-DDT) in white-tailed sea eagle eggs in 1964. It wasn´t until after further studies two years later that these compounds were identified to be PCB congeners (Anonymous, 1966; Hutzinger *et al.*, 1974; Jensen *et al.*, 1972).

1.1.2.3 Toxicity

Twelve of the PCB congeners have either none or one chlorine atom in the ortho position. These have the potential for a planar three-dimensional structure and are therefore termed dioxin-like PCBs or coplanar PCBs. Among them is one of the PCB7 i.e. PCB-118. As

reviewed by de Boer and Law (2003) it was in the 1980s that researchers discovered that the coplanar PCB congeners had toxicities similar to the extremely toxic dioxin compounds and because of that they are grouped with the dioxins. The same research showed that even if the concentration were 1000 times lower for these congeners than for the PCB7 the toxicity was perhaps 1000 times higher for the dioxin-like PCBs, than for PCB7.

Animal studies have shown that exposure to PCBs can have substantial impact on organisms such as reproductive and developmental effects, effects on the immune system, the thyroid and retinol binding proteins and some of them are also carcinogenic. (AMAP, 2009a).

Exposure to PCBs can have many and various effects on different fish species. Research shows that PCBs can cause: increased egg mortality and reduction in embryo survival ratio in Japanese medaka (Wisk & Cooper, 1990), reduced gonad size, reduced sexual maturation and increased larva mortality in white perch (Monosson *et al.*, 1994).

Birds have also shown various symptoms that have been found related to PCB exposure such as in decreased egg production in chickens (Lillie *et al.*, 1974). It has been demonstrated that there is a correlation between concentration of PCBs and DDE and eggshell-thickness, reproduction and nestling brood among white-tailed sea eagles *(Haliaeetus albicilla)* (Helander *et al.*, 2008; Helander *et al.*, 2002). Relationships have been found between the concentration of PCBs in liver and fluctuating wing asymmetry in European shag *(Phalacrocorax aristotelis)* chicks (Jenssen *et al.*, 2010).

Mammals are often at the top of the trophic level so they are very likely to be affected by PCBs. Research shows that these compounds can influence the hormonal system in animals like the estrogen levels in rats (Romkes *et al.*, 1987), and the progesterone levels and thyroxin concentration in minks (Byrne *et al.*, 1975). Reduction in the population of minks and otters in N-America is traced to pollution of PCBs (Wren, 1991). Immunosuppression is one of the consequences of exposure to PCBs and it may have increased mortality of seals, dolphins and beluga whales (De Guise *et al.*, 1995; Hall *et al.*, 1992; Lahvis *et al.*, 1995).

Because of all the observed effects that exposure to PCBs has on different animals, it is safe to assume that these compounds may have identical or similar effects in humans.

1.1.2.4 Effects of PCBs on humans

It has been demonstrated by studies that PCBs may have significant health effects on humans as well as on animals. These can be a suppressed immune system, cancer, a disrupted endocrine system, reproductive and developmental effects, liver disease, diabetes, asthma and other health problems (AMAP, 2009a; WHO, 1993). U.S Environmental Protection Agency (EPA) and International Agency for Research on Cancer (IARC) has listed PCBs as probable human carcinogens (EPA, 2009; IARC, 1998). There is a relationship between lower birth weight and abnormal fetal development and PCB concentration in the mother's milk (Jacobson *et al.*, 1990). It has been suggested that PCBs have adverse effects on neural development (Ribas-Fito *et al.*, 2001), and increased risk of endometriosis (Louis *et al.*, 2005).

Acceptable or Tolerable Daily Intake (ADI or TDI) or Tolerable Weekly intake (TWI) have been set by WHO for some of these substances. TDI is 0.17µg/kg body weight (bw) for HCB (ATSDR, 2002), for total sum of DDT and its degradation products PTDI (Provisional Tolerable Daily Intake) is 10 µg/kg bw (JMPR, 2000).

1.1.3 Pesticides

Pesticides are compounds widely used by the agricultural and food industry during both production and storage. They include chemicals like herbicides, fungicides and insecticides. The use of pesticides has decreased in part because of restrictions set by authorities on the production and use of the chemicals, but no less because consumers make greater demands on food purity. Consumer demands for cleaner food are evident by the increase in organically grown food, which is produced entirely without the use of pesticides. Levels have been established in many countries including Iceland for the allowable concentration of many pesticides in food, based on consumption surveys and suspected toxicities to humans [Reglugerð um hámarksgildi varnarefnaleifa í matvælum og fóðri, nr. 672/2008].

Use of pesticides for production of food is not the main reason why they are found in most food such as fish, dairy products and meat products, especially in the arctic area. The main reason is the ability of the chemicals for long range transport (Wania & Mackay, 1993) and biomagnification (Mackay & Fraser, 2000), it should be obvious that we are not using pesticides on our wild fish products.

As previously mentioned (chapter 1.1.1), 14 of the 21 substances listed as POPs by the Stockholm Convention are pesticides or have been used as pesticides.

1.1.3.1 Dichlorodiphenyltrichloroethane (DDT)

One of the best known pesticides is DDT, where p,p´-DDT is the main isomer. It was one of the most widely used insecticide in the world and is now found as a contaminant worldwide, regardless of where it has been used. As reviewed by Eskenazi *et al*. (2009) DDT has been used as a pesticide since 1939 and was most extensively produced from 1945-1965 (WHO, 1979). The main use of DDT was against mosquitoes that carried malaria and its use for this purpose is believed to have saved millions of lives. It was not until in 1962 when Rachel Carson's book Silent Spring was published that people began to wake up to the detrimental effects of substances released into the environment. Carson's book was very controversial but pointed out that chemicals can have adverse effects on other organisms such as birds. She even called DDT the "elixir of death" (Carson, 1962). Research has since then confirmed that Carson was right and subsequently DDT was taken off the market in most countries in the 1970s. The use of DDT is now prohibited in most Western societies but there are still some countries in Africa, Asia and Latin America that use DDT to protect against diseases such as malaria (Baird & Cann, 2005).

Dichlorodiphenyldichloroethylene (DDE) is the main degradation product of DDT about 90% of all DDT in animals is found as DDE some time after its release. As shown in Figure 3. DDE is formed by the elimination of hydrogen chloride (HCl), and dichlorodiphenyldichloroethane (DDD) is formed by reductive dechlorination (Baird & Cann, 2005).

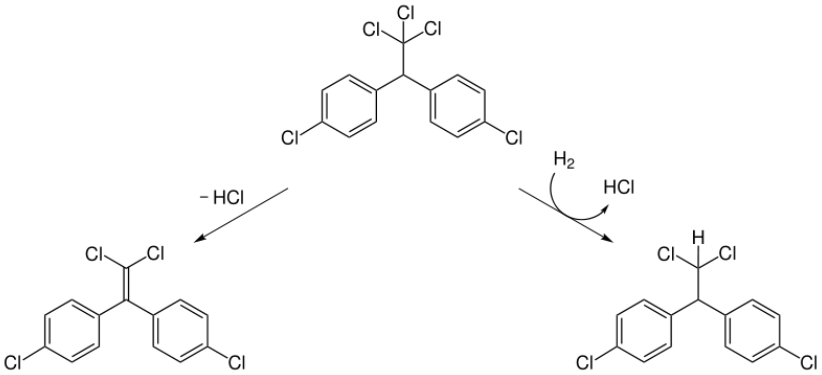

Figure 3. *Degradation of DDT to DDE (left) and DDD (right).*
Source: www.wikipedia.org

Li and Macdonald (2005) reviewed that estimated overall use of DDT from 1947 to 2000 was about 4.5 million tons, thereof about 2.6 for agricultural use only.

As reviewed by Eskenazi *et al*. (2009) many researchers have shown how the exposure to DDT and its degradation products can affect humans, e.g. evidence has been found for diabetes and effects on the fetus and the nervous system.

1.1.3.2 Hexachlorobenzene (HCB)

HCB is an organochlorine fungicide that has the molecular formula C_6Cl_6. HCB can be found ubiquitously in biota and air all over the world (Bailey, 2001). HCB has for decades been widely used as a fungicide but mostly on seeds of e.g. wheat, oat, barley, rye and onion, before it was banned. It was first used in 1933 as a fungicide (Barber *et al.*, 2005; Liu *et al.*, 2010). Today even though production of HCB is banned in most countries, it is still formed as a by-product in the production and incineration of chlorinated materials such as solvents and other chlorinated products (Liu *et al.*, 2010). Most countries have banned or implemented legislative actions about withdrawing the production and use of HCB in the 1980s and 1990s, whereas some countries regulated HCB as soon as the 1960s but others not until this century (Barber *et al.*, 2005). After the use of HCB was banned or restricted in most countries, its concentration has decreased in the environment by 75-90% since its use peaked in the late 1970s and early 1980s (Barber *et al.*, 2005; Eggesbo *et al*., 2009). Because of the uncertainty in the emission of HCB as a by-product, it is

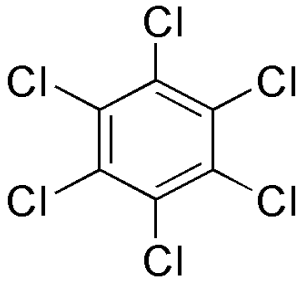

Figure 4. *The structure of Hexachlorobenzene*
Source: www.wikipedia.org

difficult to estimate how much reaches the environment every year. Bailey (2001) has estimated the global emission to be about 23,000 kg/yr with a range from 14,000 – 73,000 kg/yr. HCB can cause some adverse effects on organisms, like cancer, effects on lungs, liver, kidneys and on the nervous and immune system (WHO, 1997). EPA and International Agency for Research on Cancer (IARC) have listed HCB as carcinogenic (EPA, 2010a; IARC, 2001).

1.1.3.3 Toxaphene

Toxaphene is an organochlorine pesticide that is made from a complex combination of chemicals. It was first produced by Hercules Powder Inc. in 1945 under the name Hercules 3956 but over the years it has been produced under different names, depending on the manufacturer, such as Altox, Phene, Terphene, Huilex, Melipas, Penphene and some other (Hutzinger *et al.*, 2000). Theoretically, there are over 30,000 possible toxaphene congeners, but technical mixtures contain a few hundred, the two most persistent are TOX(8) 2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlororbornane and TOX(9) 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlororbornane (Vetter & Luckas, 1995) also known as toxaphene 26 and toxaphene 50 but also as Parlar 26 and Parlar 50, respectively (Buser *et al.*, 2000).

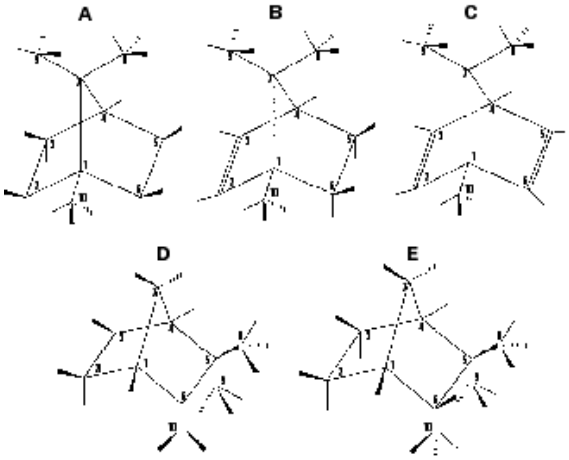

Figure 5. *Different structures of toxaphene (A) bornane, (B) bornene, (C) bornadiene, (D) camphene and (E) dihydrocamphene*
Source: http://ehp.niehs.nih.gov

Total use of toxaphene in the world was estimated to be 1.33 million tons from 1950 to 1993 and about 670,000 tons from 1970 to 1993 (Voldner & Li, 1995). Toxaphene was used instead of DDT as an insecticide in the 1960s after reduction of DDT usage (Paasivirta *et al*., 2009). The main use of toxaphene was for cotton production (Hutzinger *et al.*, 2000). Toxaphene was also used on other crops such as tobacco, vegetables, soybeans, peanuts and corn as well as on livestock like cattle, goats, sheep and pigs to control parasites that caused mange (Hutzinger *et al.*, 2000; Li, 2001). Because of the toxicity of toxaphene, it was used in the 1960s to control fish species in lakes and rivers prior to introduction of desirable fish

species, such as for sport fishing (Hutzinger *et al.*, 2000). IARC and EPA have listed toxaphene as probably carcinogenic (EPA, 2010b; IARC, 2001)

1.2 Icelandic environment

We Icelanders are very proud of our clean environment and talk about having the cleanest water and air, the best fish and lamb etc., but is our environment and food as pure as we would like it to be? Are we sufficiently aware to be awake in monitoring all the new compounds that man has put on the market and what impact they could have on the environment if released into it?

Pesticides were not in general use in Iceland but γ-HCH was used by farmers until in the late 1990 to bathe sheep against sheep ked and mange. Studies were carried out on the concentration of POPs in the Icelandic environment in the 1970s and 1980s by sampling mammals (Skaftason & Johannesson, 1979). From the late 1980s a number of studies has been performed on several bird species, such as seabirds and predatory birds as well as humans in Iceland (Magnusdottir *et al.*, 2005; Olafsdottir *et al.*, 2001, 2005).

Measurements of POPs in body fat of different animals, such as sheep, reindeer and butter made from cow´s milk in addition to trout and salmon fry from Icelandic fresh water showed what the authors referred to as very low concentrations of some of the substances (Skaftason & Johannesson, 1979, 1982). The detection limits were very high at that time compared to those we have now, and the levels found then would be considered high today.

Around 1990 organochlorine residues were analysed in both grey and harbour seals from Western Iceland, which gave a somewhat different result but a good evidence of how these compounds biomagnify. The results showed much higher concentrations than might be expected based on the use of these POPs, especially PCBs and DDT, in Iceland so there was an indication for long range transport of these compounds (Vetter *et al.*, 1995).

Temporal trends of POP concentrations analysed in black guillemots *(Cepphus grylle)* 1976-1996 showed evidence of slowly decreasing levels in West-Iceland (Olafsdottir *et al.*, 2005). Investigation on the gyrfalcon *(Falco rusticolus)* one of Iceland´s top predators showed that the concentration of POPs were at a much higher level in gyrfalcons than in the prey species such as black guillemot and ptarmigan *(Lagopus mutus)*. Furthermore, PCBs were found to be the most dominating organochlorine compounds in the Icelandic

environment (Olafsdottir *et al.*, 2001; Olafsdottir *et al.*, 1995; Olafsdottir *et al.*, 1998). Comparing concentration of POPs in guillemot *(Uria aalge)* eggs from Iceland, Faroe Island, Norway and Sweden showed no statistical difference between eggs sampled from the coasts of the Atlantic Ocean. A comparison with eggs from Sweden, sampled on an island in the Baltic sea, showed much higher concentrations than the ones from the Atlantic ocean (Jorundsdottir *et al.*, 2009a; Lofstrand *et al.*, 2008). Results on POPs in seven Icelandic bird species show extremely high concentration in great skua (*Stercorarius skua)* than in the other species and the concentration was so high that it suggested the possibility of adverse effects on the species (Jorundsdottir *et al.*, 2010a).

1.2.1 The ocean around Iceland

Systematic monitoring of pollutants, including POPs, in the marine biota around Iceland has taken place since 1989. The purpose of the monitoring is to meet the requirements of OSPAR (Oslo and Paris agreement) and AMAP agreements. Traces of heavy metals and residues of organic compounds are measured in both blue mussel and Atlantic cod livers (Jorundsdottir *et al.*, 2010b). Monitoring of undesirable contaminants in seafood began systematically in 2003, in products for human consumption as well as products of fish oil and from the fish meal industry. The objective of this monitoring is to estimate the status of Icelandic marine products based on the levels of undesirable substances (Jorundsdottir *et al.*, 2010d)

Despite the large volume of data that has been collected in these 20 years, the trend is not obvious. Statistical analysis of all the data is needed to investigate if there are spatial or temporal trends (Jorundsdottir *et al.*, 2010b).

1.2.2 Limits and regulations

There are no Icelandic maximum levels for PCB7 in fish neither in muscle nor liver. In 2004 maximum levels were set for food with less than 10% fat but that regulation was abolished in March 2010 [Reglugerð um (2.) breytingu á reglugerð nr. 411/2004 um ýmis aðskotaefni í matvælum, nr. 269/2010; Reglugerð um breytingu á reglugerð nr. 411/2004 um ýmis aðskotaefni í matvælum, nr. 056/2005] Maximum levels for pesticide residues has been set for many kinds of food and feed [Reglugerð, nr. 672/2008] and in 2010, the EU rules for

pesticides residues in food were introduced in Iceland [Reglugerð um (3.) breytingu á reglugerð nr. 672/2008 um hámarksgildi varnarefnaleifa í matvælum og fóðri, nr. 758/2010].

1.3 Research objectives

The main research objective for the project was to analyse PCB7 and four organochlorine pesticides in both liver and muscle of Atlantic cod (*Gadus morhua* L.) to see if there was any variation in the concentration of the congeners with respect to age, sex, length, weight and various other factors in individual fish. The aim was further to study whether there was a relationship between the concentration of the substances in liver and muscle of the cod and to estimate if there were any changes in the concentration in muscle through processing in a processing plant. One part of this study was furthermore to assess whether the analytical method used to analyse PCBs in liver and muscle of the Atlantic cod meets quality requirements and to evaluate whether it is possible to use the method to analyse POPs in different matrixes, such as in chicken.

2 Materials and methods

2.1 Selection and treatment of samples

2.1.1 Samples from the project: Factors influencing the quality and value of the Icelandic cod; a value chain perspective

As mentioned previously the present work was a part of a larger project "Factors influencing the quality and value of the Icelandic cod; a value chain perspective" for detail see the final report from that work (Gunnlaugsdottir *et al.*, 2010). Cod flesh and livers analysed in this study were collected and tagged with individual numbers onboard fishing trawlers and the tagged fish then went through a normal commercial processing line were the final products was a frozen fillet. When the fish was caught and tagged the liver from each individual was also collected, weighed, labelled and then frozen. The otoliths were also collected from each individual, and the age determined by the scientists of the Marine Research Institute. In addition, detailed data was registered about the fishing ground, date of capture, sex and sexual maturity of the fish. Furthermore, a total of 26 whole cods from scientific expeditions were collected for comparison, scientist at the laboratory filleted these fish samples.
The sexual maturity was also classified by the same scientists into four levels:

1) Not sexually mature; eggs/milt not yet formed
2) Pubescent; eggs/milt starting to form
3) Time for spawning
4) Spawning over; in spawning or equipped to breed

After the individual has reached level 2 for the first time, which is usually at age 5 - 6 years, levels 2 - 4 are repeated once a year.

Approximately 390 cods were collected from the processing plants following 13 fishing trips (~30 fish/trip) over a three year period (2007-2009). Further information on the samples taken in the different fishing trips and their pitch location can be shown in Table 1 and on Figure 6.

For analysis of persistent organic pollutants, muscle samples were collected from 64 cods randomly selected (~5 fish/trip) from the total of 390 cods. Liver samples were collected from 38 of these cods.

