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Dioxin and PCB concentrations in salmon and herring from the Baltic Sea – impact of cooking methods, uncertainty of chemical analyses and differences between parts of the fish

Hadi Soroosh, Hannes Waldetoft, Joakim Hållén & Magnus Karlsson

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Author: Hadi Soroosh, Hannes Waldetoft, Joakim Hållén & Magnus Karlsson, IVL Swedish Environmental Research Institute
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IVL Swedish Environmental Research Institute Ltd.
P.O Box 210 60, S-100 31 Stockholm, Sweden
Phone +46-(0)10-7886500 // Fax +46-(0)10-7886590 // www.ivl.se

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Contents

Summary

Since 2014, IVL Swedish Environmental Research Institute investigates prevalence of dioxin-like substances in fish from the Baltic Sea, Lake Vänern and Lake Vättern. The work is financed through funds allocated to small-scale commercial fishing in the northern Baltic Proper, the Gulf of Bothnia and the major lakes in Sweden with complementary funding from the IVL Foundation (SIVL).The main purpose is to clarify the existence of spatial and temporal variation, variations between different populations, fishing methods, cooking procedures, parts of the fish etc., that can be used by small scale professional fishing and associated industries to provide consumers with foodstuffs low in dioxin and PCB.

This report quantifies variations in fat content, dioxin and PCB between different parts of the fish and for different cooking procedures. It also investigates the accuracy of the chemical analysis measuring fat content, dioxin-like compounds and non-dioxin-like PCBs. All concentrations are measured on wet weight (ww).

The comparisons between cooked and raw fish are based on a relatively small number of samples showing a high degree of variability. Therefore, the results do not give a suitable setting for drawing any general conclusions but provide indications of the effects on concentrations of dioxins, PCB (and PAH) of fish preparation that can serve as the basis for discussions and decisions of future more comprehensive studies. On the other hand, the results regarding the variations in different parts of the fish are based on a relatively large sample, which allows for a higher degree of confidence in the results.

For herring, the following cooking procedures are investigated; smoking (*böckling*), fermenting (*surströmming*) and frying. For salmon, the cooking procedures are hot-smoking, cold-smoking and curing (*gravning*). Comparisons for salmon are made pairwise. The same cut from both sides of the fish is compared. One is cooked and one is kept raw. For example, if the tail part from one side of the fish is smoked, the tail part from the other side is kept raw. Regarding herring, composite samples are used, meaning that muscle tissue from several individuals are homogenized into one sample. The raw and cooked samples are composite samples of individuals from the same catch. Using pairwise observations and composite samples from the same catch minimizes variability in concentrations of dioxin-like compounds that are not a consequence of the cooking procedure.

The testing has not shown any clear results, but an indication that curing can reduce the content of dioxin-like compounds have emerged. The reduction in this sample was about 28%. For hot smoking and cold smoking, no reduction was seen. A possible explanation can be that the reduction of fat, and hence reduction dioxins and PCBs bound to the fat are compensated for by the evaporation of water, increasing the dry matter content. It is therefore likely that the content of dioxin-like compounds, measured on wet weight is about the same. Smoking herring resulted in a reduction of dioxin-like compounds of 30%, but since the lab analysis reports a 25% error margin for these substances, it is not

concluded that smoking leads to a reduction. However, the fat content was reduced, and the measure of fat content has been shown to be more exact than dioxins and PCB, which can indicate that smoking reduces content of dioxin-like compounds.

It is well established that smoking of foodstuffs leads to formation of PAH:s, of which some are deemed hazardous for humans. For these samples, a clear increase of PAH:s was observed for the smoked samples. One out of three hot-smoked samples of salmon exceeded the PAH-limit for sales set by the European Union.

The table below summarizes the results from different cooking procedures. The relative changes indicate the average change in percent for the cooked samples when compared to the corresponding raw samples.

Summary of results from comparing levels of dioxin, PCB and PAH:s between cooked and raw samples of salmon and herring. The number of observations are denoted as "n" and the percentage changes are when compared to the corresponding raw sample. Substantial.(Yes/No) indicates if changes are large enough to be suspected not only to be a consequence of measurement error and inherent variation between the pairwise and composite samples. Due to small sample sizes, no formal tests are made. Substantial differences act merely as an indication that the cooking procedure could have an effect on prevalence of dioxin, PCB or PAH.

Species	Preparation	n	Delta-fat (%) Substantial. (Yes/No)	Delta-∑PCDD (%) Substantial. (Yes/No)	Delta-∑PCDD+dl-PCB (%) Substantial. (Yes/No)	Delta-∑PCB6 (%) Substantial. (Yes/No)	Delta-PAH4 (%) Substantial. (Yes/No)
Salmon	Cold-smoked	2	+8, No	+23, No	+5, No	+4, No	-5, No
Salmon	Cured	3	+14, No	-28, Yes	-38, Yes	-28, Yes	-19, No
Salmon	Hot-smoked	4	+26, No	+20, No	+22, No	+17, No	+960, Yes
Herring	Smoked	1	-34, No	-46, No	-34, No	-6,6, No	+1300, Yes
Herring	Fried	1	+147, Yes	-3,4, No	-13, No	-7, No	-45, No
Herring	Fermented	1	+0,6, No	+15, No	-2, No	-11, No	-15, No

Analysis of fat content and dioxin-like compounds in different parts of salmon and trout showed that levels of dioxin-like compounds are approximately 8% higher in muscle tissue from the neck compared to muscle tissue from the middle section, and approximately 21,5% lower in muscle tissue from the tail compared to the middle.

Prevalence of dioxin-like compounds were shown to be strongly connected to fat content. It is estimated that for a one percent (not percentage) increase of fat content, the increase of dioxin-like compounds is 0,84%. Inclusion of belly fat in the analyzed sample resulted in an estimated 16,4% increase of dioxin-like compounds and in samples of subcutaneous fat, levels of dioxin-like compounds were at least twice as high as in its corresponding muscle tissue. When comparing the fillet (which is the part most often consumed) to the middle section prepared in accordance with EU-regulations, it was found that the fillets, on average, in this sample have 29% lower fat content than the middle part. This indicates that possibly, levels of dioxin-like compounds are lower in the fillet than in the middle part. The results from the comparison between different parts, with/without belly fat and of subcutaneous fat are summarized in the table below. The analysis that compared differences between different parts of the fish and with/without belly-fat is based on 17 individual salmon and 3 individual trout. In total, the analysis is based on 75 observations. The comparison between fillet/middle was based on 5 observations and there were 2 samples of subcutaneous fat.