Table 1. *Samples collected for measurement of POPs in cod muscle and liver*

Year	Trip nr.	Month	Ship	Samples	Location fo fishing ground
2007	1	August	Sturlaugur H. Böðv. AK	30 fish and livers	66°37' -24°46'
	2	September	Sturlaugur H. Böðv. AK	31 fish and livers	63°13,691' -24°14,528'
	3	October	Bjarni Sæmundsson RE	32 fish and livers	66°52,96'-24°30,72' & 67°02,14' -23°53,48'
	4	December	Hringur SH	33 fish and livers	65°23'-24°58'
2008	5	March	Hringur SH	34 fish and livers	64°34' - 23°55' V
	6	March	Páll Pálsson ÍS	35 fish and livers	66°16,04' N - 23°10,39' V
	7	May	Helgi SH	36 fish and livers	65°43´ N - 24°54´ V
	8	August	Hringur SH	37 fish and livers	64°33,929' N 24°41,08' V
	9	September	Helgi SH	38 fish and livers	66°57'N -21°27'W
	10	October	Bjarni Sæmundsson RE	39 fish and livers	66°16,40' N - 25°07,91' V
	11	December	Hringur SH	40 fish and livers	64°50,45' - 24°26,80'
2009	12	February	Helgi SH	41 fish and livers	64°34' N -23°44' V
	13	March	Hafró-Rall	42 fish and livers	65°47,16' N - 25°17,63' V

The whole fish, fillets and the livers were transferred frozen to the laboratory of Matís ohf. - Icelandic Food and Biotech R&D where the fish and liver samples were stored until analysis, at this point the fillets were stilled tagged with the original tags from the date of capture and the livers carefully labelled, which ensured that all further analysis could be traced back to the individual cod sample.

Figure 6. *Location of the fishing grounds where the cod were caught for the processing plants*

The fish muscles were homogenized and then freeze dried with VirTis, Genesis SQ 25 EL (SP Industries; Gardiner, Gardiner, NY, USA) freeze dryer for three days as shown in Table 2. After freeze drying the tissue was grinded and then stored at room temperature until chemical analysis. The liver from the same fish was homogenized and frozen at -20°C for later analysis. The fat content of the liver was determined by an accredited method by the staff of Matís (AOCS, 1997), the fat content of the muscles was not analysed.

Table 2. *The method used on VirTis to freeze dry the samples*

Step	Rate/ Hold	Temp. (°C)	Time (min)	Pressure (mT)
1	Hold	-15.00	220	400
2	Hold	-10.00	220	350
3	Hold	-5.00	220	300
4	Hold	-2.00	400	250
5	Hold	5.00	400	200
6	Hold	10.00	400	150
7	Hold	15.00	400	100
8	Hold	20.00	2200	50

2.1.2 Oven baked cod

To investigate the effect of cooking on the content of the eleven POPs, samples of cod were oven baked. From 15 of the 64 muscle samples, a subsample was minced and then weighed accurately into a beaker, the beaker sealed with aluminium foil, weighed again and then baked in Despatch oven (Despatch Industries, Minneapolis, MN, USA) for 15 minutes at 150 °C. After chilling the fish to room temperature the excess water was removed and the beakers weighed again and then put into a freezer (-18°C) over night. After freezing, the cooked fish was freeze dried using the same method as described in Table 1 and weighed again to estimate the dry weight. The same method as described in chapter 2.2 and 2.3 for the fish muscle was used for the extraction, cleaning and analyses.

2.1.3 Chicken breast

Part of the project was to evaluate the possibility of using the same method to analyse POPs in different matrixes such as chicken. Chicken breasts were obtained from three different producers, two Icelandic and one from Denmark, five breasts from each. The breasts were minced and a sample of all breasts from each producer pooled together into one sample. The pooled samples were freeze dried using the method shown in Table 2. After freeze drying

the samples the same method was used for the extraction, cleaning and analysing as for the fish muscle as described in chapter 2.2 and 2.3.

2.2 Pressurised solvent extraction (PSE)

POPs were extracted from the samples using a pressurised solvent extraction method utilising an Accelerated Solvent Extraction instrument (ASE®) (Dionex Corporation, Sunnyvale, CA, USA), using 97% n-hexane (Sigma-Aldrich, 34859) as extraction solvent.

Figure 7. *The ASE300® instrument*

All glassware was heated in Carbolite LHT5/60 high temperature oven (Carbolite, Hope Valley, England) for 4 hours at 450°C, prior to extraction, to reduce the risk for external contamination that may affect the results. To increase the permeability and remove excess water, the samples were mixed with Hydromatrix (Varian, 0019-8004) at different ratios depending of the sample type. A mixture of silica gel (Fluka, 60742) and sulphuric acid (Fluka, 84720) in the ratio 1:1 (w/w) was prepared. The Hydromatrix and silica gel were heated to 400°C for 4 hours prior to use, the Hydromatrix for minimum of 6 hours and the silica gel for at least 12 hours.

For quantification of the persistent organic pollutants, two PCB congeners were used as internal standards, 2,2´,5,6´-tetrachlorinated biphenyl (PCB-53) (AccuStandard, Inc., USA C-053S-TB) and 2,2′,3,3′,4,4′,5,5′,6-nonachlorinated biphenyl (PCB-198) (AccuStandard, Inc., USA C-0198S-TB). Prior to use, the internal standards were diluted using isooctane (Riedel – deHaën, 34918).

2.2.1 Preparation of samples for analysis

Approximately 2.5 g (weighed to the accuracy of 0.0001 g) of freeze dried fish muscle was weighed into a glass bowl, 80 µl of internal standard (0.5 ng/µl) added and Hydromatrix finally added up to a total weight of 5.0 g (weighed to the accuracy of 0.0001 g) followed by mixing. A 30 mm cellulose filter (Dionex Corporation, Sunnyvale, CA, USA) was placed in the bottom of a 33 ml ASE-cell. ~1 g of Hydromatrix was used to cover the filter, followed

by adding ~17 g of silica gel: sulphuric acid mixture to the cell, the mixture was used to remove fat and other matrix components from the samples. The sample mixed with Hydromatrix (5.0 g weighed to the accuracy of 0.0001 g) was then transferred to the cell and Hydromatrix added to completely fill up the cell in order to avoid any air space. A filter pad was then placed on top and filter insertion tool used to press down. The cell was then closed and placed in the ASE300 instrument.

For the analysis of fish livers, 19 g of the silica gel: sulphuric acid mixture were added on top of 1 g Hydromatrix in the cell. The cell was then filled with Hydromatrix and 0.05 – 1.0 g (weighed to the accuracy of 0.0001 g) of the homogenized liver sample and 80 µl of internal standard (0.5 ng/µl) was added to the cell on top of the Hydromatrix. The content was then mixed in the cell using a glass rod and Hydromatrix added on top for filling up the cell. A filter pad was placed on top and filter insertion tool used to press down prior to closing the cell and placing it in the ASE300 instrument.

2.2.2 Extraction using ASE300 Accelerated solvent extraction

Extraction was carried out with n-hexane as a solvent. The cell was loaded in the oven and filled with solvent prior to heating to 90°C and adjusting the pressure to 1500 psi. The heating temperature and the pressure were held for 10 min, the cell then flushed with clean solvent, using 60% of the cell volume. This process represents one cycle. If the extraction was carried out for two cycles, the ASE300 extraction instrument will automatically refill the cell with solvent and hold the pressure for another 10 min period at 90°C prior to repeated flushing. Finally, the system is purged with nitrogen (N_2) for cleaning. A schematic diagram of the process is shown in Figure 2.

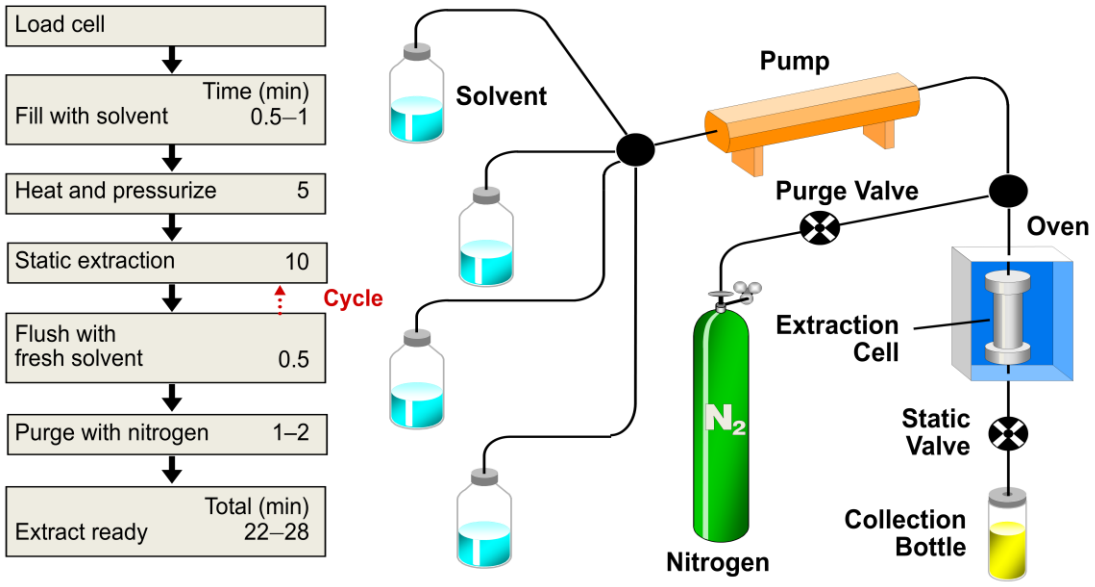

Figure 8. *A schematic diagram of the ASE method and a flow diagram of the analytical process*

2.2.3 Cleaning of samples following extraction

Following extraction, the extraction solvent in the ASE collection bottle contains POPs from the samples. The solvent from the collection bottle was transferred into a 200 ml TurboVap glass bottle. The collection bottle was rinsed twice with 5-10 ml n-hexane and the solvent then poured into the same TurboVap glass bottle. The samples were evaporated to 1 ml in a TurboVap® II Evaporation System (Caliper Life Science, Hopkinton, MA, USA) followed by rinsing the bottle with 4-5 ml of n-hexane and finally evaporating to 0.5 ml. A Pasteur pipette was then used to transfer each sample to 10 ml test tubes followed by rinsing the TurboVap glass bottle with 2 ml of n-hexane that was transferred to the same test tube. Concentrated sulphuric acid (2 ml) was added to each tube, gently mixed and let rest for 20-30 min at room temperature, with a gentle mixing 2-3 times during the resting time. The sulphuric acid removes any remains of fat from the sample. The samples were then centrifuged for 30 min at 15°C and 4500 rpm in a Sorvall® legend centrifuge (MACH 1.6R, Kendro Laboratory Products Inc., Asheville, NC, USA). The hexane phase was transferred to a graded centrifuge tube using a Pasteur pipette and evaporated to 0.5 ml in TurboVap. Isooctane (1 ml) was then added to each tube and evaporated to 0.2 ml, prior to transferring to 2 ml GC vials with micro inserts.

2.3 Gas chromatography

Analysis of persistent organic pollutants was performed on a Clarus500 gas chromatograph (Perkin Elmer, Waltham, MA, USA), equipped with one injector, splitting to two columns Equity-5 and Equity-1701 (Supelco, Bellefonte, PA, USA) and two ECD detectors used. In order to run samples through both columns at the same time using one injector, a universal Y splitter (Perkin Elmer, Waltham, MA, USA) was used. When Y splitter is used the sample injected is divided in half between the two columns right after the injection.

Helium (He) was used as carrier gas with splitless injection of 2.0 µl for 1.5 min, the flow was 4 ml/min for 1.5 min then adjusted to 1 ml/min, for the rest of the chromatopraphic run. The injection temperature was 270°C and detectors temperature 370°C. The oven was set to 85°C and temperature held for 2 min, then the temperature is raised for 30°C/min up to 200°C, kept for 28 min, then raised for 2°C/min up to 250°C, raised for 7°C/min to 280°C and kept there for 10 min.

Standard solutions were prepared from standards purchased from various suppliers (Table 3) for quantification of the selected POPs using calibration curves. Prior to use the standards were diluted with isooctane. For each standard, five different dilutions were prepared of the selected congeners with concentration of 0.02 – 0.3 ng/µl of the selected congeners and 0.2 ng/µl of the internal standards.

CB-53 (100 ng/µl) and CB-198 (100 ng/µl) were used as an internal standards. CB-53 was used for quantification of CB-28, -52, -101, HCB and p,p'-DDE, while CB-198 was used to quantify CB-138, -153, -180, Tox26 and Tox50.

Table 3. *Overview of the reference standards used in the study*

Standard type	**Standard supplier and their reference**	**concentration**
Congener Calibration Mix (contains: CBs:18, 28, 31, 44, 52, 101, 118, 138, 149, 153, 170, 180, 194 and 209)	AccuStandard, Inc., AE00061	10 ng/µl
PCB-53	AccuStandard, Inc., C-053S-TB	100 ng/µl
PCB-198	AccuStandard, Inc., C-0198S-TB	100 ng/µl
HCB	Dr. Ehrenstorfer GmbH, A14160000IO	100 ng/µl
p,p'-DDE	Dr. Ehrenstorfer GmbH, A12041000CY	100 ng/µl
Toxaphene 26	LGC Standards GmbH, DE-TOX 401	5 ng/µl
Toxaphene 50	LGC Standards GmbH, DE-TOX 402	5 ng/µl

2.4 Quality assurance

Quality assurance is very important in all chemical analysis where evaluations of concentrations are made as various factors can influence the quality of the results.

2.4.1 Reference material

To verify the accuracy of the extraction and cleaning methods it is important to analyse reference material of similar type as the samples. In this study, three types of certified reference material was used for different matrixes or substances all three of them were obtained from Quasimeme (Quality assurance of information for marine environmental monitoring in Europe). Quantification of PCBs, HCB and p,p´-DDE levels in cod muscle and chicken breasts were determined by comparison to QOR068BT reference material from plaice, PCBs, HCB and p,p´-DDE in cod liver by comparison to QOR086RT from cod liver and toxaphene by using QTX027BT reference material from herring. The conclusions of the analysis are considered acceptable if the calculated z score is > -2.0 and < 2.0, the measured z score during this project was in the range from -1.37 to 0.17 for individual substances (Appendix 1).

2.4.2 Prevention of contamination between samples

It is very important to give good attention to cleaning. All glassware was heated in oven at 450 ° C for at least 4 hours for removal of all organic residues. This is done in order to minimise cross contamination and the use of solvents. Parts that consist not only out of glass like the ASE cells, all plastic and metal parts were rinsed well with n- hexane after cleaning in dishwasher or by hand.

Blank solvent samples were prepared and analysed simultaneously with the samples to identify any interfering contamination. If evidence of the compounds is detected the amount in the bland is subtracted from the amount detected in the sample.

2.4.3 Limits of detection

Limits of detection (LOD) of POPs for the method was determined to be three times the standard deviation of the levels detected in the blank samples or 3-5x the noise level when nothing is detected in the blank samples. LOD for the instrument has been determined to be between 4.4 x 10^{-5} ng/µl and 5.0 x 10^{-5} ng/µl.

2.5 Calculation

Response factors were calculated for each substance using the average of the five different standard concentrations used for the quantification. The response factor indicates the response of a single substance versus the response of the internal standard and is calculated using the following equation (Swackhamer & Trowbridge, 1996).

$$RF = \frac{A_C/A_{IS}}{C_C/C_{IS}}$$

Where A_C = area of the congener
A_{IS}= area of the internal standard
C_C = concentration of the corresponding congener
C_{IS}= concentration of the corresponding internal standard

When the area of each substance has been found individually, the quantification of the compound in the sample is performed using the following equation where both response factor and internal standard are taken into account and assumed that the response factor in the sample is the same as in the standard (Swackhamer & Trowbridge, 1996).

$$SUB_{mass} = \frac{\frac{A_{sub}/A_{IS} * IS_{sample}}{RF}}{Sample_{mass}} * 1000$$

Where SUB_{mass} = Concentration (µg/kg) of substance in sample
A_{sub} = Area of substance detected in sample
A_{IS} = Area of internal standard detected in sample
IS_{sample} = Internal standard in ng put in sample
RF = Response factor calculated as shown above
$Sample_{mass}$ = Amount of sample in mg

2.6 Statistical analysis

Statistical analysis were performed using SigmaStat® 3.5 (Systat Software, Inc., Chicago, IL, USA). If normality test failed Mann-Whitney U test was used to compare two groups. When more than two groups were compared and normality test failed, Kruskal-Wallis one way analysis of variance on ranks or Equal variance test were used instead of one way analysis of variance (ANOVA).

Differences were considered statistically significant when $P < 0.05$.

3 Results

3.1 Persistent organic pollutants in cod muscle

Very low levels of persistent organics were found in Icelandic cod muscles (Table 4). The most abundant chemicals were the PCBs. The mean ∑PCB7 in cod muscle was 0.51 µg/kg with a range of 0.24-1.2 µg/kg. The mean ∑Tox 26&50 was 0.05 µg/kg, of HCB it was 0.04 µg/kg and mean of p,p´-DDE was 0.067 µg/kg. The entire dataset for the analysis of POPs in the cod muscle are shown in Appendix II.

Table 4. *POPs in cod muscle (µg/kg (ww))**

	∑PCB7	HCB	p,p´-DDE	∑Tox 26&50
Geom	0.506	0.0443	0.0668	0.0480
min	0.242	0.0189	0.0174	0.0143
max	1.19	0.127	0.299	0.195

*n= 64 for PCBs and 52 for pesticides

The results are shown in more detail in Figure 9 where the levels of each individual chemical measured in cod muscle can be compared. The mean of individual substances is in the range of 0.02 – 0.1 µg/kg (ww).

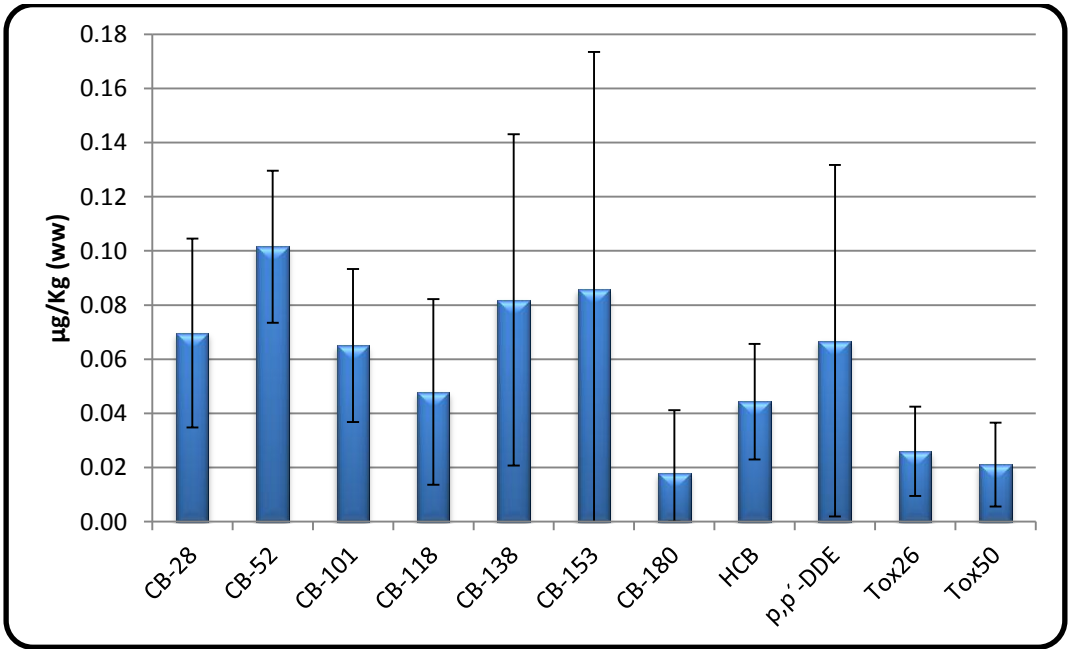

Figure 9. *Geom. ± SD value of the eleven persistent compounds analysed in cod muscle*

The male and female cods were divided into 6 different age groups. No statistical difference was seen between the sexes ($p = 0.119$, Kruskal-Wallis one-way analysis of variance on ranks) or different age groups ($p = 0.377$, equal variance test) (Table 5), although in some cases ($n \leq 3$) the number of individuals were too few for statistical analysis.

Table 5. *Effect of age and sex on POP levels in cod muscle (geometric mean, µg/kg ww)*

Age	**3**		**4**		**5**		**6**		**7**		**8+**	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
	(n=3, 2)	**(n=2, 2)**	**(n=3, 3)**	**(n=8, 7)**	**(n=6, 4)**	**(n=8, 7)**	**(n=8, 7)**	**(n=9, 7)**	**(n=6, 6)**	**(n=6, 4)**	**(n=3, 2)**	**(n=1, 1)**
CB-28	0.069	0.060	0.072	0.083	0.078	0.052	0.076	0.085	0.081	0.052	0.064	0.032
range	(0.047-0.143)	(0.034-0.107)	(0.044-0.093)	(0.041-0.136)	(0.030-0.135)	(0.025-0.105)	(0.034-0.117)	(0.038-0.122)	(0.030-0.110)	(0.033-0.101)	(0.044-0.120)	
CB-52	0.108	0.104	0.115	0.113	0.095	0.089	0.106	0.107	0.124	0.083	0.096	0.063
range	(0.077-0.172)	(0.086-0.125)	(0.085-0.136)	(0.078-0.169)	(0.063-0.151)	(0.072-0.122)	(0.064-0.142)	(0.066-0.139)	(0.094-0.161)	(0.071-0.102)	(0.083-0.127)	
CB-101	0.080	0.081	0.071	0.075	0.059	0.055	0.059	0.068	0.073	0.055	0.085	0.048
range	(0.054-0.118)	(0.062-0.107)	(0.050-0.139)	(0.033-0.146)	(0.025-0.107)	(0.035-0.092)	(0.035-0.110)	(0.052-0.102)	(0.053-0.112)	(0.026-0.158)	(0.069-0.095)	
CB-118	0.060	0.046	0.069	0.058	0.047	0.032	0.032	0.053	0.055	0.045	0.112	0.048
range	(0.048-0.078)	(0.019-0.110)	(0.045-0.107)	(0.030-0.137)	(0.022-0.083)	(0.011-0.051)	(0.012-0.081)	(0.030-0.173)	(0.038-0.074)	(0.016-0.106)	(0.062-0.160)	
CB-138	0.100	0.083	0.118	0.091	0.074	0.059	0.065	0.073	0.091	0.094	0.193	0.082
range	(0.065-0.131)	(0.043-0.159)	(0.068-0.245)	(0.032-0.215)	(0.028-0.154)	(0.034-0.079)	(0.032-0.157)	(0.033-0.295)	(0.054-0.151)	(0.033-0.251)	(0.131-0.253)	
CB-153	0.128	0.063	0.138	0.105	0.085	0.051	0.066	0.069	0.087	0.115	0.213	0.111
range	(0.082-0.168)	(0.023-0.172)	(0.063-0.286)	(0.026-0.303)	(0.040-0.179)	(0.023-0.085)	(0.024-0.202)	(0.027-0.359)	(0.033-0.216)	(0.026-0.354)	(0.124-0.348)	
CB-180	0.027	0.015	0.027	0.021	0.016	0.009	0.016	0.017	0.019	0.027	0.048	0.013
range	(0.014-0.040)	(0.007-0.031)	(0.010-0.074)	(0.005-0.065)	(0.006-0.039)	(0.002-0.014)	(0.008-0.047)	(0.007-0.095)	(0.008-0.054)	(0.005-0.112)	(0.024-0.085)	
HCB	0.033	0.027	0.040	0.049	0.045	0.038	0.043	0.061	0.047	0.043	0.055	0.031
range	(0.022-0.049)	(0.024-0.029)	(0.031-0.048)	(0.030-0.067)	(0.027-0.071)	(0.028-0.066)	(0.019-0.086)	(0.024-0.092)	(0.025-0.070)	(0.033-0.054)	(0.024-0.127)	
p,p´-DDE	0.064	0.031	0.088	0.074	0.077	0.050	0.054	0.068	0.082	0.055	0.241	0.058
range	(0.044-0.092)	(0.018-0.055)	(0.047-0.212)	(0.020-0.278)	0.050-0.155)	(0.022-0.084)	(0.017-0.173)	(0.034-0.239)	(0.020-0.179)	(0.028-0.102)	(0.194-0.299)	
Tox26	0.025	0.016	0.034	0.028	0.022	0.023	0.025	0.026	0.031	0.019	0.064	0.028
range	(0.021-0.029)	(0.010-0.024)	(0.021-0.059)	(0.012-0.043)	(0.006-0.040)	(0.011-0.031)	(0.011-0.033)	(0.016-0.052)	(0.013-0.073)	(0.011-0.033)	0.037-0.109)	
Tox50	0.018	0.025	0.029	0.022	0.014	0.018	0.018	0.021	0.021	0.024	0.066	0.035
range	(0.016-0.020)	(0.011-0.055)	(0.029-0.029)	(0.009-0.036)	(0.008-0.016)	(0.009-0.031)	(0.010-0.027)	(0.011-0.071)	(0.014-0.048)	(0.014-0.046)	(0.051-0.086)	

*n= count of samples that PCBs were analysed in, count of samples pesticides were analysed in

3.1.1 Factors that affect the ∑PCB7

There was further, no statistical difference found in the concentration of the substances analysed in cod muscle with respect to length, weight, sexual maturity (data not shown) and other factors except for the fat content in the liver. There was a linear correlation found between the ∑PCB7 and fat content of the liver (Figure 10). The concentrations in the muscles were highest when the fat contents of the livers were lowest.

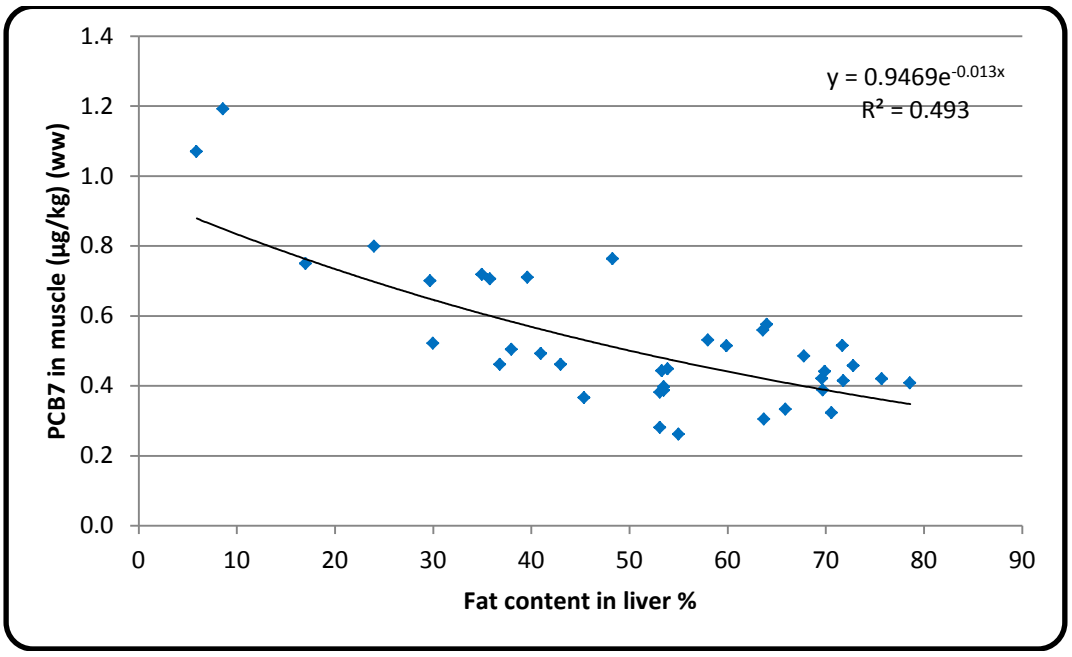

Figure 10. *The relationship between the ∑PCB7 in cod muscles (in µg/kg ww) and the fat content of the liver (%)*

Table 6 shows the relationship between individual POPs as well as the ∑PCB7 in cod muscle on a wet weight basis. A statistically significant ($p < 0.05$) positive correlation was seen between most POPs, except the lowest chlorinated PCBs (#28 and 52) and HCB.

HCB does not seem to correlate with any of the other substances in cod muscle except a little with the p,p´-DDE and Tox26 (r_s 0.26 – 0.28) and $p = 0.05$). Rather strong correlation was found between CB-28 and CB-52 and with CB-101 (r_s 0.41 – 0.78 and $p < 0.001$) but not with the other individual PCBs or the pesticides. The relationship was negative between these two substances and the pesticides except with HCB. There was moderate correlation between the following PCBs; CB-101, CB-118, CB-138, CB153 and CB-180 and the pesticides (r_s 0.40 – 0.93 and $p < 0.007$) except with HCB were it was (r_s 0.001 – 0.07 and $p < 0.64$). There

was a correlation between the ∑PCB7 and all of the substances (r_s 0.31 – 0.92 and $p < 0.004$) except with HCB were it was ($r_s = 0.1$ and $p = 0.47$).

Table 6. *Relationship between individual POPs measured in cod muscle on a wet weight basis*

	CB-52	**CB-101**	**CB-118**	**CB-138**	**CB-153**	**CB-180**	**HCB**	**p.p´-DDE**	**Tox26**	**Tox50**	**∑PCB7**
CB-28	r_s=0.78 p=<0.001	r_s=0.41 p=<0.001	r_s=0.08 p=<0.55	r_s=0.005 p=<0.97	r_s=-0.14 p=<0.25	r_s=-0.05 p=<0.71	r_s=0.24 p=<0.08	r_s=-0.04 p=<0.78	r_s=-0.005 p=<0.97	r_s=-0.22 p=<0.11	r_s=0.31 p=<0.002
	CB-52	r_s=0.55 p=<0.001	r_s=0.11 p=<0.41	r_s=0.07 p=<0.57	r_s=-0.14 p=<0.29	r_s=-0.05 p=<0.7	r_s=0.26 p=<0.06	r_s=-0.004 p=<0.98	r_s=0.21 p=<0.13	r_s=0.006 p=<0.97	r_s=0.36 p=<0.004
		CB-101	r_s=0.51 p=<0.001	r_s=0.60 p=<0.001	r_s=0.41 p=<0.001	r_s=0.5 p=<0.001	r_s=0.01 p=<0.95	r_s=0.43 0.002	r_s=0.59 p=<0.001	r_s=0.37 p=<0.007	r_s=0.71 p=<0.001
			CB-118	r_s=0.87 p=<0.001	r_s=0.81 p=<0.001	r_s=0.79 p=<0.001	r_s=-0.023 p=<0.87	r_s=0.73 p=<0.001	r_s=0.52 p=<0.001	r_s=0.47 p=<0.001	r_s=0.85 p=<0.001
				CB-138	r_s=0.93 p=<0.001	r_s=0.93 p=<0.001	r_s=-0.001 p=<0.99	r_s=0.76 p=<0.001	r_s=0.58 p=<0.001	r_s=0.60 p=<0.001	r_s=0.92 p=<0.001
					CB-153	r_s=0.93 p=<0.001	r_s=0.01 p=<0.97	r_s=0.73 p=<0.001	r_s=0.46 p=<0.001	r_s=0.49 p=<0.001	r_s=0.82 p=<0.001
						CB-180	r_s=0.07 p=<0.64	r_s=0.76 p=<0.001	r_s=0.52 p=<0.001	r_s=0.45 p=<0.001	r_s=0.85 p=<0.001
							HCB	r_s=0.26 p=<0.058	r_s=0.28 p=<0.045	r_s=0.09 p=<0.54	r_s=0.1 p=<0.47
								p.p´-DDE	r_s=0.74 p=<0.001	r_s=0.38 p=<0.006	r_s=0.69 p=<0.001
									Tox26	r_s=0.57 p=<0.001	r_s=0.56 p=<0.001
										Tox50	r_s=0.46 p=<0.001

r_s="Spearman rank correlation coefficient"

3.1.2 PCB-52 vs. PCB-153

CB-153 is generally the dominating PCB congener in the Icelandic environment. Therefore, it was unexpected that in some of the samples, CB-52 was the most abundant congener and as shown in Figure 11 the geometric mean of CB-52 is higher than for CB-153. However, the standard deviation (SD) for CB-153 is higher than for CB-52 this observation was not connected to the sex of the fish (Fig 11, $p = 0.272$).

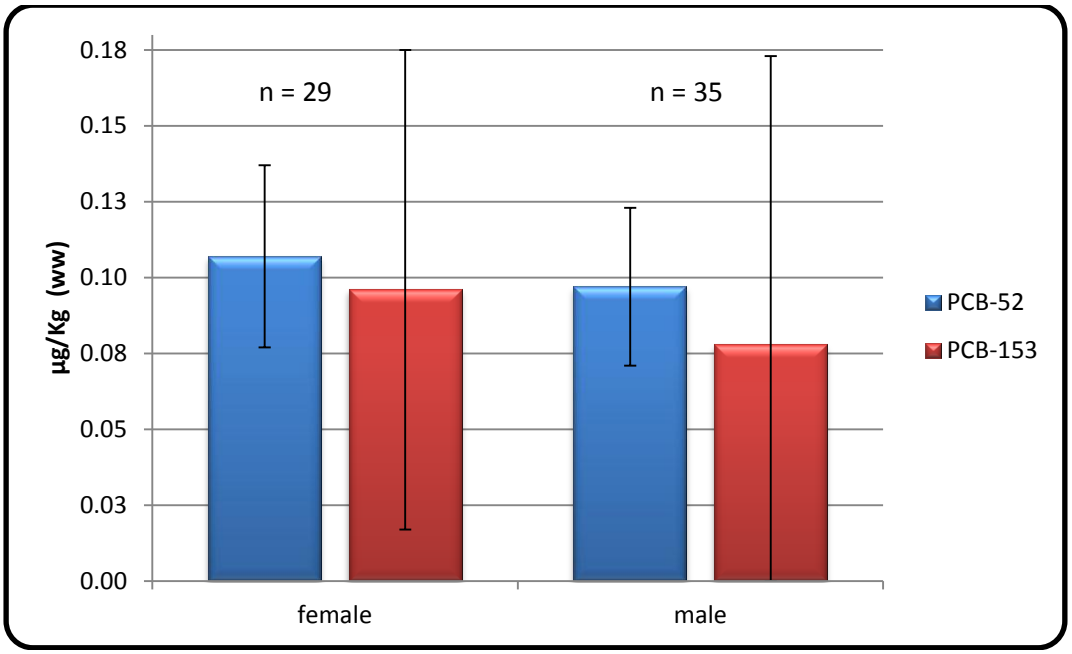

Figure 11. *Comparison of PCB-52 and PCB-153 based on the gender of the cod (geom. ± SD)*

The effect of age and sexual maturity on the levels of these two congeners was investigated and there was a significant statistical difference between the concentration of CB-52 and CB-153 between the age groups as shown in Figure 12 (P = <0.001). The geometric mean of CB-153 was only higher at the age of 8 years, CB-52 was higher for ager 3, 5 and 6 years and it was similar for age 4 and 7 years.

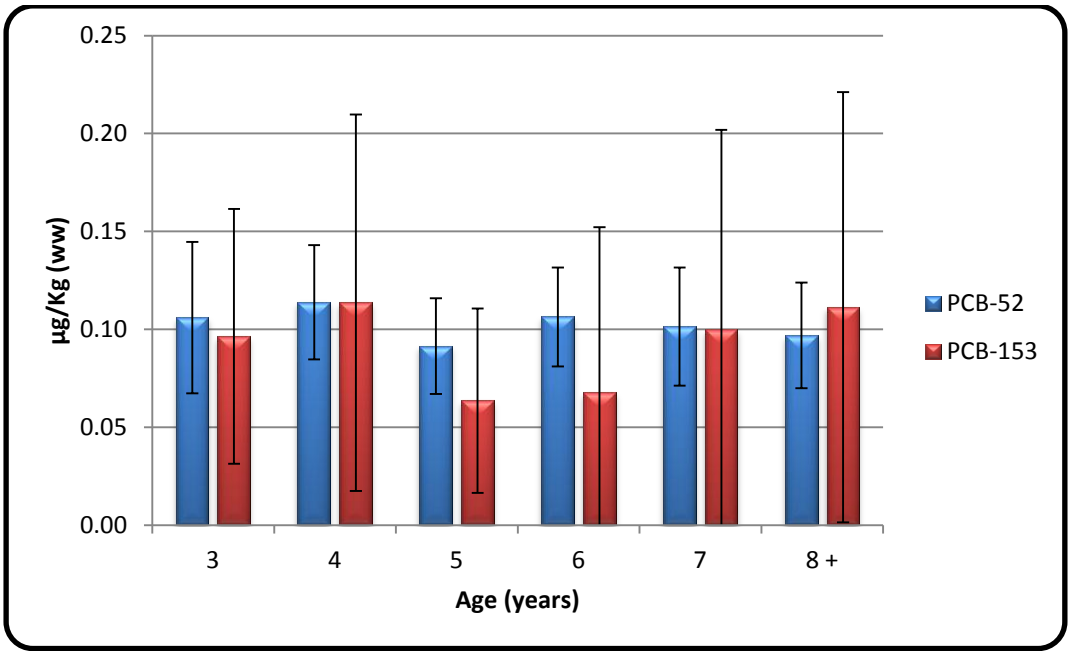

Figure 12. *Comparison of CB-52 and CB-153 regarding the age of the cod (geom.. ± SD)*

Sexual maturity also seems to affect the PCB congener concentration, since significant difference was found between different groups of sexual maturity as shown in Figure 13 ($p = <0.001$). The concentration of CB-153 was lowest for level 2 and CB-52 was always higher except for level 3 where it was a slightly higher for CB-153.