Summary of differences between different parts of salmon and trout. The percentage changes are when compared to the middle part. The results for "neck", "tail" and "with belly-fat" are based on a much larger sample than "subcutaneous fat" and "fillet", so their estimated differences are believed to apply for the population of salmon and trout, whereas changes for "subcutaneous fat" and "fillet" are weaker indications which applies mainly to this specific sample.

Part	Delta-fat (%)	Delta-∑PCDD/F (%)	Delta-∑PCDD/F+dl-PCB (%)	Delta-∑PCB6 (%)
Neck	+9,6	+8,0	+8,0	+5,4
Tail	-26	-22	-22	-16
Fillet	-29	-24	-24	-18
With belly-fat	+20	+16	+16	+11
Subcutaneous fat	+96	+190	+160	+150

About thirty duplicate samples was sent to two independent laboratories. When comparing the results from the labs, it was justified that lab analysis of dioxin-like compounds are associated with relatively large measurement errors. Also, it was found that for dioxin-like compounds and non-dioxin-like PCBs, some bias between the labs is present. One lab, on average, reported higher values than the other. The values reported were about 10-20% higher for this lab, but this investigation cannot tell if these differences are consistent over time.

About forty duplicate samples was analyzed by the same lab. Results for dioxin-like compounds showed a higher accuracy when the same lab analyzed replicates. 80- 83% of the variance in one of the duplicates could be explained by the other, compared to 64- 73%

when two labs analyzed the duplicates. For non-dioxin-like PCBs, an indication of bias was found. The average value was 13% higher for one set of the duplicates. Regarding fat content for both within a lab and between labs, 93- 95% of the variance in one set of the duplicates could be explained by values for the other set.

A summary of the results from the lab comparisons are seen in the table below.

Summary of results from Wilcoxon signed rank test of replicates from Lab1 and Lab2. A p-value smaller than 0,05 is significant. In case of significance, it is concluded that at the 5%-level, bias exists.

	Fat	**∑PCDD/F**	**∑PCDD/F+dl-PCB**	**∑PCB6**
	Bias. (Yes/No) (p-val)	Bias. (Yes/No) (p-val)	Bias. (Yes/No) (p-val)	Bias. (Yes/No) (p-val)
Lab1 vs Lab2	No 0,22	No 0,29	Yes 0,00078	Yes 0,013
Lab2 vs Lab2	No 0,19	No 0,99	No 0,25	Yes 0,0035

Sammanfattning

IVL Svenska Miljöinstitutet genomför sedan 2014 undersökningar av halter av dioxinlika ämnen i fisk från Östersjön, Vänern och Vättern. Arbetet finansieras genom medel som tillställts det småskaliga yrkesfisket i norra Egentliga Östersjön, Bottniska viken och de stora sjöarna i Sverige med motfinansiering från Stiftelsen IVL. Det övergripande syftet är att klarlägga om det finns variationer i tid och rum, fiskbestånd, val av fiskemetod, beredningsmetod eller andra faktorer som det småskaliga yrkesfisket, och därtill associerad beredningsindustri, kan utnyttja för att tillhandahålla livsmedelsprodukter med så låga halter som möjligt av dioxinlika ämnen.

I denna rapport kvantifieras hur olika beredningsmetoder påverkar innehållet av dioxinlika ämnen i fiskens muskelkött, den variation som föreligger mellan olika delar av fisken med avseende på fetthalt och halter av klororganiska ämnen samt statistiska aspekter när det gäller mätnoggrannhet och den osäkerhet som följer av att kemiskt analysera aktuella ämnen.

Jämförelserna mellan beredd och rå fisk baseras på ett relativt litet antal prover som uppvisade en hög grad av variabilitet. Rapporten kan därför inte användas för att dra några allmänna slutsatser men ger indikationer på effekterna på koncentrationer av dioxiner, PCB (och PAH) av olika beredning som kan tjäna som bas för diskussioner och underlag för att utforma framtida mer omfattande studier. Däremot är resultaten avseende skillnader i förekomst av dioxin och PCB mellan fiskens olika delar baserat på ett relativt stort urval, vilket medger en högre grad av generaliserbarhet.

För strömming undersöktes följande beredningsmetoder; rökning (böckling), fermentering (surströmming) och stekning. Beredningsmetoderna för lax var varmrökning, kallrökning och gravning. Jämförelserna för lax gjordes parvis. Samma del från samma lax, men motsatt sida av kroppen användes. Delen från ena sidan analyserades efter tillagning och den andra analyserades rå. Till exempel, om stjärten från ena sidan av en lax röktes, behölls stjärten från motsatt sida rå. För strömmingen användes samlingsprov, vilket betyder att muskelvävnad från flertalet individer homogeniseras till ett prov. De råa och tillagade proven var samlingsprov från samma fångst. Att göra parvisa jämförelser utifrån samlingsprov från samma fångst minskar den variation i halter av dioxinlika ämnen mellan det råa och tillagade provet som inte är en konsekvens av tillagningen.

Jämförelserna visar inte på någon tydlig reducering av halter, men en indikation på att gravning kan reducera halter av dioxinlika ämnen framkom. Minskningen efter gravning var ca 28%. För varm- och kallrökning av lax syntes ingen minskning. Sannolikt kompenseras den avgång av fett och föroreningar bundna till fettet som sker i samband med rökningen med att även vattenånga avgår och att torrhalten därmed ökar. Dioxinhalten mätt på färskviktsbasis blir då ungefärligen densamma. Rökning av strömming till böckling visade på en minskning av halter av dioxinlika ämnen på ca 30%,

men på grund av liten mängd data och felmarginalen på ca 25% kopplat till laboratorie-analysen dras inte slutsatsen att rökning leder till en reduktion av dioxinlika ämnen. Däremot syntes en minskning av fetthalten vid rökning, och då analyser av fetthalt i denna rapport visas vara mer exakta än analyser av dioxinlika ämnen finns en indikation på att rökning kan reducera halter av dioxinlika ämnen. Stekning och fermentering till surströmming visade inte på någon minskning av halter av dioxinlika ämnen.

Det är väl känt att rökning av livsmedel leder till bildning av PAH:er, av vilka några anses farliga för människor. För dessa data syntes en tydlig ökning av PAH:er vid rökning av lax och strömming. Ett av de varmrökta laxproven överskred EU:s gränsvärde för saluföring. I nedanstående tabell summeras resultaten från jämförelserna som gjorts mellan råa och tillagade prover. De relativa förändringarna indikerar genomsnittlig förändring i procent för tillagade prover jämförda med motsvarande råa prover.