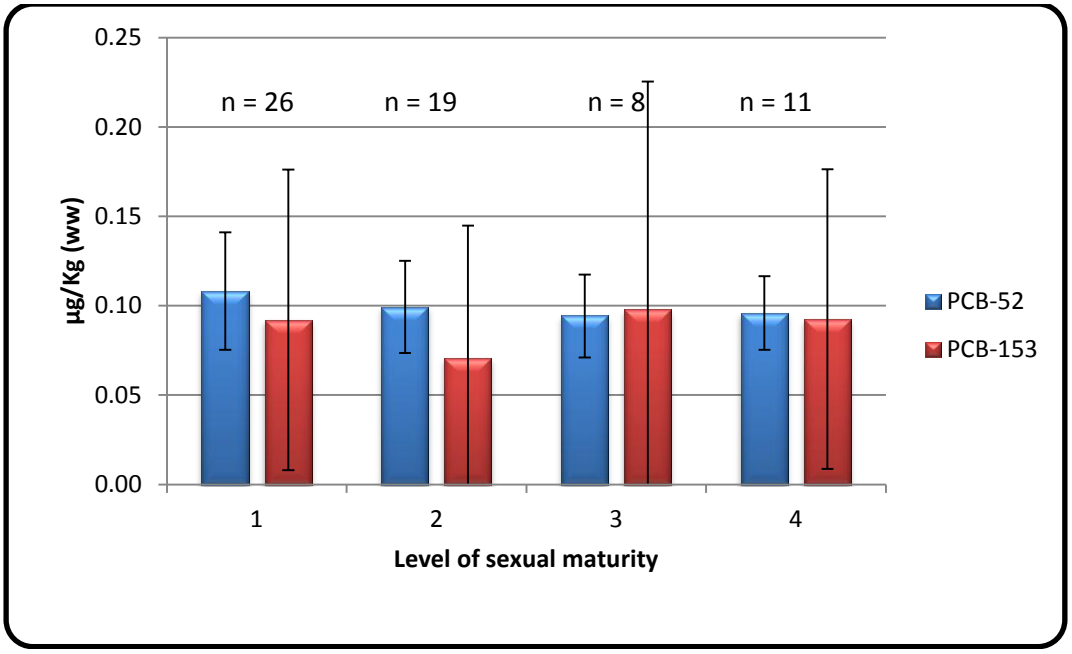

Figure 13. *Comparison of PCB-52 and PCB-153 in cod muscle based on the sexual maturity of the cods (geom. ± SD)*

3.2 Persistent organic pollutants in cod liver

Persistent organics were also measured in the liver from 38 of the 64 cods analysed (Table 7), in the livers the mean ∑PCB7 was 158 µg/kg with a range from 73 – 325 µg/kg (ww). The mean ∑Tox 26&50 was 44.5 µg/kg, for HCB it was 15.1 µg/kg and mean of p,p´-DDE it was 55.8 µg/kg. The entire dataset for the analysis of POPs in the cod liver are shown in Appendix III.

Table 7. *POPs in cod liver (µg/kg (ww))**

	∑PCB-7	HCB	p,p´- DDE	∑Tox 26&50	Fat content (%)
Geom	158	15.1	55.8	44.5	45.9
min	73.1	3.33	24.2	9.32	5.90
max	325	31.1	134	128	78.6

*n=38

Figure 14 shows the levels of the individual POPs in cod liver. Mean of individual substances was in the range of 8 – 56 µg/kg (ww) or about 400x higher than in the muscles. The most abundant chemicals in the livers were p,p´- DDE and CB-153.

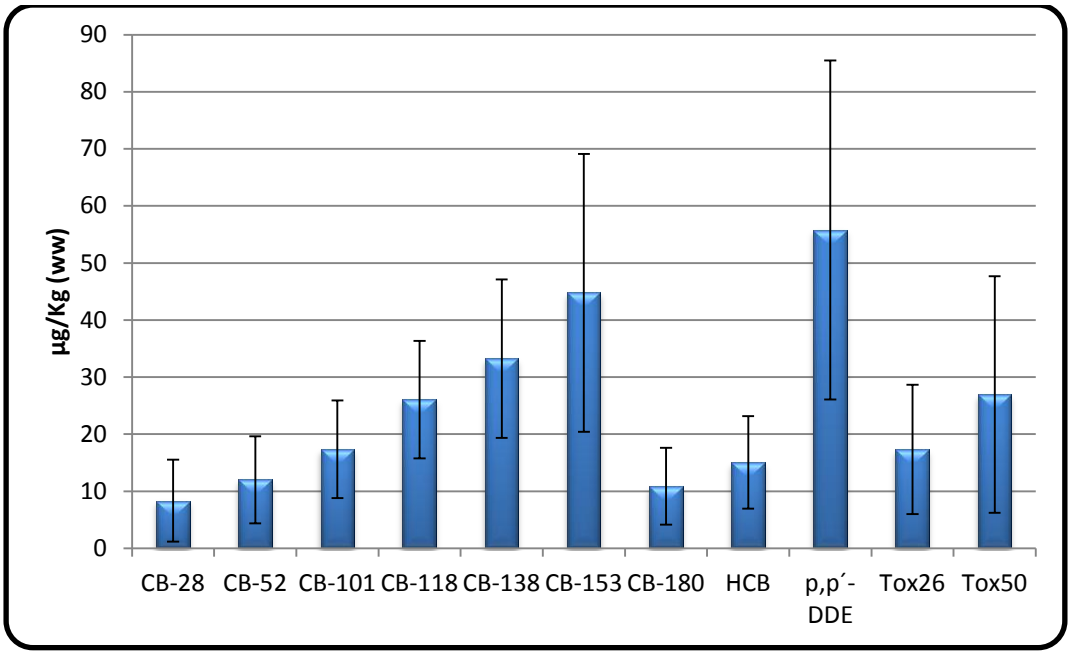

Figure 14. *Geom. ± SD value of the eleven persistent compounds analysed in cod liver*

The effects of age and sex on the POP levels in the cod livers can be seen in Table 8. The cod livers from males and females were divided into 6 different age groups. No statistical difference was seen between the sexes ($p = 0.687$, equal variance test) or different age groups ($p = 0.151$, equal variance test), although in some cases the number of individuals were too few for statistical analysis ($n \leq 2$).

Further more, no statistical difference was found in the concentration of the substances analysed in cod muscle with respect to length, weight, sexual maturity (data not shown) or other factors analysed.

Table 8. *Effects of age and sex of POP levels in cod liver (geom., µg/kg (ww))*

Age	**3**		**4**		**5**		**6**		**7**		**8+**	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
	(n=1)	**(n=2)**	**(n=2)**	**(n=4)**	**(n=3)**	**(n=4)**	**(n=7)**	**(n=4)**	**(n=5)**	**(n=4)**	**(n=1)**	**(n=1)**
CB-28	27.7	13.4	3.92	4.46	6.70	16.0	10.1	5.16	15.1	6.16	1.65	12.4
range		(11.4-15.9)	(2.31-6.66)	(3.05-9.99)	(3.63-16.2)	(13.6-19.0)	(3.11-17.7)	(2.97-6.77)	(5.21-33.1)	(3.52-14.1)		
CB-52	21.3	16.1	10.3	8.67	7.85	22.8	12.1	7.22	19.5	10.8	2.65	22.9
range		(14.9-17.4)	(10.1-10.6)	(5.81-18.5)	(2.83-19.8)	(20.8-25.3)	(2.53-23.8)	(2.62-11.9)	(9.65-35.2)	(7.14-16.5)		
CB-101	16.9	18.4	12.5	14.5	13.1	30.6	15.2	12.2	23.8	19.5	9.94	34.8
range		(12.7-26.7)	(11.3-13.9)	(12.1-20.1)	(4.59-34.4)	(28.7-32.8)	(8.05-27.7)	(7.77-19.2)	(16.7-34.0)	(14.9-26.1)		
CB-118	18.6	17.7	26.8	27.0	20.0	33.2	27.2	15.7	38.0	27.5	21.4	43.9
range		(12.3-25.5)	(26.6-27.1)	(21.8-32.8)	(15.6-27.5)	(28.0-40.5)	(19.2-45.1)	(10.9-21.9)	(31.2-59.0)	(17.0-47.2)		
CB-138	27.6	31.6	32.7	37.3	23.4	44.0	31.0	20.5	44.0	35.8	28.4	66.4
range		(19.7-50.5)	(30.6-35.0)	(30.7-51.2)	(18.3-36.3)	(40.6-53.8)	(22.9-45.3)	(14.3-28.2)	(27.6-73.5)	(22.0-63.7)		
CB-153	38.0	40.9	51.6	58.4	30.2	56.0	36.4	27.9	56.4	53.2	40.4	106
range		(26.2-63.6)	(45.2-58.8)	(45.2-89.9)	(20.5-49.6)	(52.4-67.4)	(18.9-63.8)	(20.0-38.1)	(30.9-117)	(33.3-115)		
CB-180	9.41	8.38	10.9	13.0	7.48	13.4	10.0	6.27	14.5	13.2	8.86	28.1
range		(5.85-12.0)	(8.97-13.2)	(9.37-21.4)	(5.75-10.8)	(12.8-14.3)	(6.69-14.8)	(4.49-9.24)	(8.46-35.3)	(8.18-31.0)		
HCB	16.4	12.0	10.5	10.4	10.6	25.8	16.9	14.7	22.6	15.2	3.33	19.2
range		(10.1-14.3)	(10.2-10.8)	(7.30-20.5)	(5.92-16.7)	(20.1-29.1)	(4.70-27.4)	(4.57-23.7)	(10.2-31.1)	(10.9-25.3)		
p,p´-DDE	36.6	29.9	40.9	51.3	46.8	101	53.1	38.3	89.0	58.3	38.4	92.2
range		(24.2-36.9)	(40.8-41.0)	(37.0-71.1)	(37.0-52.8)	(90.8-112)	(27.0-98.4)	(25.1-67.0)	(54.1-134)	(36.2-81.2)		
Tox26	19.4	12.4	13.4	13.9	10.1	38.8	16.9	13.3	27.9	17.0	4.74	30.9
range		(9.3-16.4)	(12.1-14.8)	(9.08-26.0)	(2.66-20.76)	(36.2-42.8)	(3.96-34.23)	(4.82-24.3)	(15.6-47.5)	(11.6-26.1)		
Tox50	33.6	22.8	18.6	18.2	20.3	72.2	24.0	21.4	40.0	25.7	6.93	48.7
range		(15.9-32.5)	(15.7-22.1)	(6.43-40.2)	(7.53-35.5)	(62.0-82.0)	(5.36-52.0)	(7.36-39.4)	(21.5-80.3)	(16.5-44.6)		

Table 9 shows the relationship between individual POPs as well as the ∑PCB7 in cod liver on a wet weight basis as well as the ∑PCB7 lipid weight.

A statistically significant ($p < 0.05$) positive correlation was seen between most POPs, except for PCB-28, HCB and ∑PCB7 based on lipid weight.

CB-28 correlates with the PCBs with lower chlorination (CB-52, 101 and 118) and the pesticides but not with the PCBs with higher chlorination such as CB-138, 153 and 180. CB-52 correlates with all of the substances except CB-153 although it is almost significant (r_s 0.31 and $p = 0.062$). HCB correlates with the lower PCBs (CB-28, 52 and 101) and the pesticides but not with the PCBs with higher chlorination (CB-118, 138, 153 and 180). The

correlation between the two toxaphenes and CB-153 is also close to being significant, Tox26 (r_s 0.29 and p = 0.076) and Tox50 (r_s 0.32 and p = 0.053).

∑PCB7 wet weight correlates with ∑PCB7 lipid weight and all of the individual substances (r_s 0.46-0.96 and p < 0.001) except HCB (r_s 0.29 and p = 0.083).

No relationship was observed between ∑PCB7 lipid weight and the lower chlorinated PCBs (CB-28, 52 and 101) or three of the pesticides (p,p´-DDE, Tox26 and Tox50). The relationship with HCB was negative (r_s -0.44 and p = 0.006) but there was a moderate positive relationship with the individual PCBs with higher chlorination (CB-118, 138, 153 and 180) as well as the ∑PCB7 wet weight (r_s 0.50-0.71 and p < 0.002).

Table 9. *Relationship between individual POPs measured in cod liver on a wet weight basis unless marked otherwise*

	CB-52	**CB-101**	**CB-118**	**CB-138**	**CB-153**	**CB-180**	**HCB**	**p.p´-DDE**	**Tox26**	**Tox50**	**∑PCB7**	**∑PCB7 (lw)**
CB-28	r_s=0.90 p<0.001	r_s=0.64 p<0.001	r_s=0.33 p=0.044	r_s=0.28 p=0.088	r_s=0.14 p=0.39	r_s=0.19 p=0.26	r_s=0.64 p<0.001	r_s=0.40 p=0.013	r_s=0.68 p<0.001	r_s=0.68 p<0.001	r_s=0.46 p<0.001	r_s=-0.15 p=0.38
	CB-52	r_s=0.84 p<0.001	r_s=0.47 p=0.003	r_s=0.48 p=0.002	r_s=0.31 p=0.062	r_s=0.36 p=0.029	r_s=0.73 p<0.001	r_s=0.62 p<0.001	r_s=0.87 p<0.001	r_s=0.88 p<0.001	r_s=0.63 p<0.001	r_s=-0.05 p=0.78
		CB-101	r_s=0.64 p<0.001	r_s=0.72 p<0.001	r_s=0.57 p<0.001	r_s=0.62 p<0.001	r_s=0.59 p<0.001	r_s=0.79 p<0.001	r_s=0.85 p<0.001	r_s=0.88 p<0.001	r_s=0.80 p<0.001	r_s=0.14 p=0.40
			CB-118	r_s=0.86 p<0.001	r_s=0.80 p<0.001	r_s=0.84 p<0.001	r_s=0.30 p=0.069	r_s=0.76 p<0.001	r_s=0.53 p<0.001	r_s=0.47 p=0.003	r_s=0.90 p<0.001	r_s=0.50 p=0.002
				CB-138	r_s=0.93 p<0.001	r_s=0.95 p<0.001	r_s=0.13 p=0.42	r_s=0.70 p<0.001	r_s=0.47 p=0.003	r_s=0.49 p=0.002	r_s=0.96 p<0.001	r_s=0.66 p<0.001
					CB-153	r_s=0.93 p<0.001	r_s=-0.07 p=0.68	r_s=0.56 p<0.001	r_s=0.29 p=0.076	r_s=0.32 p=0.053	r_s=0.89 p<0.001	r_s=0.71 p<0.001
						CB-180	r_s=0.07 p=0.68	r_s=0.66 p<0.001	r_s=0.38 p=0.018	r_s=0.39 p=0.016	r_s=0.91 p<0.001	r_s=0.66 p<0.001
							HCB	r_s=0.60 p<0.001	r_s=0.86 p<0.001	r_s=0.79 p<0.001	r_s=0.29 p=0.083	r_s=-0.44 p=0.006
								p.p´-DDE	r_s=0.79 p<0.001	r_s=0.77 p<0.001	r_s=0.75 p<0.001	r_s=0.19 p=0.25
									Tox26	r_s=0.97 p<0.001	r_s=0.59 p<0.001	r_s=-0.11 p=0.50
										Tox50	r_s=0.60 p<0.001	r_s=-0.12 p=0.48
											∑PCB7	r_s=0.53 p<0.001

r_s="Spearman´s rank correlation coefficient"

3.2.1 PCBs in cod liver

Looking at the average of each of the seven PCB congeners in cod liver where related to age of the fish much higher levels were observed, than in the muscle and almost the same pattern emerges for all of the PCBs. The levels of the more persistent PCBs (CB-118, 138 and 180)

follow the same pattern as PCB-153, whereas the lower chlorinated PCBs (CB-28 and 101) show a pattern more similar to that of PCB-52.

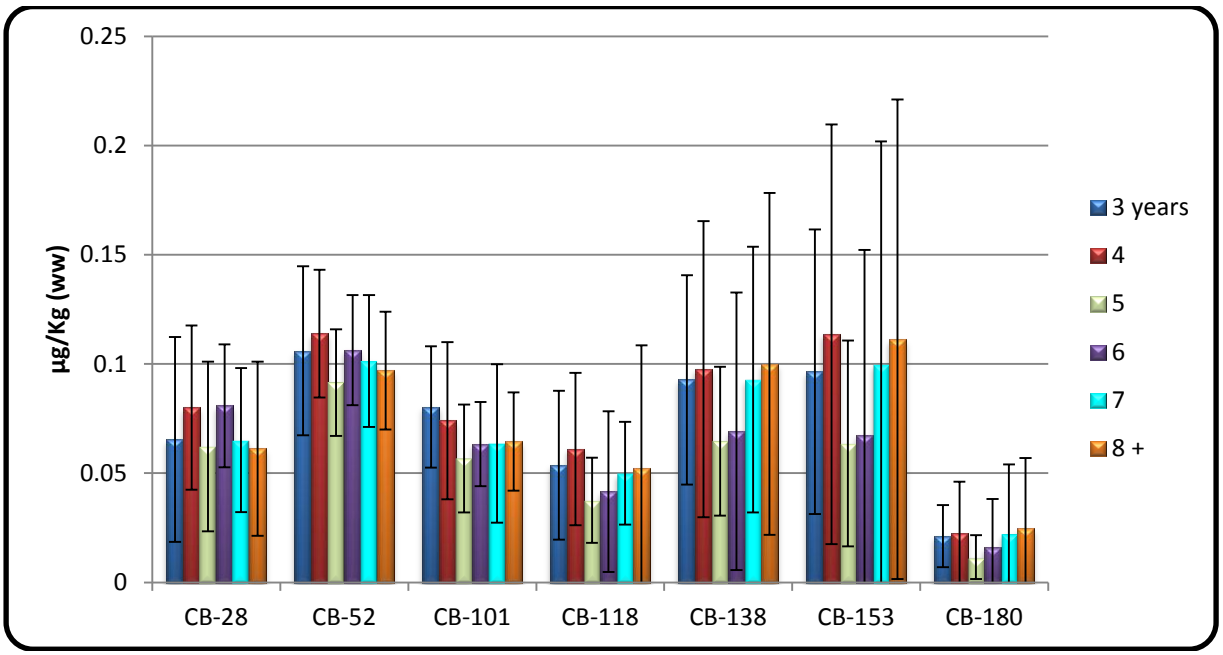

Figure 15. *Geom. ± SD of the seven PCB congeners in cod liver, with respect to age*

3.2.2 Pesticides in cod liver

When looking at the mean of each of the four pesticides analysed in cod liver with respect to age much higher levels than in the muscle were observed and the same pattern emerges for all of the pesticides.

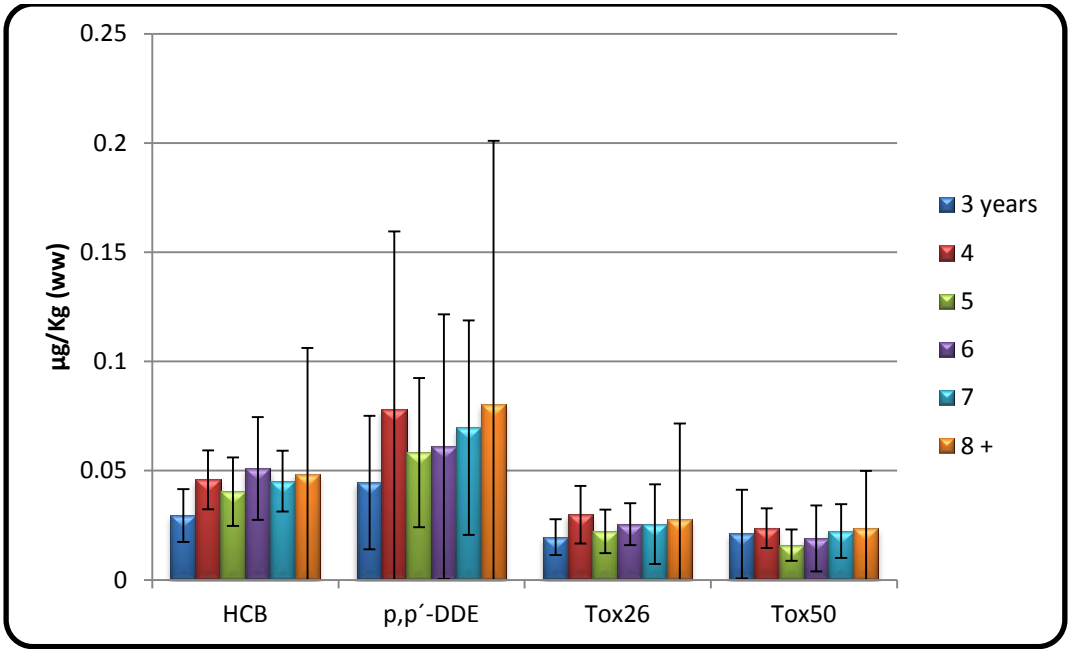

Figure 16. *Geom. levels (µg/kg ww) ± SD of the four pesticides analysed in cod liver*

3.2.3 ∑PCB7 in cod liver compared to cod muscle

There was a linear relationship between the ∑PCB7 in the liver based on lipid weight and the fat content of the liver (%) as shown in Figure 17 similar to the relationship between ∑PCB7 in cod muscle based on wet weight with fat content in liver (Fig. 10). On the other hand, there was not a linear relationship between the ∑PCB7 based on wet weight and the fat content of the liver (%) in the liver as shown in Figure 18.

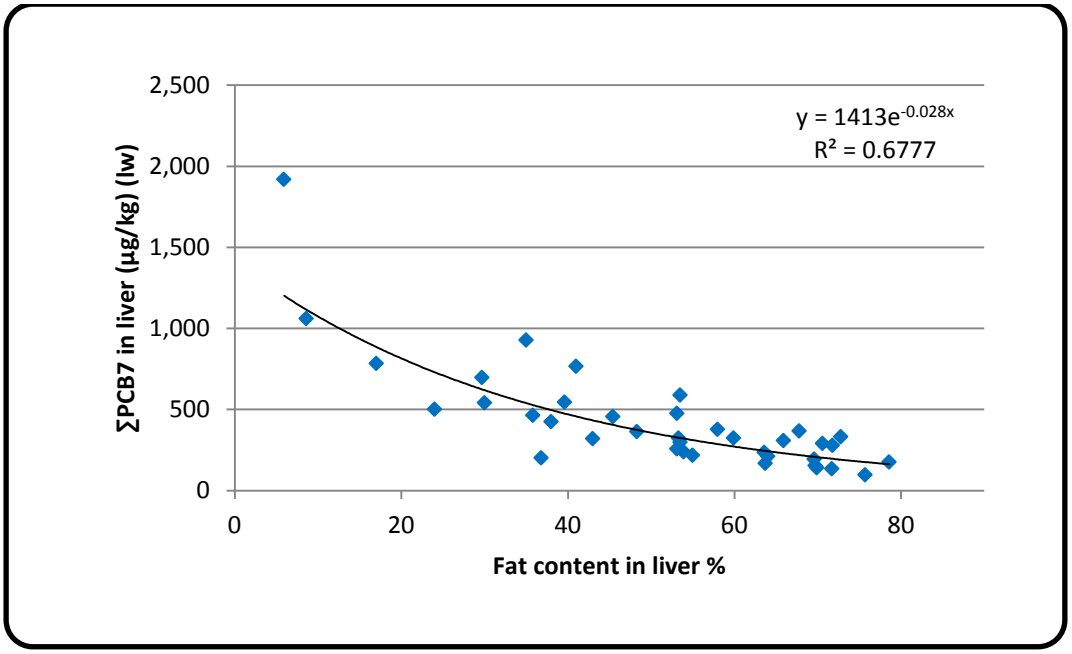

Figure 17. *The relationship between the ∑PCB7 in cod liver (µg/kg lw) and the fat content of the liver (%).*

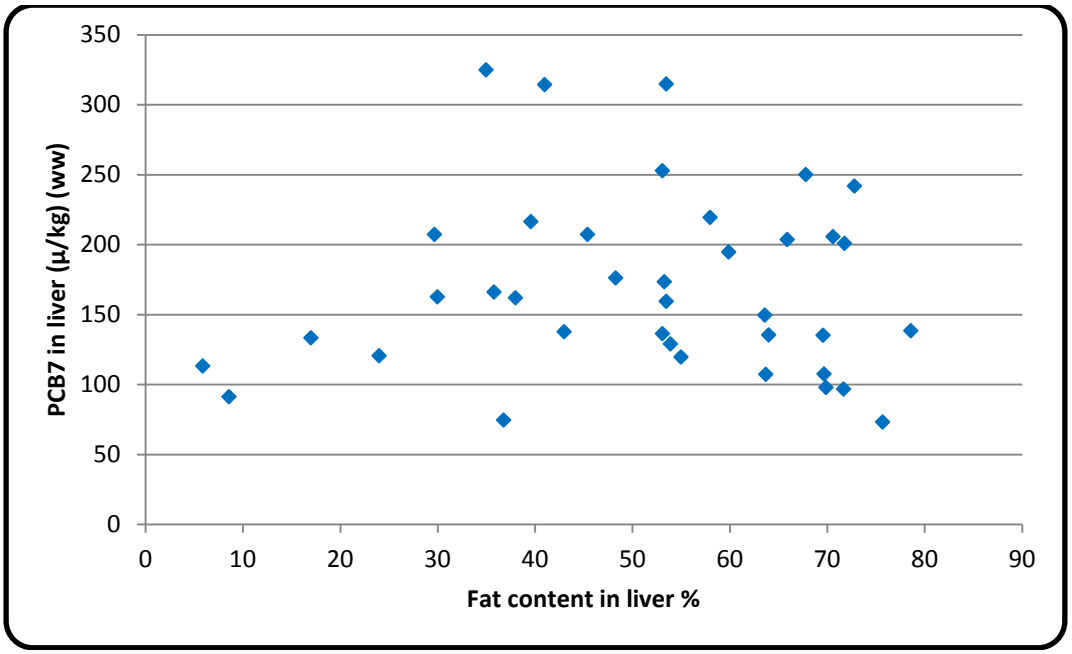

Figure 18 *The relationship between the ∑PCB7 in cod liver (in µg/kg ww) and the fat content of the liver (%).*

Figure 19 shows that there was no relationship between ∑PCB7 in muscle and liver based on wet weight but if the concentration in the liver was based on lipid weight, a relationship was observed (Figure 20).

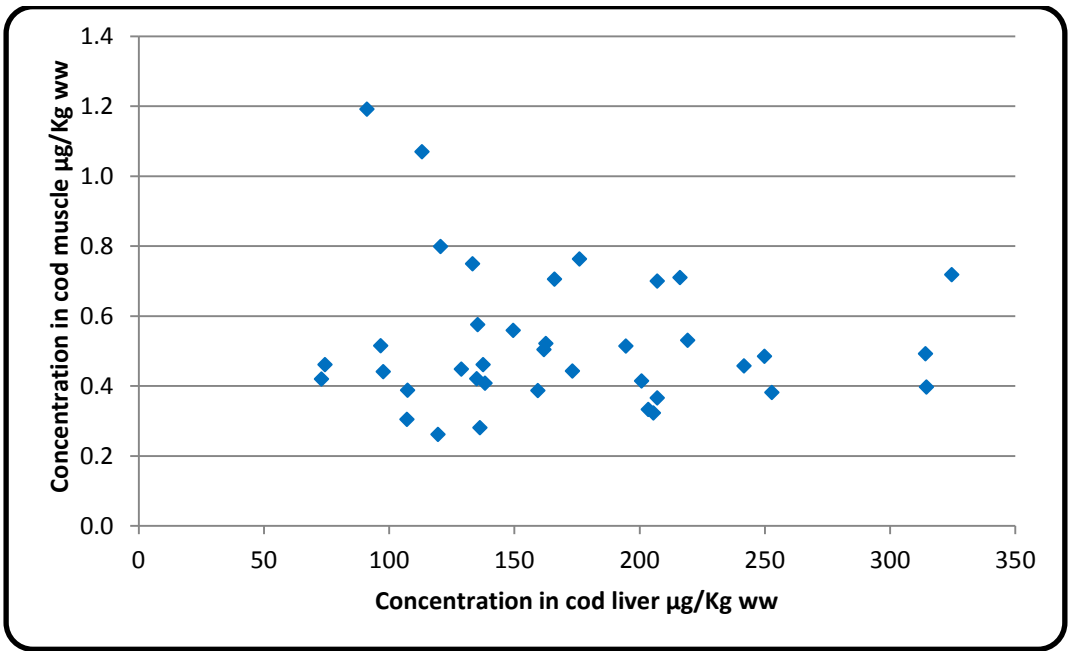

Figure 19 *The relationship between concentration of ∑PCB7 in cod muscle (ww) and liver (ww)*

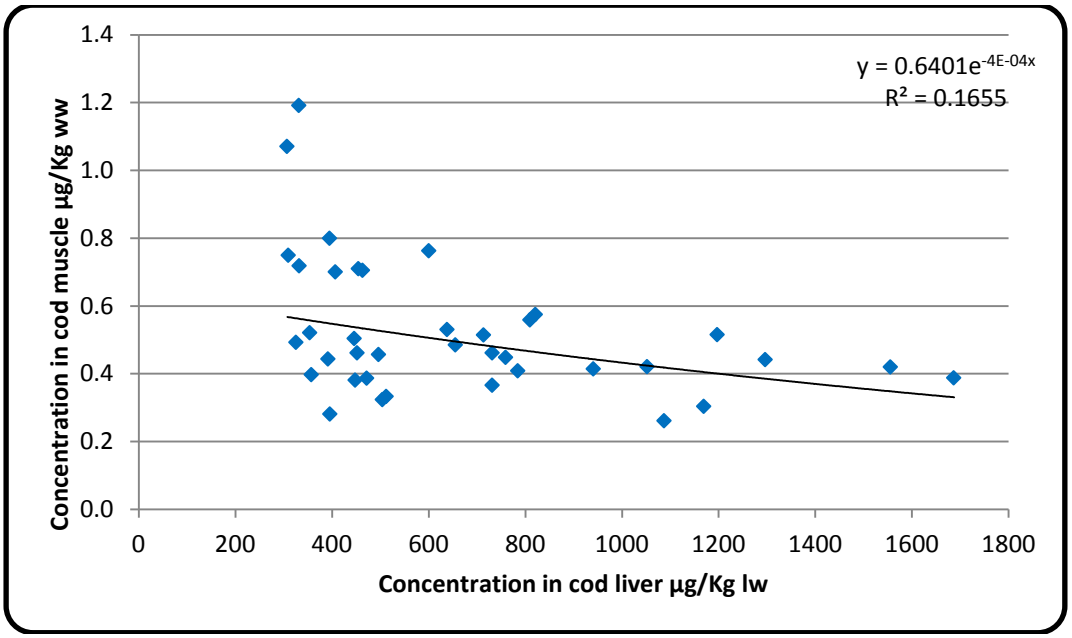

Figure 20. *The relationship between concentration of ∑PCB7 in cod muscle (ww) and liver (lw)*

3.2.4 PCBs in muscles from whole fish vs. fish from the processing plants

The level of PCBs in muscle from whole fish, filleted in the laboratory, was compared to levels found in fish fillets that were processed in different factories. The mean of ∑PCB7 in muscle from whole fish was slightly lower than in processed fish as seen in fig 21. However, the difference was not significant (p = 0.330, Mann-Whitney U-test).

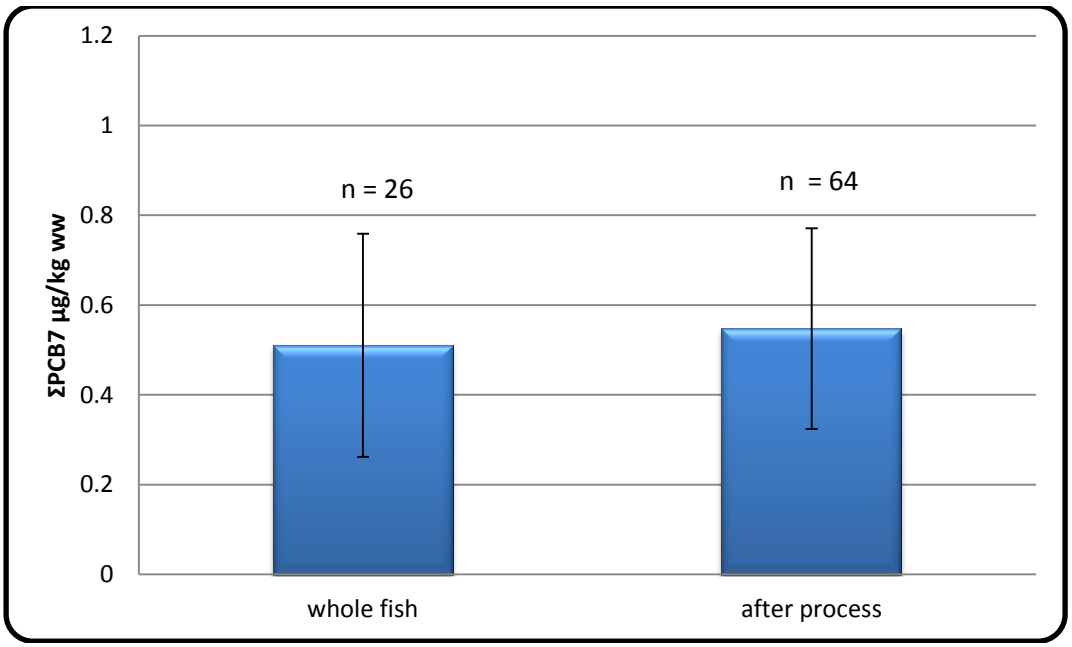

Figure 21. *Geom. ± SD of ∑PCB7 in muscle from the whole fish compared to fish that have been through the processing plant*

3.3 Persistent organic pollutants in oven baked cod muscle

As shown in Table 10, the concentration of both ∑PCB7 and the pesticides was very low in oven baked cod muscle. The concentrations of each chemical analysed are shown in Appendix IV. Comparing the amount of the POPs in the same fish samples before and after the cooking process, the amount after cooking had decreased to 21 – 31 % of what is was in the raw fish for all compounds except for HCB where the concentration after cooking was about 65 – 87% of what is was prior to cooking.

Table 10. *Concentration of POPs in oven baked cod muscle (µg/kg ww)**

	∑PCB7	%**	HCB	%**	p,p'-DDE	%**	∑Tox 26&50	%**
Geom	0.113	24	0.028	72	0.012	29	0.011	25
Min	0.055	21	0.016	87	0.004	26	0.004	17
Max	0.219	31	0.050	65	0.036	29	0.038	32

*n = 14; ** percent of substances in oven baked compared to fresh cod

3.4 Persistent organic pollutants in chicken breast

As shown in Table 11, the concentration of POPs in chicken breasts were very low, ∑PCB7 was 0.128-0.173 µg/kg (ww) and 0.005-0.047 µg/kg (ww) for individual congeners. The quantity in the Icelandic chicken was slightly higher than in the Danish chicken. However, quantification of the compounds found in the chicken was difficult because there was a different kind of interference in the chromatograms that made it harder to evaluate the amount in the chicken compared to cod muscle.

Table 11. *POPs in chicken breast µg/kg ww*

Sample ID	CB-28	CB-52	CB-101	CB-118	CB-153	CB-138	CB-180	∑PCB7	HCB	p,p'-DDE	Tox26	Tox50
Danish*	0.047	0.007	0.042	0.015	0.005	0.005	0.007	**0.128**	0.031	0.009	0.002	0.006
Kjörfugl*	0.037	0.017	0.018	0.013	0.021	0.021	0.011	**0.138**	0.026	0.017	0.008	0.018
Ísfugl*	0.040	0.023	0.005	0.019	0.035	0.030	0.022	**0.173**	0.027	0.045	0.010	0.028

*Pooled samples from at least five chicken breasts

4 Discussion

One of the objectives of the study was to investigate whether there is a difference in the concentration of persistent organic pollutants in individual cods from what is found in pooled samples. The problem is that monitoring programmes may involve different numbers of individuals in the pooled samples and extreme values disappear in the mean of all the individuals. Another aspect is the presentation of the results. It varies between laboratories how many PCBs are analysed but most or all of them analyse at least the PCB7. However, typically they publish the ∑PCB7 but not the concentration of individual congeners. Some investigators also report on dioxin like PCBs and the sum of all PCBs they analyse, which can vary greatly in numbers so the sum can represent very different levels. When it comes to pesticides the same problem arises, when comparing own results with those of other researchers. It is very common to publish only the sum for DDT and its degradation products but not the quantity for each chemical. The same goes for the Toxaphenes, it is common to publish the sum for all of them but not for individual substances, however, the most common thing is to analyse three of them i.e. Tox26, 50 and 62.

There are no Icelandic or EU limits for the maximum concentration of PCBs in food. There was a now abolished Icelandic maximum limit between 2004 and 2010, for each of the individual PCB7 congeners, which was 10-60 µg/kg and a total sum of 200 µg/kg for all PCBs and 170 µg/kg for ∑PCB7 wet weight in food with fat content under 10%. The highest concentration of the ∑PCB7 found in cod muscle in this study was 1.2 µg/kg (ww) which was less than 1% of the previous maximum level set for ∑PCB7. Research from Denmark show that about 90% of the human intake of POPs is from food and the main source is fish for both PCBs and DDT (50%) but for HCB it is more distributed. The estimated intake for the PCB7 and three more (CB-105, 156 and 170) was 0.9 µg per day (Fromberg *et al.*, 2011).

Taking into account that the adult Icelander consumes about 40 g of fish each day (Steingrimsdottir *et al.*, 2003) the daily consumption of the PCB7 from fish like cod would be less than 10% of the estimated amount consumed in Denmark.

Results of this study show that the concentration of ∑PCB7 in cod muscle (n = 64) from individuals was between 0.24 – 1.2 µg/kg (ww). This compares well to the results from the ongoing monitoring programme for undesirable substances in the edible part (i.e. muscle) of cod caught in Icelandic waters where the concentration of ∑PCB7 in three pooled samples of cod muscle in 2009 was 0.41; 0.50 and 1.6 µg/kg (ww) (Jorundsdottir *et al.*, 2010c). In

2008 it was 0.3 and 0.32 µg/kg (ww) in two pooled samples (Jorundsdottir *et al.*, 2010d), and in 2005 it was 0.17 and 0.43 µg/kg (ww) in two pooled samples (Asmundsdottir & Gunnlaugsdottir, 2006). There are no signs of change in the levels of ∑PCB7 in cod muscle around Iceland. However, no statistical analysis on temporal trends in the concentration of PCBs in Atlantic cod caught around Iceland has been made so far. The concentration in pooled samples from cod muscle from the monitoring programmes from 2005 – 2009 is in the range of 0.17 – 1.6 µg/kg (ww) which is similar to the concentration found in individuals in this study.

Reported levels of POPs in Atlantic cod caught in Norwegian waters in the years 1990-2000 (n = 240) were 1.2 µg/kg for ∑PCB7, 0.33 µg/kg p,p´-DDE and 0.10 µg/kg of HCB (mean, ww) (Green & Knutzen, 2003). That tend to be higher levels than were observed in the Icelandic cod and the mean concentration of PCB in cod from Iceland is about half of what was found in the Norwegian cod (Green & Knutzen, 2003; Karl *et al.*, 1999). In muscle of cod (*Gadus morhua*) caught in the more polluted southern Baltic Sea in the years 1997 – 2003 the mean concentration of ∑PCB7 was from 0.7 – 2.1 µg/kg (ww) with a range from 0.4 – 2.9 µg/kg (ww) (Szlinder-Richert *et al.*, 2009) which is of the same order of magnitude as in the Icelandic cod. In cod caught in the North Sea in 2001 the concentration of ∑PCB7 was from 0.51 – 1.01 µg/kg (ww) (Baeyens *et al.*, 2007).