Summering av resultat från jämförelse av halter av dioxin, PCB och PAH mellan tillagade och råa lax- och strömmingsprover. Antal observationer betecknas som "n" och de procentuella skillnaderna visar på skillnad gentemot motsvarande rått prov. Om "n">1 avser den procentuella skillnaden ett medelvärde. Substantiell (Ja/Nej) indikerar om en skillnad är så pass stor att den misstänks vara en konsekvens av tillagningsmetoden. De små urvalsstorlekarna möjliggör inte statistiska hypotestester. En "substantiell" skillnad agerar som en indikation på att tillagningsmetoden i fråga påverkar halter av aktuella ämnen.

Art	Tillagning	n	Delta-fett (%) Substantiell. (Ja/Nej)	Delta-∑PCDD (%) Substantiell. (Ja/Nej)	Delta-∑PCDD+dl-PCB (%) Substantiell. (Ja/Nej)	Delta-∑PCB6 (%) Substantiell. (Ja/Nej)	Delta-PAH4 (%) Substantiell. (Ja/Nej)
Lax	Varmrökt	2	+8 Nej	+23 Nej	+5 Nej	+4 Nej	-5 Nej
Lax	Gravad	3	+14 Nej	-28 Ja	-38 Ja	-28 Ja	-19 Nej
Lax	Kallrökt	4	+26 Nej	+20,0 Nej	+22 Nej	+17 Nej	+960 Ja
Strömming	Rökt	1	-34 Nej	-46 Nej	-34 Nej	-6,6 Nej	+1300 Ja
Strömming	Stekt	1	+147 Ja	-3,4 Nej	-12,7 Nej	-6,5 Nej	-45 Nej
Strömming	Fermenterad	1	+0,59 Nej	+15 Nej	-2,3 Nej	-11 Nej	-15 Nej

Statistiska analyser av fetthalt och halter av dioxinlika ämnen visade på att halten av dioxinlika ämnen i lax och öring i genomsnitt är ca 8% högre i nacken jämfört med mittbiten, och ca 22 % lägre i stjärten än mittbiten. Förekomsten av dioxinlika ämnen visade sig starkt kopplad till fetthalten. Det estimerades att en ökning av fetthalten med en procent (obs. inte procentenhet) i genomsnitt leder till en ökning av dioxinlika ämnen med 0,84%. Inkludering av buklisten (fettvävnaden i bukens underkant) i provet estimerades leda till en genomsnittlig ökning av dioxinlika ämnen med cirka 16 %. I prover av endast subkutant fett var halten av dioxinlika ämnen åtminstone dubbelt så hög som i motsvarande muskelvävnad. Vid jämförelse av hela filén med motsvarande mittbit fanns en indikation på att filén generellt sett har lägre halter av dioxinlika ämnen än

mittbiten. I de prover som studerats här var fetthalten ca 28% lägre i filén jämfört med i mittbiten, vilket indikerar att halten av dioxinlika ämnen kan vara lägre i filén än i mittbiten. Resultaten från jämförelsen mellan olika delar, med/utan buklist, subkutant fett med muskel och filé mot mittbit summeras i nedanstående tabell. Den statistiska analysen som jämför olika delar och med/utan buklist baseras på 17 laxar och 3 öringar. Totalt utgörs analysen av 75 observationer. Jämförelsen av filé och mittbit baseras på fem observationer (fem filéer med motsvarande mittbit) och jämförelsen av subkutant fett och muskelvävnad baseras på två observationer.

Summering av resultat avseende skillnader mellan olika delar av lax och öring. Procentuella skillnader avser skillnader för aktuell del jämförd med mittbiten. Resultaten för "nacke", "stjärt" och "med buklist" är baserade på ett relativt stort urval, så dessa resultat anses i högre grad gälla generellt, medan "filé" och "subkutant fett" baseras på mindre urval, varför de resultaten anses vara svagare indikationer.

Del	Delta-fett (%)	Delta-∑PCDD/F (%)	Delta-∑PCDD/F+ dl-PCB (%)	Delta-∑PCB6 (%)
Nacke	+9,6	+8,0	+8,0	+5,4
Stjärt	-26	-22	-22	-16
Filé	-29	-24	-24	-18
Med buklist	+20	+16	+16	+11
Subkutant fett	+96	+190	+160	+150

Drygt 30 duplikat skickades till två av varandra oberoende ackrediterade laboratorier för analys av fetthalt, dioxin och PCB. Vid jämförelse av laboratoriernas provsvar förstärktes uppfattningen att kemisk analys av dioxin och PCB är förknippat med relativt stor felmarginal. Systematiska fel upptäcktes även för dioxinlika ämnen och icke-dioxinlika PCB mellan de två laboratorierna, där det ena tycks rapportera i genomsnitt ca 10–20% högre värden än det andra. Det är däremot oklart om denna skillnad är konstant över tid.

Drygt 40 duplikat analyserades av samma laboratorium. Resultaten gällande dioxinlika ämnen visade på högre träffsäkerhet då samma laboratorium analyserade replikaten, jämfört när de analyserades av två oberoende laboratorier. 80–83% av variansen i analyssvaren av ett set av duplikat kunde förklaras av analyssvaren för det andra setet av duplikat, jämfört med 64–73% då två laboratorier jämfördes. För icke-dioxinlika-PCBer hittades systematiskt fel för analysen av duplikat. Analyssvaren från ett set av duplikat var i genomsnitt 13% högre än för det andra setet. Gällande analyser av fetthalt från både samma och olika laboratorier fanns att 93–95% av variansen i ett set av duplikaten kunde förklaras av det andra setet. En summering av laboratoriejämförelserna presenteras i nedanstående tabell.

Sammanfattning av resultat från "Wilcoxon signed rank test" av duplikat från Lab1 och Lab2. Ett p-värde minde än 0,05 indikerar signifikans. Om testet är signifikant dras slutsatsen att det på 5%-signifikansnivå föreligger systematiskt fel. Bias=Systematiskt fel.