In the Icelandic environment, CB-153 is usually the most abundant PCB substance. When using pooled samples as done in most studies, the individual levels of each congener and the ∑PCB7 disappear in the mean of many individuals. The results from this study show that CB-153 is not always the most abundant congener at least in the muscle, in some of the samples CB-52 was the highest one. In an attempt to investigate what could explain the concentration of these congeners they were compared with respect to all the different variables like age, sex, fishing ground and sexual maturity. There was a statistical difference between these two congeners between different age groups as well as at different stages of sexual maturity. The turning point where CB-52 is no longer the most abundant congener and CB-153 takes over is at the same time as the cod becomes pubescent or gets to level 2 of sexual maturity and at age 5 – 6 years. No relationship was found with sex, fat content in liver or fishing ground. No other studies have been found that report higher levels of CB-52 than CB-153 but in most cases these publish mean concentration from pooled samples. A literature study revealed only one study from Belgium, where PCBs were detected in the muscle of five individual cods. In that study the results were presented for ∑PCB7 for individuals and the

average of individual substances for all the cods examined (Baeyens *et al.*, 2007). The difference in CB-52 and CB-153 was only found in muscle but not in the liver where CB-153 was always higher. This difference could possibly be due to uncertainty at low levels but nonetheless it could also be very real.

The concentration of the ∑PCB7 in the individual cod livers (n = 38) based on wet weight of the liver samples analysed in this study was between 73 – 325 µg/kg, with the mean 158 µg/kg. Which is in the same range that has been detected in pooled samples in the Icelandic monitoring programme last years (Jorundsdottir *et al.*, 2010b; Jorundsdottir *et al.*, 2009b; Rabieh *et al.*, 2007) and about 300x higher than was found in the muscle. The mean concentration of the ∑PCB7 in the individual cod livers based on lipid weight in this study was 345 µg/kg with a range 96 – 1,919 µg/kg, only in 2 of the 38 livers analysed the concentration was over 1,000 µg/kg (lw). The concentration of the pesticides found in the cod livers in this study was for HCB 3 – 31 µg/kg (ww) with the mean 15 µg/kg (ww), 24 – 134 µg/kg (ww) with the mean 56 µg/kg (ww) for p,p´ - DDE and 9 – 128 µg/kg (ww) with the mean 45 µg/kg (ww) for the ∑Tox 26&50 based on wet weight.

The mean concentration for ∑PCB7 in Icelandic cod livers caught in the years 2005-2009 was 20 – 377 µg/kg, HCB was 4 – 26 µg/kg, p,p´ - DDE was 15 - 76 µg/kg and ∑Tox 26&50 was 29 – 84 µg/kg in pooled samples, based on wet weight (Jorundsdottir *et al.*, 2010b; Jorundsdottir *et al.*, 2009b; Rabieh *et al.*, 2007).

In cod caught at different locations around Norway in the years 1990-2000 the mean concentration of ∑PCB7 in livers (n>1000) was found to be 328 µg/kg (ww) the mean concentration of HCB were 13 µg/kg (ww) and p,p´-DDE was 87 µg/kg (ww) (Green & Knutzen, 2003). Concentration of ∑PCB7 in cod livers caught in the southern Baltic Sea in the years 1998 – 2003 was 574 – 1,727 µg/kg (lw) with a range from 351 – 2,681 µg/kg (lw) (Szlinder-Richert *et al.*, 2009). In cod caught in the Baltic Sea in the year 2007 the mean concentration of ∑PCB7 in livers were 266 – 664 µg/kg (ww) in pooled samples (Dabrowska *et al.*, 2009). In polar cod *(Boreogadus saida)* from Greenland the mean concentration of ∑PCB7 in livers was found to be 22 µg/kg (ww) the mean concentration of HCB was 11 µg/kg (ww) and p,p´-DDE was 15 µg/kg (ww) in pooled samples (Cleemann *et al.*, 2000). This shows that the concentration in the cod livers from Iceland is low compared to what has been detected in other cods except for cod from Greenland where it was found to be very similar.

Looking at the correlation between individual substances, in general the different contaminants correlate very well, indicating that their source in the cod is the same, namely the food chain. However, the correlation in the muscle is much better between the congeners with higher chlorination (CB-101, 118, 138, 153, 180, p,p´-DDE and the toxaphenes) than between the lighter and more volatile chemicals (HCB, CB-28 and 52). The reason could be that there is more uncertainty in the analysis of the lighter compounds. This could also be because some of the PCBs like CB-52 can be found in some solvents at low concentrations, but since the levels in the muscles are also very low, the proportion from the solvents can be substantial. Then the portion of the individual PCBs could be different between muscle and liver and may thus be found at different concentration in the organs.

When the fish loses weight for example after spawning the fat content of the liver decreases. When this happens the concentration of the POPs in the liver increases on fat weight basis, since these chemicals are found in the fat. The concentration in the muscle will also increase at the same time, due to relocation. When the fat content in the liver increases the concentration of POPs in the liver decreases again in the liver lipids and will also decrease in the muscle because of the liver's ability to receive the chemicals will increase and the substances will be transferred from the muscle back to the liver (Anonymous, 2003; Audunsson, 1999; Olafsdottir *et al.*, 1998).

The processing procedure in the processing plant does not seem to affect the concentration of the PCBs in the cod muscle, which is what was expected because these compounds are not known contaminants in the processing plant, nor would the process be expected to reduce their levels in the muscle.

It could be expected that freeze-drying the samples before analysing could affect the results. The effect would be that the substances found were at lower concentrations than in fresh or frozen fish (Wells *et al.*, 1997). This was indicated by the results from the analysis of the control samples (Appendix I). The z scores from the analysis of control samples show that they tend to be negative (-1.37 to -0.24) in the cod muscles that had been freeze-dried before extraction (QOR068BT and QTX027BT) while the trend was positive for the liver (-0.02 to 0.17) that had not been freeze-dried (QOR086BT).

As reviewed by Sherer and Price (1993) studies have in general shown a decrease of 10 - 35% of PCBs in fish muscle after using baking as a cooking method. HCB appears to remain relatively stable despite cooking but that differs between fish species (Perelló *et al.*,

2009). Compared with the results obtained in this project, even if it involved only a few samples the results are 20-30% lower than others have found. The reason is probably the method used, i.e. oven baking the cod muscle in a closed container and then pouring the broth containing most of the fat, immediately after cooking before analysing. In hindsight, the process should be started by steam boiling the fish in an open container and then freeze drying all that would be left in the container. It would also be interesting to try different cooking methods for example frying and grilling but then all the co ingredients such as oil, spices and flour needed to be taken into account and the substances measured in them as well.

The mean concentration of ∑PCB7 in chicken breasts (0.15 µg/kg) is about 30% of what is found in cod muscle (0.51 µg/kg), and about 3 times more than found in chicken in Spain (Perelló *et al.*, 2010). Sample from single chicken in Northwest Russia contained 1.21 µg/kg (ww) PCBs but that was the sum of ∑PCB7 and 9 others (Polder *et al.*, 2010) so it is difficult to compare with the results from this study. The concentration of DDE was about 0.4 µg/kg (ww) and 0.26 µg/kg (ww) for HCB (Polder *et al.*, 2010), slightly higher than in this study but on the other hand this was a sample from whole chicken with much higher fat content than the breasts. Studies have shown that if chicken are given feed containing PCBs the concentration increases in the meat (Maervoet *et al.*, 2004). That could be one of the reasons that levels in chicken from Iceland is higher than from Spain, since at least in Iceland the chicken feed normally contains some fish meal. This requires a watchful eye on the levels of POPs in chicken.

The chromatograms of the chicken breasts differed from the chromatograms of the fish samples. More background in the chicken samples compared to the fish samples made it difficult to quantitative the POPs in the chicken. Therefore, it is necessary to review the process and perhaps modify the method, especially the cleaning procedure. It may be enough to use larger cells for the ASE 300 that will make it possible to use more of the silica gel / sulphuric acid mixture for cleaning.

Human consumption of fish and chicken in Iceland has been undergoing changes in the direction that fish consumption is declining and chicken consumption increasing. From 1990 – 2002 the decline in fish consumption was 30% (Steingrimsdottir *et al.*, 2003). The increase in chicken consumption has mainly been among the younger people (Steingrimsdottir *et al.*, 2003).

5 Conclusion

The concentration of POPs found in cod caught in Icelandic waters is very low in relation to health effects and lower than what has been found in cod from other waters. It is therefore safe to recommend the consumption of Icelandic cod, especially when all the benefits such as the omega-3 fatty acids are taken into account.

Traceability of samples through the value chain shows no difference in the concentration of the PCBs, which is what could be expected as the substances originate from the food consumed by the fish.

The results from this study indicate that CB-153 is not always the most abundant congener in the environment, at least not in fish muscle, since CB-52 was found at higher levels in cod muscle. However, further studies are needed for confirmation. The results may indicate that fat can transfer between different tissues at puberty.

The analytical method proved to be satisfactory and gave good results on control samples.

The next steps will be to investigate other Icelandic fish species as well as farmed fish. It would also be interesting to look further into different cooking methods to see how the levels of POPs can be minimized in the fish muscle.

6 References

AMAP. (2009a). *AMAP Assessment 2009: Human health in the Arctic*. Oslo: Arctic monitoring and assessment programme.

AMAP. (2009b). *Arctic pollution 2009*. Oslo: Arctic monitoring and assessment programme.

Anonymous. (1966). Report of a new chemical hazard. *New Science, 32*, 612.

Anonymous. (2003). *Dioxins and PCBs in four commercially important pelagic fish stocks in the North East Atlantic. NORA project report 1-57*. from http://www.sf.is/attachments/045_Nora-lokaskyrsla.pdf

AOCS Official Method Ba 3-38 and application note Tecator no AN 301, (1997).

Asmundsdottir, A. & Gunnlaugsdottir, H. (2006). *Undesirable substances in seafood products - results from the monitoring activities in 2005*. Reykjavik: The Icelandic Fisheries Laboratories.

ATSDR. (2002). *Toxicological profile for HCB.* Atlanta, GA: U.S. Department of Health and Human Services, Public health services. Report.

Auðunsson, G. A. (1999). The effect of nutritional status of Icelandic cod *(Gadus morhua)* on macroconstituents and trace elements in the liver. *Rit Fiskideildar, 16* 111-129.

Baeyens, W., Leermakers, M., Elskens, M., Van Larebeke, N., De Bont, R., Vanderperren, H., Fontaine, A., Degroodt, J. M., Goeyens, L., Hanot, V. & Windal, I. (2007). PCBs and PCDD/FS in fish and fish products and their impact on the human body burden in belgium. *Archives of Environmental Contamination and Toxicology, 52* (4), 563-571.

Bailey, R. E. (2001). Global hexachlorobenzene emissions. *Chemosphere, 43* (2), 167-182.

Baird, C. & Cann, M. (2005). *Environmental Chemistry, third edition*. New York: W. H. Freeman and Company.

Ballschmiter, K. & Zell, M. (1980). Analysis of polychlorinated-biphenyls (PCB) by glass-capillary gas-chromatography - composition of technical aroclor-PCB and clophen-pcb mixtures. *Fresenius Zeitschrift Fur Analytische Chemie, 302* (1), 20-31.

Barber, J. L., Sweetman, A. J., van Wijk, D. & Jones, K. C. (2005). Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. *Science of the Total Environment, 349* (1-3), 1-44.

Barrie, L. A., Gregor, D., Hargrave, B., Lake, R., Muir, D., Shearer, R., Tracey, B. & Bidleman, T. (1992). Arctic contaminants - sources, occurence and pathways. *Science of the Total Environment, 122* (1-2), 1-74.

Borja, J., Taleon, D. M., Auresenia, J. & Gallardo, S. (2005). Polychlorinated biphenyls and their biodegradation. *Process Biochemistry, 40* (6), 1999-2013.

Breivik, K., Alcock, R., Li, Y. F., Bailey, R. E., Fiedler, H. & Pacyna, J. M. (2004). Primary sources of selected POPs: regional and global scale emission inventories. *Environmental Pollution, 128* (1-2), 3-16.

Burkow, I. C. & Kallenborn, R. (2000). Sources and transport of persistent pollutants to the Arctic. *Toxicology Letters, 112* 87-92.

Buser, H. R., Haglund, P., Muller, M. D., Poiger, T. & Rappe, C. (2000). Discrimination and thermal degradation of toxaphene compounds in capillary gas chromatography when using split/splitless and on-column injection. *Chemosphere, 41* (4), 473-479.

Byrne, J. J., Reineke, E. P., Ringer, R. K. & Aulerich, R. (1975). Influences of polychlorinated biphenyl mixture (Aroclor 1254) administration on reproduction and thyroid-function in mink (*Mustela vison*). *Federation Proceedings, 34* (3), 321-321.

Carson, R. (1962). *Silent spring*. Boston: Houghton Mifflin.

Cleemann, M., Riget, F., Paulsen, G. B., Klungsoyr, J. & Dietz, R. (2000). Organochlorines in Greenland marine fish, mussels and sediments. *Science of the Total Environment, 245* (1-3), 87-102.

Dabrowska, H., Bernard, E., Barska, I. & Radtke, K. (2009). Inter-tissue distribution and evaluation of potential toxicity of PCBs in Baltic cod *(Gadus morhua L.). Ecotoxicology and Environmental Safety, 72* (7), 1975-1984.

Darnerud, P. O., Atuma, S., Aune, M., Bjerselius, R., Glynn, A., Grawe, K. P. & Becker, W. (2006). Dietary intake estimations of organohalogen contaminants (dioxins, PCB, PBDE and chlorinated pesticides, e.g. DDT) based on Swedish market basket data. *Food and Chemical Toxicology, 44* (9), 1597-1606.

de Boer, J. & Law, R. J. (2003). Developments in the use of chromatographic techniques in marine laboratories for the determination of halogenated contaminants and polycyclic aromatid hydrocarbons. *Journal of chromatography A*, 223-251.

De Guise, S., Martineau, D., Beland, P. & Fournier, M. (1995). Possible mechanisms of action of environmental contaminants on St-Lawrence beluga whales (*Delphinapterus-leucas*). *Environmental Health Perspectives, 103* 73-77.

Eggesbo, M., Stigum, H., Longnecker, M. P., Polder, A., Aldrin, M., Basso, O., Thomsen, C., Skaare, J. U., Becher, G. & Magnus, P. (2009). Levels of hexachlorobenzene (HCB) in breast milk in relation to birth weight in a Norwegian cohort. *Environmental Research, 109* (5), 559-566.

EPA. (2009, 24 March). Polychlorinated biphenyls (PCBs). Retrieved 1 September, 2010, from http://www.epa.gov/wastes/hazard/tsd/pcbs/pubs/about.htm

EPA. (2010a, 16 March). Hexachlorobenzene. Retrieved 23 August, 2010, from http://www.epa.gov/NCEA/iris/subst/0374.htm

EPA. (2010b, 16 March). Toxaphene. Retrieved 23 August, 2010, from http://www.epa.gov/ncea/iris/subst/0346.htm

Erickson, M. D. (1997). *Analytical Chemistry of PCBs, second edition*. Florida: Lewis publishers.

Eskenazi, B., Chevrier, J., Rosas, L. G., Anderson, H. A., Bornman, M. S., Bouwman, H., Chen, A. M., Cohn, B. A., de Jager, C., Henshel, D. S., Leipzig, F., Leipzig, J. S., Lorenz, E. C., Snedeker, S. M. & Stapleton, D. (2009). The Pine River Statement: Human Health Consequences of DDT Use. *Environmental Health Perspectives, 117* (9), 1359-1367.

Fromberg, A., Granby, K., Hojgard, A., Fagt, S. & Larsen, J. C. (2011). Estimation of dietary intake of PCB and organochlorine pesticides for children and adults. *Food Chemistry, 125* (4), 1179-1187.

Green, N. W. & Knutzen, J. (2003). Organohalogens and metals in marine fish and mussels and some relationships to biological variables at reference localities in Norway. *Marine Pollution Bulletin, 46* (3), 362-374.

Gunnlaugsdottir, H., Vidarsson, J. R., Asmundsdottir, A. M., Garate, C., Jorundsdottir, H., Jonsdottir, I. G., Arason, S., Baldursdottir, V., Sigurdsson, T. & Margeirsson, S. (2010). *Factors influencing the quality and value of the Icelandic cod; a value chain perspective* (No. 31-10). Reykjavik: Matis ltd.

Hall, A. J., Law, R. J., Wells, D. E., Harwood, J., Ross, H. M., Kennedy, S., Allchin, C. R., Campbell, L. A. & Pomeroy, P. P. (1992). Organochlorine levels in common seals (*Phoca-vitulina*) which were victims and survivors of the 1988 phocine distemper epizootic. *Science of the Total Environment, 115* (1-2), 145-162.

Helander, B., Bignert, A. & Asplund, L. (2008). Using raptors as environmental sentinels: Monitoring the white-tailed sea eagle *Haliaeetus albicilla* in Sweden. *Ambio, 37* (6), 425-431.

Helander, B., Olsson, A., Bignert, A., Asplund, L. & Litzen, K. (2002). The role of DDE, PCB, coplanar PCB and eggshell parameters for reproduction in the white-tailed sea eagle *(Haliaeetus albicilla)* in Sweden. *Ambio, 31* (5), 386-403.

Hutzinger, O., Paasivirta, J., Vetter, W. & Oehme, M. (2000). Toxaphene. Analysis and Environmental Fate of Congeners. In *Volume 3 Anthropogenic Compounds Part K* (Vol. 3K, pp. 237-287): Springer Berlin / Heidelberg.

Hutzinger, O., Safe, S. & Zitko, V. (1974). *Chemistry of PCBs*. Cleveland, Ohio: CRC Press, Inc.

IARC. (1998). *IARC monographs on the evaluation of carcinogenic risks to humans. Polychlorinated biphenyl and polybrominated biphenyls*. Retrieved 24 November 2010, from http://monographs.iarc.fr/ENG/Monographs/vol18/volume18.pdf

IARC. (2001). *IARC monographs on the evaluation of carcinogenic risks to humans*. Retrieved 24 August 2010, from http://monographs.iarc.fr/ENG/Monographs/vol79/index.php

Ishikawa, Y., Noma, Y., Mori, Y. & Sakai, S. (2007). Congener profiles of PCB and a proposed new set of indicator congeners. *Chemosphere, 67* (9), 1838-1851.

Jacobson, J. L., Jacobson, S. W. & Humphrey, H. E. B. (1990). Effects of inutero exposure to polychlorinated-biphenyls and related contaminants on cognitive-functioning in young-children. *Journal of Pediatrics, 116* (1), 38-45.

Jensen, S., Johnels, A. G., Olsson, M. & Otterlin.G. (1972). DDT and PCB in herring and cod from the Baltic, the Kattegat and the Skagerrak. *Ambio Special Report, 1*, 71-85.

Jenssen, B. M., Aarnes, J. B., Murvoll, K. M., Herzke, D. & Nygard, T. (2010). Fluctuating wing asymmetry and hepatic concentrations of persistent organic pollutants are associated in European shag *(Phalacrocorax aristotelis)* chicks. *Science of the Total Environment, 408* (3), 578-585.

JMPR. (2000). *Joint FAO/WHO meeting on pesticides residues; 2000. Report*. Atlanta, GA: U.S. Department of Health and Human Services, Public health services. Report.

Jorundsdottir, H., Bignert, A., Svavarsson, J., Nygard, T., Weihe, P. & Bergman, A. (2009a). Assessment of emerging and traditional halogenated contaminants in Guillemot (Uria aalge) egg from North-Western Europe and the Baltic Sea. *Science of the Total Environment, 407* (13), 4174-4183.

Jorundsdottir, H., Lofstrand, K., Svavarsson, J., Bignert, A. & Bergman, A. (2010a). Organochlorine Compounds and Their Metabolites in Seven Icelandic Seabird Species - a Comparative Study. *Environmental Science & Technology, 44* (9), 3252-3259.

Jorundsdottir, H. O., Desnica, N., Gudjonsdottir, S. H., Ragnarsdottir, T. & Gunnlaugsdottir, H. (2010b). *Monitoring of the marine biosphere around Iceland 2008 and 2009* (No. 30-10). Reykjavik: Matis ltd.

Jorundsdottir, H. O., Desnica, N., Ragnarsdottir, T. & Gunnlaugsdottir, H. (2010c). *Undesirable substances in seafood products. Results from the Icelandic marine monitoring activities year 2009* (No. 38-10). Reykjavik: Matis ltd.

Jorundsdottir, H. O., Hauksdottir, K., Desnica, N. & Gunnlaugsdottir, H. (2010d). *Undesirable substances in seafood products. Results from the Icelandic marine monitoring activities year 2008* (No. 16-10). Reykjavik: Matis ltd.

Jorundsdottir, H. O., Rabieh, S. & Gunnlaugsdottir, H. (2009b). *Undesirable substances in seafood products. Results from the monitoring activities in 2007* (No. 28-09). Reykjavik: Matis ltd.

Karl, H., Khandker, S. & Alder, L. (1999). Variation of toxaphene indicator compounds in fish from single fishing grounds: Conclusions for sampling. *Chemosphere, 39* (14), 2497-2506.

Lahvis, G. P., Wells, R. S., Kuehl, D. W., Stewart, J. L., Rhinehart, H. L. & Via, C. S. (1995). Decreased lymphocyte-responses in free-ranging bottle-nosed dolphins (*Tursiops-truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral-blood. *Environmental Health Perspectives, 103* 67-72.

Li, Y. F. (2001). Toxaphene in the United States 1. Usage gridding. *Journal of Geophysical Research-Atmospheres, 106* (D16), 17919-17927.

Li, Y. F. & Macdonald, R. W. (2005). Sources and pathways of selected organochlorine pesticides to the Arctic and the effect of pathway divergence on HCH trends in biota: a review. *Science of the Total Environment, 342* (1-3), 87-106.

Lillie, R. J., Cecil, H. C., Bitman, J. & Fries, G. F. (1974). Differences in response of caged white leghorn layers to various polychlorinated biphenyls (PCBs) in diet. *Poultry Science, 53* (2), 726-732.

Liu, C. Y., Jiang, X., Yang, X. L. & Song, Y. (2010). Hexachlorobenzene dechlorination as affected by organic fertilizer and urea applications in two rice planted paddy soils in a pot experiment. *Science of the Total Environment, 408* (4), 958-964.

Lofstrand, K., Jorundsdottir, H., Tomy, G., Svavarsson, J., Weihe, P., Nygard, T. & Bergman, A. (2008). Spatial trends of polyfluorinated compounds in guillemot (Uria aalge) eggs from North-Western Europe. *Chemosphere, 72* (10), 1475-1480.

Lohmann, R., Breivik, K., Dachs, J. & Muir, D. (2007). Global fate of POPs: Current and future research directions. *Environmental Pollution, 150* (1), 150-165.

Louis, G. M. B., Weiner, J. M., Whitcomb, B. W., Sperrazza, R., Schisterman, E. F., Lobdell, D. T., Crickard, K., Greizerstein, H. & Kostyniak, P. J. (2005). Environmental PCB exposure and risk of endometriosis. *Human Reproduction, 20* (1), 279-285.

Mackay, D. & Fraser, A. (2000). Bioaccumulation of persistent organic chemicals: mechanisms and models. *Environmental Pollution, 110* (3), 375-391.

Maervoet, J., Chu, S. G., De Vos, S., Covaci, A., Voorspoels, S., De Schrijver, R. & Schepens, P. (2004). Accumulation and tissue distribution of selected polychlorinated biphenyl congeners in chickens. *Chemosphere, 57* (1), 61-66.

Magnusdottir, E. V., Thorsteinsson, T., Thorsteinsdottir, S., Heimisdottir, M. & Olafsdottir, K. (2005). Persistent organochlorines, sedentary occupation, obesity and human male subfertility. *Human Reproduction, 20* (1), 208-215.

Ministry for the Environment. (2004, February 18th). *Alþjóðlegur samningur um þrávirk lífræn efni tekur gildi*, from http://www.umhverfisraduneyti.is/frettir/nr/464

Monosson, E., Fleming, W. J. & Sullivan, C. V. (1994). Effects of the planar PCB 3,3',4,4'-tetrachlorobiphenyl (TCB) on ovarian development, plasma-levels of sex steroid-hormones and vitellogenin, and progeny survival in the white perch (Morone-americana). *Aquatic Toxicology, 29* (1-2), 1-19.

Muir, D. & Sverko, E. (2006). Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: a critical appraisal. *Analytical and Bioanalytical Chemistry, 386* (4), 769-789.

Olafsdottir, K., Petersen, A. E., Magnusdottir, E. V., Bjornsson, T. & Johannesson, T. (2001). Persistent organochlorine levels in six prey species of the gyrfalcon *Falco rusticolus* in Iceland. *Environmental Pollution, 112* (2), 245-251.

Olafsdottir, K., Petersen, A. E., Magnusdottir, E. V., Bjornsson, T. & Johannesson, T. (2005). Temporal trends of organochlorine contamination in Black Guillemots in Iceland from 1976 to 1996. *Environmental Pollution, 133* (3), 509-515.

Olafsdottir, K., Petersen, A. E., Thordardottir, S. & Johannesson, T. (1995). Organochlorine residues in gyrfalcons *(Falco-rusticolus)* in Iceland. *Bulletin of Environmental Contamination and Toxicology, 55* (3), 382-389.

Olafsdottir, K., Skirnisson, K., Gylfadottir, G. & Johannesson, T. (1998). Seasonal fluctuations of organochlorine levels in the common elder *(Somateria mollissima)* in Iceland. *Environmental Pollution, 103* (2-3), 153-158.

Paasivirta, J., Sinkkonen, S., Nikiforov, V., Kryuchkov, F., Kolehmainen, E., Laihia, K., Valkonen, A. & Lahtinen, M. (2009). Long-range atmospheric transport of three toxaphene congeners across Europe. Modeling by chained single-box FATEMOD program. *Environmental Science and Pollution Research, 16* (2), 191-205.

Perelló, G., Martí-Cid, R., Castell, V., Llobet, J. M. & Domingo, J. L. (2009). Concentrations of polybrominated diphenyl ethers, hexachlorobenzene and polycyclic aromatic hydrocarbons in various foodstuffs before and after cooking. *Food and Chemical Toxicology, 47* (4), 709-715.

Perelló, G., Martí-Cid, R., Castell, V., Llobet, J. M. & Domingo, J. L. (2010). Influence of various cooking processes on the concentrations of PCDD/PCDFs, PCBs and PCDEs in foods. *Food Control, 21* (2), 178-185.

Polder, A., Savinova, T. N., Tkachev, A., Loken, K. B., Odland, J. O. & Skaare, J. U. (2010). Levels and patterns of Persistent Organic Pollutants (POPS) in selected food items from Northwest Russia (1998-2002) and implications for dietary exposure. *Science of the Total Environment, 408* (22), 5352-5361.

Rabieh, S., Schmeisser, E., Yngvadóttir, E., Jónsdóttir, I., Ragnarsdóttir, u. & Gunnlaugsdóttir, H. (2007). *Monitoring of the marine biosphere around Iceland in 2005 - 2006*. Reykjavík: Matís ohf.

Reglugerð um (2.) breytingu á reglugerð nr. 411/2004 um ýmis aðskotaefni í matvælum, nr. 269/2010.

Reglugerð um (3.) breytingu á reglugerð nr. 672/2008 um hámarksgildi varnarefnaleifa í matvælum og fóðri, nr. 758/2010.

Reglugerð um breytingu á reglugerð nr. 411/2004 um ýmis aðskotaefni í matvælum, nr. 056/2005.

Reglugerð um hámarksgildi varnarefnaleifa í matvælum og fóðri, nr. 672/2008.

Ribas-Fito, N., Sala, M., Kogevinas, M. & Sunyer, J. (2001). Polychlorinated biphenyls (PCBs) and neurological development in children: a systematic review. *Journal of Epidemiology and Community Health, 55* (8), 537-546.

Riget, 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.

Romkes, M., Piskorskapliszczynska, J. & Safe, S. (1987). Effects of 2,3,7,8-tetrachlorodibenzo-para-dioxin on hepatic and uterine estrogen-receptor levels in rats. *Toxicology and Applied Pharmacology, 87* (2), 306-314.

Sherer, R. A. & Price, P. S. (1993). The effect of cooking processes on PCB levels in edible fish tissue. *Quality assurance: Good practice, regulation and law, 2* (4), 396-407.

Sinkkonen, S. & Paasivirta, J. (2000). Degradation half-life times of PCDDs, PCDFs and PCBs for environmental fate modeling. *Chemosphere, 40* (9-11), 943-949.

Skaftason, J. F. & Johannesson, T. (1979). Organochlorine compounds (ddt, hexachlorocyclohexane, hexachlorobenzene) in Icelandic animal body-fat and butter fat - local and global sources of contamination. *Acta Pharmacologica Et Toxicologica, 44* (2), 156-157.

Skaftason, J. F. & Johannesson, T. (1982). Organochlorine compounds in Icelandic lake trout and salmon fry - local and global sources of contamination. *Acta Pharmacologica Et Toxicologica, 51* (4), 397-400.

Steingrimsdottir, L., Thorgeirsdottir, H. & Olafsdottir, A. S. (2003). *The Diet of Icelanders. Dietary Survey of the Icelandic Nutrition Council 2002. Main findings*. from http://www.lydheilsustod.is/media/manneldi/rannsoknir/skyrsla.pdf

Stockholm Convention on Persistent Organic Pollutants. Retrieved 10th January, 2010, from http://chm.pops.int/Home/tabid/36/language/en-US/Default.aspx

Swackhamer, D. L. & Trowbridge, A. G. (1996). *Standard operating procedure for the analysis of PCB congeners byr GC/ECD and Trans-nonachlor byr GC/MS/ECNI*. Minneapolis: University of Minnesota.

Szlinder-Richert, J., Barska, I., Mazerski, J. & Usydus, Z. (2009). PCBs in fish from the southern Baltic Sea: Levels, bioaccumulation features, and temporal trends during the period from 1997 to 2006. *Marine Pollution Bulletin, 58* (1), 85-92.

UNEP. (1999). *Guidelines for the identification of PCBs and materials containing PCBs*.

Vetter, W., Hummert, K., Luckas, B. & Skirnisson, K. (1995). Organochlorine residues in 2 seal species from Western Iceland. *Science of the Total Environment, 170* (3), 159-164.

Vetter, W. & Luckas, B. (1995). Theoretical aspects of polychlorinated bornanes and the composition of toxaphene in technical mixtures and environmental-samples. *Science of the Total Environment, 160-61*, 505-510.

Voldner, E. C. & Li, Y. F. (1995). Global usage of selected persistent organochlorines. *Science of the Total Environment, 160-61,* 201-210.

Wania, F., Haugen, J. E., Lei, Y. D. & Mackay, D. (1998). Temperature dependence of atmospheric concentrations of semivolatile organic compounds. *Environmental Science & Technology, 32* (8), 1013-1021.

Wania, F. & Mackay, D. (1993). Global fractionation and cold condensation of low volatility organochlorine compounds in polar-regions. *Ambio, 22* (1), 10-18.

Wells, D. E., Chiffoleau, J. F. & Klungsoyr, J. (1997). QUASIMEME: a preliminary study on the effects of sample handling on the measurement of trace metals and organochlorine residues in mussels. *Marine Pollution Bulletin, 35* (1-6), 109-124.

WHO. (1979). *Environmental health criteria 9. DDT and its Derivatives*. Geneva: World Health Organization.

WHO. (1993). *Environmental health criteria 140. Polychlorinated biphenyls and terphenyls*. Geneva: World Health Organization.

WHO. (1997). *Environmental health criteria 195. Hexachlorobenzene*. Geneva: World Health Organization.

Wisk, J. D. & Cooper, K. R. (1990). Comparison of the toxicity of several polychlorinated dibenzo-para-dioxins and 2,3,7,8-tetrachlorodibenzofuran in embryos of the Japanese medaka (Oryzias-latipes) *Chemosphere, 20* (3-4), 361-377.

Wren, C. D. (1991). Cause-effect linkages between chemicals and populations of mink (*Mustela-vison*) and otter (*Lutra-canadensis*) in the Great-Lakes Basin. *Journal of Toxicology and Environmental Health, 33* (4), 549-585.

7 Appendices

Appendix I

Reference material: levels of POPs analysed at Matis, Akureyri in µg/kg (ww)

QOR068BT	Plaice											
chemical	anal. 1	anal. 2	anal. 3	anal. 4	anal. 5	anal. 6	anal. 7	**mean**	**SD**	assigned value	assigned error %	**I Z I**
CB28*	0.10	0.12	0.12	0.10	0.04	0.04	0.11	**0.09**	**0.035**	0.08		
CB52	0.14	0.15	0.11	0.17	0.07	0.09	0.09	**0.12**	**0.038**	0.14	49.36	**-0.32**
CB101	0.24	0.19	0.20	0.24	0.19	0.18	0.17	**0.20**	**0.027**	0.3	29.25	**-1.10**
CB118	0.32	0.27	0.30	0.22	0.35	0.33	0.27	**0.29**	**0.043**	0.37	25.92	**-0.80**
CB138	0.64	0.68	0.66	0.65	0.74	0.76	0.67	**0.68**	**0.048**	0.73	19.38	**-0.33**
CB153	0.82	0.75	0.74	0.78	0.86	0.87	0.79	**0.80**	**0.052**	1.05	17.26	**-1.37**
CB180	0.16	0.14	0.16	0.16	0.19	0.17	0.16	**0.16**	**0.014**	0.18	40.3	**-0.24**
HCB						0.10	0.16	**0.13**	**0.042**	0.23	34.49	**-1.25**
p,p'-DDE						0.74	0.80	**0.77**	**0.042**	1	17.49	**-1.29**

* Quasimeme does not assign %error and thus Z-score can not be calculated

QOR086BT	Cod liver						
chemical	anal. 1	anal. 2	**mean**	**SD**	assigned value	assigned error %	**I Z I**
CB28	12.3	10.9	**11.62**	**0.96**	9.64	1.22	**0.17**
CB52	30.1	28.8	**29.44**	**0.92**	23.8	2.99	**0.08**
CB101	72.4	69.9	**71.13**	**1.78**	65.3	8.18	**0.01**
CB118	78.4	76.2	**77.32**	**1.57**	73.1	9.15	**0.01**
CB138	156	146	**151.32**	**7.1**	136	17.1	**0.01**
CB153	243	235	**238.96**	**6.1**	220	27.6	**0.00**
CB180	59.4	52.8	**56.12**	**4.64**	45.5	7.71	**0.03**
HCB	15.2	15.5	**15.35**	**0.17**	13.5	1.7	**0.08**
p,p'-DDE	73.8	64.3	**69.05**	**6.72**	83.3	10.42	**-0.02**

QTX027BT	Herring						
chemical	anal. 1	anal. 2	**mean**	**SD**	assigned value	assigned error %	**I Z I**
Tox26	0.88	0.76	**0.82**	**0.08**	0.96	17.0	**-0.01**
Tox50	2.02	1.92	**1.97**	**0.07**	1.77	27.2	**0.89**

Appendix II

Concentration of POPs in cod muscle in µg/kg (ww)

Sample ID	Trip no.	fishing ground	Length (cm)	Weight (kg)	Sex	Maturity	Liver weigth (kg)	Weight gutted (kg)	Age (y)	Fat content Liver (%)	CB-28	CB-52	CB-101	CB-118	CB-153	CB-138	CB-180	∑PCB7	HCB	p,p´-DDE	Tox26	Tox50
M-2007-3027	2	SW	83	4.300	female	4	0.100	3.70	8	31.0	0.120	0.127	0.094	0.143	0.224	0.217	0.053	0.978	0.127	0.299	0.109	0.086
M-2007-3034	2	SW	76	3.400	female	3	0.100	3.26	6	24.0	0.117	0.139	0.057	0.081	0.202	0.157	0.047	0.799	0.086	0.173	0.027	0.027
M-2007-3036	2	SW	84	5.480	female	4	0.160	4.70	7	35.0	0.093	0.094	0.053	0.074	0.216	0.133	0.054	0.718	0.050	0.179	0.028	0.016
M-2007-3041	2	SW	72	3.300	female	4	0.100	2.90	5	36.8	0.135	0.087	0.025	0.032	0.102	0.064	0.016	0.461	0.061	0.050	0.006	0.008
M-2007-3043	2	SW	58	1.520	male	3	0.050	1.32	4	4.2	0.103	0.105	0.070	0.137	0.303	0.215	0.065	0.999	0.067	0.278	0.043	0.034
M-2007-3049	2	SW	66	3.180	male	3	0.180	2.69	7	63.7	0.101	0.097	0.026	0.016	0.026	0.033	0.005	0.304	0.050	0.028	0.011	0.014
M-2008-745	1	NW	58	1.606	female	1	0.097	1.33	6	53.9	0.091	0.122	0.074	0.022	0.059	0.065	0.016	0.448	0.039	0.039	0.030	0.021
M-2008-746	1	NW	68	2.385	female	1	0.108	2.04	7	48.3	0.110	0.161	0.112	0.074	0.127	0.151	0.028	0.763	0.061	0.123	0.073	0.048
M-2008-747	1	NW	81	4.345	female	1	0.406	3.44	7	63.6	0.101	0.139	0.102	0.047	0.069	0.087	0.013	0.559	0.055	0.059	0.025	0.024
M-2008-748	1	NW	50	0.964	male	1	0.071	0.86	4	59.6	0.129	0.169	0.146	0.068	0.075	0.097	0.014	0.697	0.030	0.038	0.029	0.024
M-2008-749	1	NW	70	3.100	female	1	0.231	2.54	6	64.0	0.097	0.132	0.110	0.066	0.070	0.081	0.019	0.575	0.040	0.058	0.033	0.021
M-2008-750	3	NW	85	5.224	male	2	0.402	4.32	5	56.4	0.105	0.122	0.077	0.051	0.053	0.079	0.014	0.501	0.036	0.055	0.023	0.022
M-2008-751	3	NW	74	3.354	male	2	0.257	2.88	6		0.105	0.119	0.052	0.046	0.041	0.055	0.010	0.427	0.067	0.048	0.025	0.018
M-2008-752	3	NW	66	2.328	male	1	0.138	2.04	5		0.079	0.094	0.036	0.027	0.023	0.034	0.005	0.297	0.036	0.022	0.011	0.009
M-2008-753	3	NW	61	1.833	male	1	0.113	1.56	6		0.088	0.115	0.062	0.061	0.065	0.085	0.015	0.491	0.076	0.098	0.031	0.017
M-2008-754	3	NW	49	1.354	male	1	0.093	1.08	4		0.136	0.146	0.057	0.034	0.026	0.037	0.005	0.441	0.039	0.020	0.012	0.013
M-2008-755	4	W	89	7.950	female	2	0.515	6.94	7	48.4	0.103	0.143	0.059	0.038	0.033	0.054	0.008	0.438	0.025	0.020	0.013	0.014
M-2008-756	4	W	79	4.202	female	2	0.113	3.73	6	58.0	0.108	0.142	0.061	0.047	0.083	0.079	0.011	0.530	0.027	0.050	0.028	0.026
M-2008-757	4	W	70	2.777	female	2	0.059	2.53	4	43.0	0.090	0.133	0.052	0.045	0.063	0.068	0.010	0.461	0.042	0.047	0.031	0.029
M-2008-758	4	W	60	2.212	female	2	0.063	1.99	6	55.0	0.058	0.074	0.035	0.030	0.024	0.032	0.008	0.261	0.019	0.017	0.011	0.010
M-2008-759	4	W	47	1.008	male	1	0.026	0.928	3	69.7	0.107	0.125	0.062	0.019	0.023	0.043	0.007	0.388	0.024	0.018	0.010	0.011