	Fett	**∑PCDD/F**	**∑PCDD/F+dl-PCB**	**∑PCB6**
	Bias. (Ja/Nej) (p-värde)	Bias. (Ja/Nej) (p-värde)	Bias. (Ja/Nej) (p-värde)	Bias. (Ja/Nej) (p-värde)
Lab1 vs Lab2	Nej 0,22	Nej 0,29	Ja 0,00078	Ja 0,013
Lab2 vs Lab2	Nej 0,19	Nej 0,99	Nej 0,25	Ja 0,0035

Delar av denna studie har sitt ursprung i att det funnits skilda uppfattningar mellan yrkesfiskarkåren och Livsmedelsverket, den myndighet som är ansvarig för den nationella livsmedelskontrollen, gällande tolkningar och hur man ska förhålla sig till divergerande analysresultat samt hur väl analyser av en specifik del av lax, som enligt en EU-förordning ska analyseras vid dioxinkontroll, speglar det som konsumenter exponeras för. Övergripande har denna studie, utan att för den skull hävda att resultaten är statistiskt säkerställda, indikerat att:

- Beredning av produkter av lax och strömming har i vissa fall resulterat i att halterna av dioxinlika ämnen minskat jämfört med den ursprungliga råvaran. Några tydliga indikationer på att beredning generellt leder till betydande minskningar av dioxininnehållet har emellertid inte påvisats.

- Det finns skillnader mellan halter av dioxinlika ämnen i olika delar av laxen som i princip följer skillnader i fetthalt, och där halter i nackpartiet är högre i stjärtpartiet. Skillnaderna är dock mindre än vad som påvisats i en äldre studie utförd av Livsmedelsverket, baserat på ett väsentligt mindre dataunderlag.

- Om den så kallade buklisten, fettvävnaden i bukens nederkant, tas med i ett prov som i övrigt speglar muskelköttet, så ökar halten av dioxinlika ämnen med storleksordningen 15 %.

- Det så kallade subkutana fettet eller underhudsfettet, som enligt EU-förordningen ska inkluderas i prover för dioxinkontroll, innehåller föga förvånande höga halter av dioxinlika ämnen. Mängden underhudsfett utgör dock bara ett par procent av den totala mängden vävnad som ingår i ett prov. Smärre skillnader i metodik vid provberedning kan därför sannolikt inte till någon högre grad förklara divergerande analysresultat.

- Ett prov från mittbiten preparerad enligt EU-förordningen överskattar halten i ett prov berett från hela filén.

- Jämförelse mellan oberoende laboratorier indikerar att felmarginalen i bestämningar av dioxinlika ämnen är behäftad med relativt stora felmarginaler och att det kan finnas systematiska skillnader mellan analyslaboratorier trots att man är ackrediterade (kvalitetssäkrade) för analyserna ifråga.

- Även bestämningar av den till synes betydligt enklare variabeln fetthalt kan variera mellan laboratorier, vilket kan var en orsak till divergerande uppfattningar gällande korrelationer mellan fetthalt och halt av dioxinlika ämnen.

Sammanfattningsvis är det mer troligt att divergerande uppfattningar gällande halter av dioxinlika ämnen i fisk har sitt ursprung i osäkerheter gällande den kemiska analysen snarare än att provberedningen utförts på olika sätt. Det är uppenbart att kemiska analyser av en ämnesgrupp som förekommer i så pass låga halter som pg/g (10^{-12} g/g eller miljondelars miljondelar) ligger på gränsen för vad som är tekniskt möjligt att utföra. Det är således nödvändigt med en ömsesidig ödmjukhet och respekt för att analysresultat från samma matriser kan skilja sig åt utan att någon för den skull har fel. Detta är också en viktig aspekt att ta hänsyn till för att på ett rättssäkert sätt kunna under- eller godkänna ett parti för saluföring.

Introduction

Due to misuse and lack of understanding about their harmful effects, many persistent organic pollutants (POPs) were released in the environment from the 1930's in the form of pesticides, solvents or other industrial products. These compounds are resistant against degradation and therefore, traces of them are observable worldwide to this day. Polychlorinated biphenyls (PCBs), polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs) are POP compounds with carcinogenic and immunotoxic effects on human health. Despite of the ban of use and storage in the 1970's, PCBs and dioxins are still found in the environment although it is reported that their concentration follows a downward trend. Notable is that dioxins forms unintentionally as a rest product, for example when burning waste, so it is still added to the environment. In aquatic ecosystems, bioaccumulation of PCBs and dioxins is found to be more severe in piscivorous fish, including fish from Swedish lakes, the Baltic Sea and the Gulf of Bothnia (Kelly, et al., 2007; Bignert, et al., 2016).

Salmon, herring, trout, arctic char and European whitefish are examples of species with relatively high fat-content, that are an attractive resource of food and protein, especially in Nordic countries. However, due to accumulation of PCBs in fat (Aune, et al., 2003; Persson, et al., 2007), catch and distribution of fatty fish are more likely to be subject to restrictions. In this regard, local fishermen from the Baltic Sea and the two biggest lakes in Sweden; Lake Vänern and Lake Vättern, are limited in their marketing of fatty fish due to high levels of dioxin-like compounds. Even though the levels of these compounds are decreasing in the environment due to restrictions, bans and abatement methods, e.g. improved incineration methods , the dioxin and PCB content in fish from Lake Vänern, Lake Vättern and the Baltic Sea often exceeds the existing marketing limits set by the EU (European Union, 2011). However, an exception in the regulation have been agreed on, in order to allow marketing of wild herring, salmon, arctic char and trout from Lake Vänern, Lake Vättern and the Baltic Sea on the Swedish market. This exception is compensated for through dietary advices specified by the Swedish National Food Agency.

In the project "Dioxiner i fet fisk – hot och utvecklingsmöjligheter för svensk småskaligt kust- och insjöfiske", that runs from 2017 to 2019, variations in levels of dioxin and dioxin-like compounds in fatty fish over different seasons, populations etc., are studied. The project targets fatty fish from the Baltic Sea, Lake Vänern and Lake Vättern. Its purpose is to, by increasing knowledge about factors related to dioxins and PCBs, assist local fishermen and associated industries with supplying the market with foodstuffs as low in dioxins and PCBs as possible.

Also, since these compounds are lipophilic, a possible way of reducing the levels of PCBs and dioxins could be practicing the cooking methods which would reduce the fat content.

Possibly, some cooking procedure could reduce content of dioxin-like compounds without a reduction of fat. Mechanisms of how dioxin-like components reduce because of cooking are largely unknown. Due to this, as a part of this project, some regular cooking

and processing methods were examined on samples of salmon and herring from the Gulf of Bothnia (the northern part of the Baltic Sea).

For studies regarding dioxin-like compounds to be credible, it is of high relevance that there is no substantial bias between different laboratories. Possibly, this could lead to erroneous conclusions. It is also relevant that a laboratory performs consistently. Results from a laboratory should be independent of e.g. time of the analysis. Bias within a laboratory could also lead to erroneous conclusions about dioxin-like compounds in fatty fish.

In the project "Dioxiner i fet fisk – hot och utvecklingsmöjligheter för svensk småskaligt kust- och insjöfiske", two accredited well-recognized multi national laboratories are used; Eurofins and ALS, hereby denoted Lab1 and Lab2. Because of this, it is of interest to see if these laboratories perform similarly, or if bias is found. It is also of interest to see how consistent lab-results from the same laboratory are.

Throughout the report, when "dioxins" are mentioned, chlorinated furans are included as well.

Methodology

Prepared samples

To study the impact of different cooking methods on levels of PCDD/Fs and PCBs in fatty fish from the Bothnian Bay, sample preparation was conducted according to Table 1. The studied species are salmon (*Salmo salar*) and herring (*Cluphea harengus*). Two samples, one raw and one cooked, was analysed from each fish. Samples were also analysed as duplicates, to study possible variations in the chemical analyses. For the duplicates, some of the samples are of European whitefish.

Samples of salmon and herring were delivered from fishermen along the Bothnian Bay in 2014 and 2015. The samples were then prepared at IVL Swedish Environmental Research Institute in Stockholm and the chemical analyses were made by ALS Scandinavia. Each sample was analysed with regards to fat, PCDD/Fs (17 congeners), dioxin like PCBs (12 congeners), non-dioxin like PCBs (6 congeners) and PAH (PAH4 and PAH16).

Table 1 Cooking procedures for herring and salmon.

Herring	**Salmon**
Smoked (*böckling*)	Cured (*gravad*)
Fermented (*surströmming*)	Cold-smoked
Fried	Hot-smoked

As seen, herring was prepared in three different ways: smoked, fermented (*surströmming*), a traditional way to prepare herring in Sweden) and fried (Figure 1 and Appendix A).

From the same catch, approximately fifteen herring were frozen raw, and approximately fifteen was smoked and then frozen. Then, at IVL:s laboratory, composite samples were prepared. The procedure was the same for all types of preparations (smoking, fermenting and frying). Levels of fat, PCDD/Fs and PCBs where then compared before and after cooking. This means that comparisons with regards to dioxin-like compounds should be made between the prepared herring and the raw herring from the same catch.

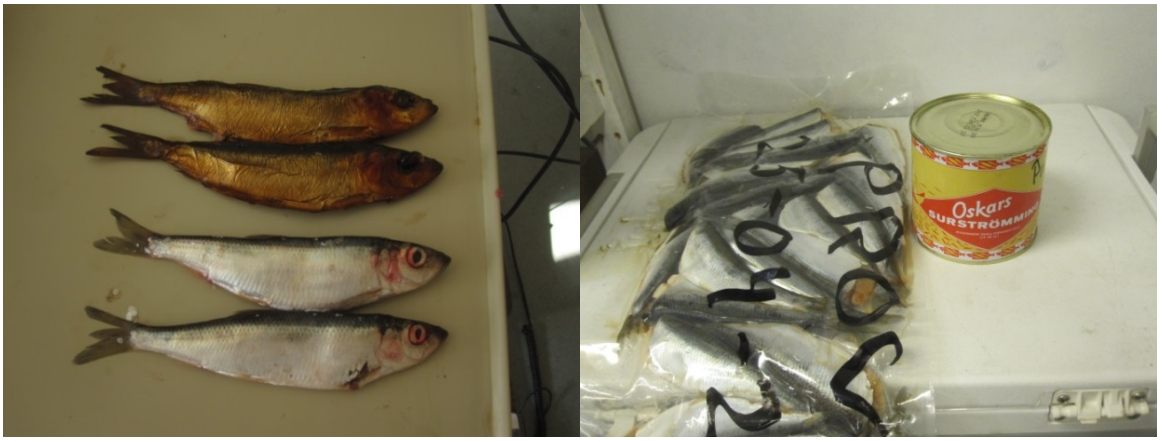

Figure 1 **To the left: smoked herring (above) and raw herring (below). To the right: raw herring (left) and surströmming , i.e. fermented herring (right).**

The salmon was prepared in three ways: hot-smoked, cold-smoked and cured (**Error! Reference source not found.** and Appendix A). For each sample representing a cooking method, a raw sample from the opposite side of the same individual was analyzed.

Figure 2 **To the left: raw salmon tail (above) and smoked salmon tail (below). To the right: cured salmon middle (above) and raw salmon middle (below).**

Different parts of salmon and trout

In addition, from salmon and trout, a sample was taken from the neck, middle and tail, respectively. This was done with the purpose of examining possible variations in fat-, PCDD/Fs- and PCB-levels in different parts of the fish. These cuts are shown in Figure 3.

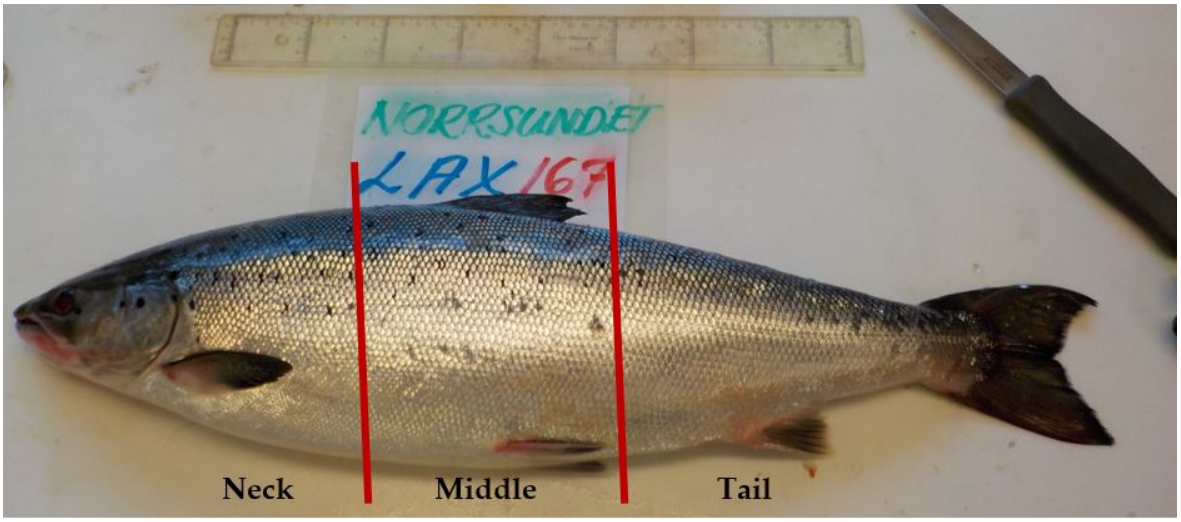

Figure 3 **Visualization of the different parts of salmon and trout that were analysed.**