In µg/kg wet weight (w.w)

Sample ID	Trip no.	fishing ground	Length (cm)	Weight (kg)	Sex	Maturity	Liver weigth (kg)	Weight gutted (kg)	Age (y)	Fat content Liver (%)	CB-28	CB-52	CB-101	CB-118	CB-153	CB-138	CB-180	∑PCB7	HCB	p,p´-DDE	Tox26	Tox50
M-2008-1147	5	SW	92	8.860	male	2	0.298	6.80		60.8	0.052	0.088	0.046	0.029	0.070	0.052	0.011	0.348				
M-2008-1148	5	SW	74	3.896	male	2	0.068	3.24	7	31.3	0.037	0.073	0.044	0.106	0.354	0.173	0.112	0.899				
M-2008-1153	5	SW	96	8.650	female	2	0.711	6.89	9		0.049	0.083	0.069	0.062	0.124	0.131	0.024	0.542				
M-2008-1162	5	SW	62	2.033	female	1	0.036	1.77	5	15.2	0.044	0.063	0.065	0.069	0.179	0.132	0.033	0.585				
M-2008-1173	5	SW	64	2.421	male	2	0.150	1.45	6	75.9	0.068	0.084	0.056	0.037	0.053	0.050	0.007	0.354				
M-2008-1347	6	NW	44	0.908	female	1	0.011	0.77	4	30.0	0.044	0.085	0.050	0.069	0.147	0.099	0.027	0.521	0.048	0.068	0.021	0.029
M-2008-1349	6	NW	68	2.912	male	1	0.038	2.48	6	8.6	0.064	0.102	0.102	0.173	0.359	0.295	0.095	1.191	0.055	0.239	0.052	0.071
M-2008-1353	6	NW	74	4.532	female	3	0.048	3.38	8	5.9	0.044	0.084	0.095	0.160	0.348	0.253	0.085	1.070	0.024	0.194	0.037	0.051
M-2008-1356	6	NW	53	1.429	male	1	0.022	1.19	4	17.0	0.041	0.079	0.068	0.104	0.242	0.164	0.050	0.749	0.040	0.114	0.031	0.033
M-2008-1366	6	NW	83	5.778	male	2	0.231	4.64	7	53.3	0.038	0.082	0.049	0.056	0.113	0.084	0.021	0.443	0.054	0.054	0.026	0.046
M-2008-2269	7	W	86	5.162	male	4	0.132	4.63	7	41.4	0.044	0.102	0.158	0.039	0.278	0.251	0.069	0.942				
M-2008-2275	7	W	65	2.013	female	4	0.075	1.74	6	49.0	0.058	0.093	0.055	0.016	0.069	0.055	0.016	0.363				
M-2008-2277	7	W	73	3.244	female	4	0.084	2.90	6	53.1	0.034	0.064	0.043	0.012	0.071	0.046	0.012	0.281	0.056	0.044	0.020	0.013
M-2008-2278	7	W	49	0.905	male	4	0.024	0.83	4	38.0	0.050	0.114	0.092	0.030	0.113	0.086	0.019	0.504	0.055	0.073	0.032	0.036
M-2008-2282	7	W	54	1.335	male	3	0.034	1.14	5	45.4	0.057	0.091	0.066	0.019	0.050	0.070	0.014	0.366	0.066	0.034	0.030	0.031
M-2008-3450	8	SW	82	5.144	male	1	0.193	4.64	8	53.5	0.032	0.063	0.048	0.048	0.111	0.082	0.013	0.397	0.031	0.058	0.028	0.035
M-2008-3454	8	SW	78	4.340	male	1	0.120	3.96	7	41.0	0.033	0.071	0.050	0.063	0.151	0.089	0.037	0.492	0.033	0.059	0.014	0.021
M-2008-3458	8	SW	66	2.426	male	1	0.043	2.26	5	53.1	0.035	0.074	0.061	0.048	0.085	0.065	0.013	0.381	0.038	0.056	0.020	0.014
M-2008-3461	8	SW	56	1.515	male	1	0.018	1.42	3	29.7	0.034	0.086	0.107	0.110	0.172	0.159	0.031	0.700	0.029	0.055	0.024	0.055
M-2008-4095	9	NW	77	3.476	male	2	0.138	3.11	5	70.6	0.025	0.072	0.046	0.049	0.064	0.055	0.012	0.323	0.041	0.084	0.030	0.017
M-2008-4096	9	NW	47	0.815	female	2	0.034	0.74	3	53.5	0.047	0.077	0.054	0.048	0.082	0.065	0.014	0.387	0.022	0.044	0.021	0.020
M-2008-4098	9	NW	53	1.343	male	1	0.072	1.18	4		0.043	0.078	0.033	0.030	0.040	0.032	0.007	0.263				
M-2008-4104	9	NW	90	5.730	female	2	0.307	5.06	7	72.8	0.030	0.097	0.063	0.066	0.092	0.087	0.021	0.457	0.036	0.101	0.035	0.019
M-2008-4112	9	NW	66	2.303	male	2	0.091	2.10	5	65.9	0.036	0.074	0.050	0.041	0.065	0.055	0.012	0.333	0.032	0.060	0.022	0.017
M-2008-4763	10	NW	70	2.775	female	4	0.271	2.35	5		0.030	0.078	0.036	0.022	0.040	0.028	0.006	0.242				
M-2008-4767	10	NW	62	1.800	male	4	0.131	1.58	5		0.036	0.079	0.035	0.011	0.043	0.053	0.002	0.257				
M-2008-4774	10	NW	81	4.978	male	3	0.158	4.39	6		0.038	0.066	0.058	0.057	0.170	0.116	0.041	0.546				
M-2008-4777	10	NW	48	0.915	female	1	0.015	0.75	3		0.049	0.094	0.079	0.057	0.153	0.118	0.035	0.584				

In µg/kg wet weight (w.w)

Sample ID	Trip no.	fishing ground	Length (cm)	Weight (kg)	Sex	Maturity	Liver weigth (kg)	Weight gutted (kg)	Age (y)	Fat content Liver (%)	CB-28	CB-52	CB-101	CB-118	CB-153	CB-138	CB-180	∑PCB7	HCB	p,p´-DDE	Tox26	Tox50
M-2009-500	11	SW	80	4.410	male	2	0.346	3.77	5	17.6	0.104	0.117	0.092	0.039	0.049	0.072	0.012	0.484	0.028	0.066	0.031	0.028
M-2009-507	11	SW	89	4.658	male	2	0.092	4.18	6	47.4	0.122	0.115	0.087	0.066	0.114	0.103	0.029	0.636	0.024	0.063	0.016	0.011
M-2009-509	11	SW	72	3.360	female	2	0.049	3.12	5	50.0	0.100	0.110	0.107	0.083	0.171	0.154	0.039	0.763	0.027	0.155	0.033	0.014
M-2009-516	11	SW	55	1.548	female	1	0.023	1.37	3	56.4	0.143	0.172	0.118	0.078	0.168	0.131	0.040	0.851	0.049	0.092	0.029	0.016
M-2009-517	11	SW	65	2.259	female	1	0.025	2.12	4	69.5	0.093	0.136	0.139	0.107	0.286	0.245	0.074	1.081	0.031	0.212	0.059	0.029
M-2009-1820	12	SW	67	2.622	male	3	0.195	2.01	6	69.9	0.121	0.115	0.074	0.039	0.034	0.045	0.011	0.441	0.067	0.044	0.024	0.021
M-2009-1821	12	SW	61	2.004	male	1	0.020	1.85	4	39.6	0.119	0.132	0.089	0.064	0.156	0.113	0.037	0.710	0.067	0.077	0.024	0.009
M-2009-1828	12	SW	56	1.568	male	2	0.108	1.25	6	75.7	0.113	0.139	0.070	0.030	0.027	0.033	0.008	0.420	0.092	0.034	0.022	0.020
M-2009-1831	12	SW	70	2.784	female	1	0.092	2.51	5	59.9	0.106	0.106	0.084	0.053	0.072	0.078	0.014	0.514	0.035	0.069	0.028	0.016
M-2009-1836	12	SW	88	7.425	male	3	0.670	5.24	7	78.6	0.098	0.076	0.065	0.038	0.052	0.066	0.013	0.408	0.038	0.102	0.033	0.023
M-2009-2251	13	W	86	4.933	female	4	0.228	3.93	6	71.8	0.090	0.112	0.061	0.034	0.044	0.058	0.015	0.414	0.066	0.095	0.032	0.013
M-2009-2254	13	W	68	2.483	male	1	0.138	2.18	6	69.6	0.092	0.128	0.062	0.039	0.040	0.051	0.009	0.421	0.077	0.065	0.028	0.016
M-2009-2260	13	W	55	1.275	female	1	0.064	1.14	5	71.7	0.123	0.151	0.083	0.049	0.043	0.056	0.010	0.515	0.071	0.065	0.040	0.014
M-2009-2262	13	W	60	1.778	male	1	0.021	1.65	4	35.8	0.124	0.113	0.095	0.069	0.152	0.119	0.034	0.705	0.057	0.095	0.043	0.020
M-2009-2267	13	W	74	2.905	female	4	0.083	2.68	7	67.8	0.090	0.127	0.068	0.043	0.074	0.071	0.013	0.485	0.070	0.116	0.041	0.020

In µg/kg wet weight (w.w)

d

Appendix III

Concentration of POPs in cod liver samples in µg/kg (ww)

Sample ID	CB-28	CB-52	CB-101	CB-118	CB-138	CB-153	CB-180	∑PCB7	∑PCB7 (lw)	HCB	p,p'-DDE	Tox26	Tox50
M-2007-3034	3.11	2.53	8.05	22.4	28.7	44.4	11.4	**121**	**502**	4.70	40.12	3.96	5.36
M-2007-3036	5.21	9.65	24.6	59.0	73.5	117	35.3	**325**	**928**	10.2	134.1	15.6	21.5
M-2007-3041	3.63	2.83	4.59	18.8	18.3	20.5	5.75	**74.5**	**202**	5.92	37.02	2.66	7.53
M-2007-3049	4.61	7.14	14.9	17.0	22.0	33.3	8.18	**107**	**168**	11.0	36.2	11.6	16.5
M-2008-745	13.8	12.9	12.7	30.6	24.1	28.2	6.69	**129**	**239**	20.5	37.9	18.4	21.8
M-2008-746	16.2	18.4	18.1	33.1	35.9	44.2	10.1	**176**	**364**	24.1	76.6	31.5	43.9
M-2008-747	15.8	18.0	16.7	32.2	27.5	30.9	8.46	**150**	**235**	29.7	54.1	22.0	27.5
M-2008-749	10.2	15.1	16.0	25.2	27.9	32.9	8.24	**135**	**212**	27.3	58.8	20.5	30.0
M-2008-756	9.57	16.9	23.8	45.1	45.3	63.8	14.8	**219**	**378**	22.9	90.0	34.2	47.6
M-2008-757	2.31	10.1	13.9	26.6	30.6	45.2	8.97	**138**	**320**	10.2	40.8	14.8	22.1
M-2008-758	14.8	11.6	8.5	19.2	22.9	34.7	7.96	**120**	**217**	11.3	27.0	10.4	15.2
M-2008-759	15.9	14.9	12.7	12.3	19.7	26.2	5.85	**107**	**154**	14.3	24.2	9.30	15.9
M-2008-1347	6.66	10.6	11.2	27.1	35.0	58.8	13.2	**163**	**542**	10.8	41.0	12.1	15.7
M-2008-1349	2.97	2.62	7.77	16.2	22.3	32.2	7.00	**91.2**	**1,060**	4.57	29.28	4.82	7.36
M-2008-1353	1.65	2.65	9.94	21.4	28.4	40.4	8.86	**113**	**1,919**	3.33	38.38	4.74	6.93
M-2008-1356	3.67	7.49	12.1	21.8	30.7	48.2	9.54	**133**	**785**	7.30	37.00	9.14	6.43
M-2008-1366	3.52	7.51	17.3	32.7	40.4	58.0	13.8	**173**	**325**	17.8	51.3	17.2	22.1
M-2008-2277	9.51	16.8	20.1	25.2	34.9	18.9	10.9	**136**	**257**	24.3	55.6	23.6	35.0
M-2008-2278	9.99	18.4	20.1	25.6	33.2	45.2	9.37	**162**	**426**	20.5	71.1	26.0	40.2
M-2008-2282	17.2	21.1	30.5	31.5	41.0	52.5	13.3	**207**	**456**	26.5	112.2	39.9	78.5

In µg/kg wet weight (w.w) unless otherwise marked

lw* lipid weight

Sample ID	CB-28	CB-52	CB-101	CB-118	CB-138	CB-153	CB-180	∑PCB7	∑PCB7 (lw)	HCB	p,p'-DDE	Tox26	Tox50
M-2008-3450	12.4	22.9	34.8	43.9	66.4	106	28.1	**315**	**588**	19.2	92.2	30.9	48.7
M-2008-3454	14.1	16.5	26.0	47.2	63.7	116	31.0	**314**	**766**	10.9	76.7	16.2	27.0
M-2008-3458	19.0	25.2	32.8	40.5	53.8	67.4	14.3	**253**	**476**	20.1	97.1	41.8	82.0
M-2008-3461	11.4	17.4	26.7	25.5	50.5	63.6	12.0	**207**	**697**	10.1	37.0	16.4	32.5
M-2008-4095	13.6	20.8	28.7	34.1	42.0	53.1	13.3	**206**	**291**	28.6	105	36.2	68.0
M-2008-4096	27.7	21.3	16.9	18.6	27.6	38.0	9.4	**159**	**298**	16.4	36.6	19.4	33.6
M-2008-4104	33.1	35.2	30.0	31.2	46.1	53.1	13.3	**242**	**332**	25.9	74.9	33.3	49.3
M-2008-4112	14.8	24.6	30.4	28.0	40.6	52.4	12.8	**204**	**309**	29.1	90.8	37.4	62.0
M-2009-1820	6.77	11.3	14.8	15.5	19.4	24.7	5.3	**97.8**	**140**	23.7	43.5	20.1	34.2
M-2009-1821	3.05	5.81	12.2	32.8	51.2	89.9	21.4	**216**	**546**	7.4	51.3	9.1	14.7
M-2009-1828	5.40	7.71	10.2	11.0	14.3	20.0	4.49	**73.1**	**96.5**	18.8	25.1	13.5	21.3
M-2009-1831	16.2	19.8	34.4	27.5	36.3	49.6	10.8	**195**	**325**	12.1	52.6	20.8	35.5
M-2009-1836	6.28	15.2	21.7	21.8	28.9	35.9	8.58	**138**	**176**	25.3	81.2	26.1	44.6
M-2009-2251	17.7	23.8	27.7	29.6	39.8	49.3	12.8	**201**	**280**	23.6	98.4	30.7	52.0
M-2009-2254	6.53	11.9	19.2	21.9	28.2	38.1	9.24	**135**	**194**	22.7	67.0	24.3	39.4
M-2009-2260	5.11	8.63	14.3	15.6	19.2	27.1	6.74	**96.7**	**135**	16.7	52.8	18.6	31.4
M-2009-2262	3.54	7.03	15.1	29.1	37.0	59.2	15.1	**166**	**464**	10.6	51.2	17.1	28.9
M-2009-2267	17.9	24.8	34.0	40.3	49.4	67.2	16.3	**250**	**369**	31.1	134	47.5	80.3

In µg/kg wet weight (w.w) unless otherwise marked

lw* lipid weight

Appendix IV

Concentration of POPs in oven baked cod muscle µg/kg (ww)

Sample ID	CB-28	CB-52	CB-101	CB-118	CB-153	CB-138	CB-180	∑PCB7	HCB	p,p'-DDE	Tox26	Tox50
M-2008-745	0.024	0.033	0.016	0.005	0.013	0.010	0.002	**0.105**	0.027	0.011	0.003	0.008
M-2008-746	0.030	0.048	0.030	0.022	0.049	0.031	0.009	**0.219**	0.050	0.036	0.015	0.023
M-2008-747	0.038	0.041	0.019	0.012	0.021	0.018	0.005	**0.154**	0.036	0.020	0.007	0.013
M-2008-748	0.034	0.049	0.022	0.013	0.021	0.018	0.002	**0.158**	0.044	0.021	0.010	0.015
M-2008-749	0.026	0.040	0.025	0.010	0.018	0.016	0.004	**0.140**	0.028	0.016	0.006	0.011
M-2008-750	0.024	0.046	0.021	0.013	0.026	0.021	0.006	**0.158**	0.029	0.023	0.007	0.011
M-2008-751	0.022	0.036	0.014	0.006	0.006	0.008	0.003	**0.095**	0.021	0.010	0.002	0.005
M-2008-753	0.021	0.012	0.027	0.012	0.024	0.014	0.001	**0.111**	0.037	0.021	0.006	0.009
M-2008-754	0.033	0.037	0.021	0.007	0.008	0.009	0.002	**0.116**	0.033	0.009	0.002	0.006
M-2008-755	0.016	0.026	0.015	0.005	0.004	0.004	0.004	**0.074**	0.019	0.005	0.001	0.004
M-2008-756	0.028	0.041	0.016	0.011	0.023	0.015	0.003	**0.138**	0.027	0.015	0.004	0.007
M-2008-757	0.025	0.030	0.008	0.006	0.012	0.008	0.001	**0.091**	0.016	0.006	0.001	0.003
M-2008-758	0.017	0.007	0.011	0.004	0.007	0.007	0.002	**0.055**	0.018	0.005	0.001	0.004
M-2008-759	0.023	0.021	0.010	0.006	0.004	0.005	0.001	**0.071**	0.023	0.004	0.001	0.003

In µg/kg wet weight (w.w)