Also, samples were prepared including or excluding the belly fat, and samples were also taken of the subcutaneous fat. The belly fat is the fatty tissue at the very bottom of the fish (the lower part from the neck fin to the tail fin in Figure 3). The subcutaneous fat is the layer of fat located between the muscle tissue and the skin. This fat can be scraped of and removed or added to the sample. The fillet is the neck, middle and tail part combined.

Between and within laboratory comparison

For the analysis with purpose of comparing how Lab1 and Lab2 perform with respect to analysis of fat content, dioxin-like compounds and non-dioxin-like PCBs, duplicates were sent to both laboratories. The procedure to obtain measurements of duplicates is the following: Sample material (muscle tissue) is taken from a number of fishes from the same species caught at the same time and place. The sample material from each fish is homogenized with a blender. Subsequently, 100 grams of the material is sent to Lab1 and 100 grams of the material is sent to Lab2. For the within laboratory comparison, both 100 gram samples were sent to Lab2 for analysis.

Statistics

On order to determine when differences of some quantities are statistically significant, some statistical tests and models are used. For the comparison of cooking procedures, there is too little data for a formal test. This data is therefore displayed and commented on descriptively. However, when all cooking procedures are combined to one category, Wilcoxon signed rank test is applied to test whether cooking, regardless of procedure, lowers content of dioxin-like compounds.

To investigate differences between different parts of salmon and trout, a linear mixed model with "subject" as the random effect is used. To assess the significance of differences between the parts, a post hoc Tukey-test for multiple comparisons is applied.

To assess the significance of differences between results of two laboratories, Wilcoxon signed rank test is used. Also, the lab-results from one of the replicates are regressed upon the other, for each substance of interest. The same procedure is made for the within-laboratory comparison.

In this report, when it refers to comparisons and differences as "significant", it implies that the null-hypothesis (H_0) is rejected and the probability of occurrence of the null-hypothesis is below 5%. When a difference is not significant, it means that we are more than 95% confident that any difference between the two groups is due to chance; i.e. there is no difference between the two groups statistically. For the mixed models, the *lme4*-package in *R* was used, and for the regressions comparing lab-results, the *lm*-function in *R* was used. For Wilcoxon signed rank test, the function *wilcox.test(..)* was used.

Results

Levels for different preparation methods

An introductory comment is that the following sections are presented under the assumption that the exactness of the laboratory analysis for fat content and dioxin like compounds are the same, regardless of if a sample is cooked or raw. If there is an unknown bias with regards to cooked or raw samples, false conclusions can be drawn.

Data for comparison between different preparations procedures are available for two types of fish: salmon and herring. Results are presented for salmon first, then herring.

Comparisons between cooked and raw samples of salmon are made pairwise. For example, if the tail part from one side of the fish is smoked, the tail part from the other side is kept raw. Regarding herring, composite samples are used, meaning that muscle tissue from several individuals are homogenized into one sample. The raw and cooked samples are composite samples of individuals from the same catch.

Salmon

For salmon, there are four hot-smoked (*varmrökt*) samples, three cured (*gravad*) samples and two cold-smoked (*kallrökt*). Cooked samples are compared to the corresponding raw sample in order to minimize the impact on the results from variability between different parts of the fish, and variability between different individuals.

Due to the small sample size, a first analysis merges smoked, cured and cold-smoked into one category which can be called "cooked". It is now tested if cooking salmon, regardless of cooking procedure, lowers levels of fat, dioxins, and PCB in salmon. Since the assumption of data coming from normally distributed populations do not hold, *Wilcoxons signed rank test* for paired values is used. The hypotheses for the tests are as follows:

$$Null\ hypothesis, H_0: The\ raw\ and\ cooked\ sample\ have\ the\ same\ mean\ rank$$

$$Alternative\ hypothesis, H_1:\ The\ cooked\ sample\ has\ a\ lower\ mean\ rank$$

The test is performed for fat content, ∑PCDD/F, ∑PCDD/F+dl-PCB and ∑PCB6. For all four substances, the null hypothesis is not rejected, giving no evidence for the hypothesis that cooking salmon would reduce levels of the mentioned substances. A comment is that for one of the smoked samples, the corresponding raw sample has been analyzed twice. Therefore, the average of the duplicates is used. Also, one of the cured samples and its corresponding raw sample has been analyzed twice, so the value for observation number 5 is an average of the duplicates. For observation number 9, the raw sample has been analyzed twice, so the raw sample for observation 9 is an average as well.

When comparing the cooking procedures separately, the sample sizes are four, three or two, and this in combination with the fact that there is substantial measurement error connected to the laboratory analysis, does not give a good setting for performing a formal test. Instead, for a visualization, data is presented descriptively. The cooked and raw values are presented, together with cooking procedure and an indicator if levels are lower for the cooked part. If values are lower for the cooked sample, the "Reduced"-column equals "Yes".

Table 2 Comparison of fat content between raw and cooked salmon.

Obs. number	Fat raw (%)	Fat prepared (%)	Preparation	Reduced (Yes/No)
1	11,2	9,78	Cold-smoked	Yes
2	11,4	14,6	Cold-smoked	No
3	7,01	8,82	Cured	No
4	16,0	12,9	Cured	Yes
5	11,2	11,5	Cured	No
6	9,11	11,7	Hot-smoked	No
7	16,0	12,1	Hot-smoked	Yes
8	12,1	10,5	Hot-smoked	Yes
9	9,08	11,2	Hot-smoked	No

Table 3 Comparison of ∑PCDD/F between raw and cooked salmon.

Obs. number	Dioxin raw	Dioxin prepared	Preparation	Reduced (Yes/No)
1	2,30	3,50	Cold-smoked	No
2	0,17	0,16	Cold-smoked	Yes
3	1,10	0,74	Cured	Yes
4	3,00	2,10	Cured	Yes
5	2,70	2,05	Cured	Yes
6	3,10	4,00	Hot-smoked	No
7	3,00	2,60	Hot-smoked	Yes
8	2,85	0,92	Hot-smoked	Yes
9	1,80	2,00	Hot-smoked	No

Table 4 Comparison of ∑PCDD/F+dl-PCB between raw and cooked salmon.

Obs. number	PCDD/F+dl-PCB raw	PCDD/F+dl-PCB raw prepared	Preparation	Reduced (Yes/No)
1	7,80	8,10	Cold-smoked	No
2	0,48	0,51	Cold-smoked	No
3	7,80	4,24	Cured	Yes
4	13,0	11,9	Cured	Yes
5	10,1	7,00	Cured	Yes
6	11,7	14,0	Hot-smoked	No
7	13,0	7,60	Hot-smoked	Yes
8	9,75	5,72	Hot-smoked	Yes
9	8,00	9,90	Hot-smoked	No

Table 5 Comparison of ∑PCB6 between raw and cooked salmon.

Obs. number	PCB6_raw	PCB6_prepared	Preparation	Reduced (Yes/No)
1	48,2	45,4	Cold-smoked	Yes
2	3,16	3,59	Cold-smoked	No
3	30,3	20,8	Cured	Yes
4	41,7	38,3	Cured	Yes
5	38,9	28,9	Cured	Yes
7	39,6	46,0	Hot-smoked	No
8	41,7	47,9	Hot-smoked	No
	47,4	23,5	Hot-smoked	Yes
11	33,0	39,1	Hot-smoked	No

Overall, the Yes and No are fairly equally distributed, reflected in the non-rejection of the null hypotheses in the Wilcoxon signed rank tests. Regarding the number of Yes/No per cooking procedure, they are all have Yes and No:s, except for when a sample is cured. For the cured samples, all had lower levels than the corresponding raw sample with respect to ∑PCDD/F, ∑PCDD/F+dl-PCB and ∑PCB6. This gives some indication that curing can reduce the levels of the mentioned substances. It should anyhow be mentioned that from a statistical viewpoint, if the three cured samples have the exact same values as the raw samples, it is not highly unlikely that all three are reported as lower due to chance. If the values are equal, the chance of the cured measurement being lower than the raw is 50%. In that case, the probability of observing three consecutive "Yes" is $0{,}5^3 = 12{,}5\%$.

Regarding fat content for the cured samples, one cooked sample has lower levels and two have higher values, giving no evidence of curing reducing the fat content in salmon.

Salmon – PAHs

To investigate how different cooking procedures affect the content of PAH:s, PAH16 and PAH4 are compared between the raw and cooked sample. The values of PAHs are the mean of the lower and upper bound. PAH:s have only been measured for one of the duplicates for observation 5 and 9 in the previous page, so no values for PAH:s are averages.

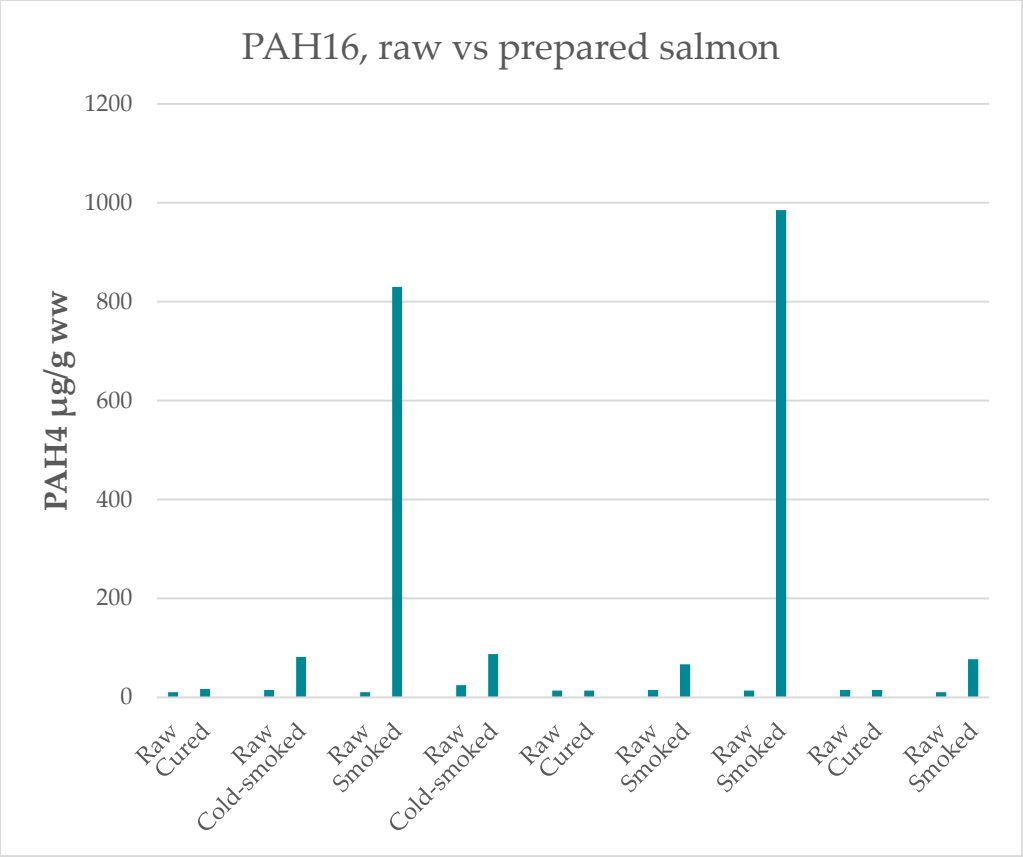

Figure 4 **Comparison of PAH16 in raw and cooked salmon.**

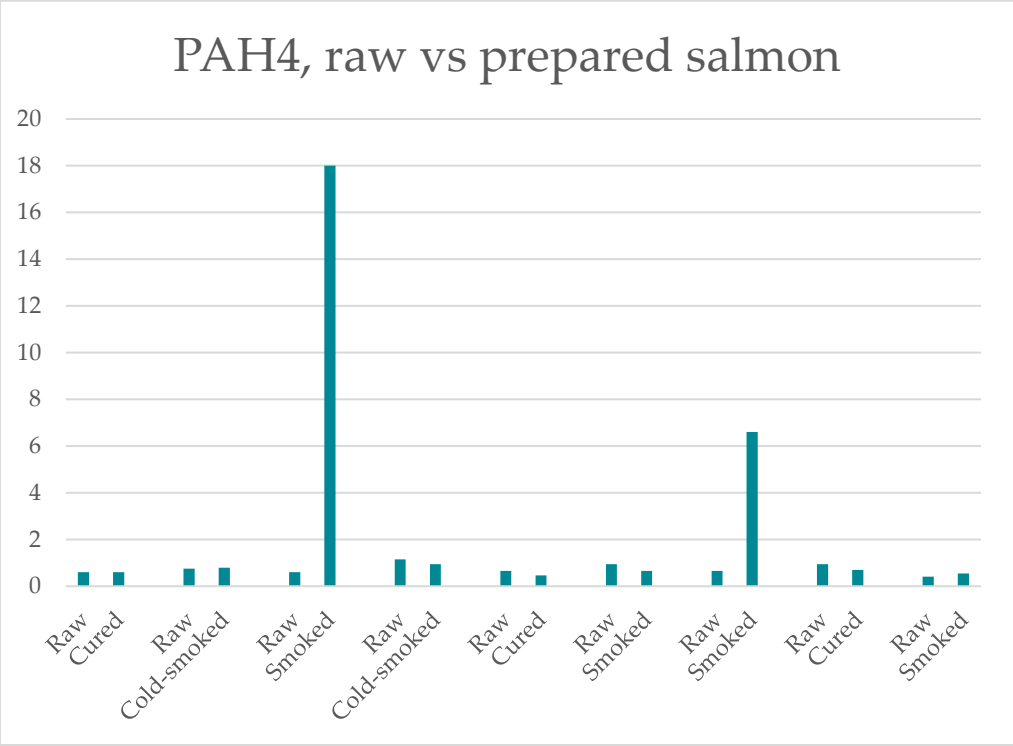

Figure 5 **Comparison of PAH4 in raw and cooked salmon.**

Notable is that in two out of three analyzed hot-smokes samples, the measured concentrations was much higher than for the corresponding raw samples. It is the same

two samples that have high values for both PAH16 and PAH4. The limit for PAH:s is only set for PAH4 and is 12,0 $\mu g/g$ (European Union, 2011). One of the smoked samples have a value higher than this.

Herring

Herring caught at the same time and place are here called a "batch". To minimize differences in the investigated substances that are due to other factors than the cooking procedure, comparisons between cooked and raw samples are made batch-wise.

The following figures display measurements of fat, dioxin like compounds and PCB6 for different raw and cooked samples. Each colour represents a batch.

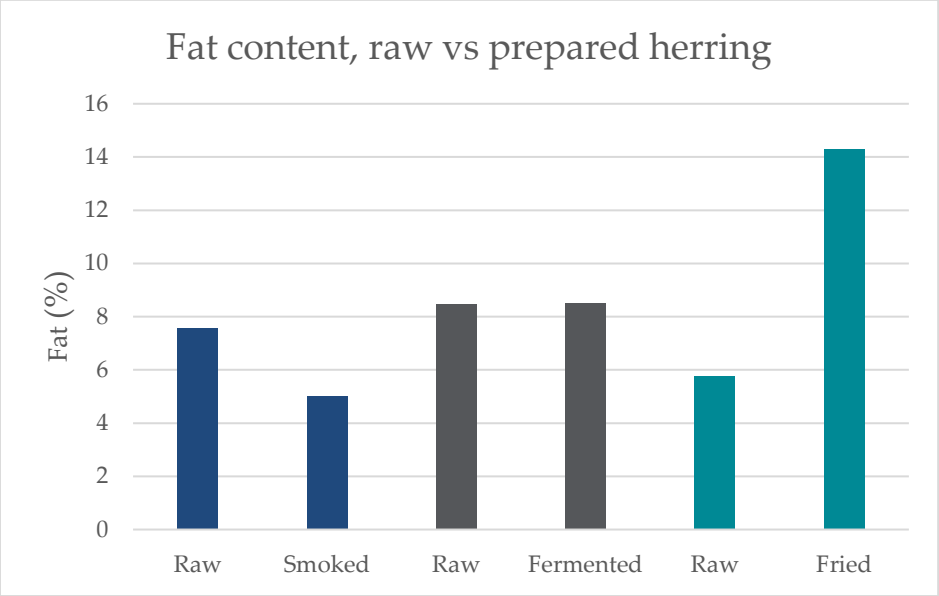

Figure 6 **Differences between measured fat content in cooked/prepared and raw herring. Each colour represents a batch, so comparisons are made colour-wise.**

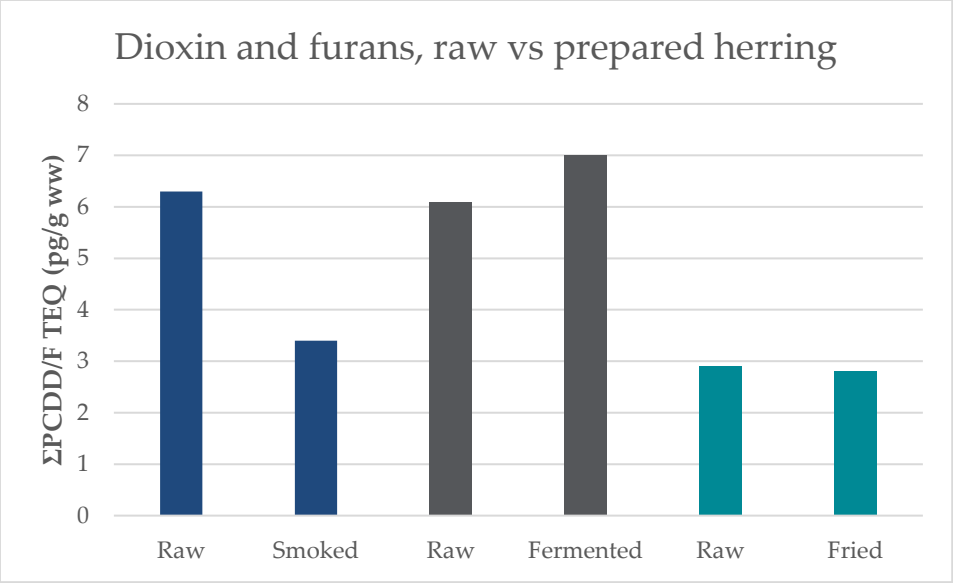

Figure 7 **Differences between measured dioxin content in cooked/prepared and raw herring. Each colour represents a batch, so comparisons are made colour-wise.**

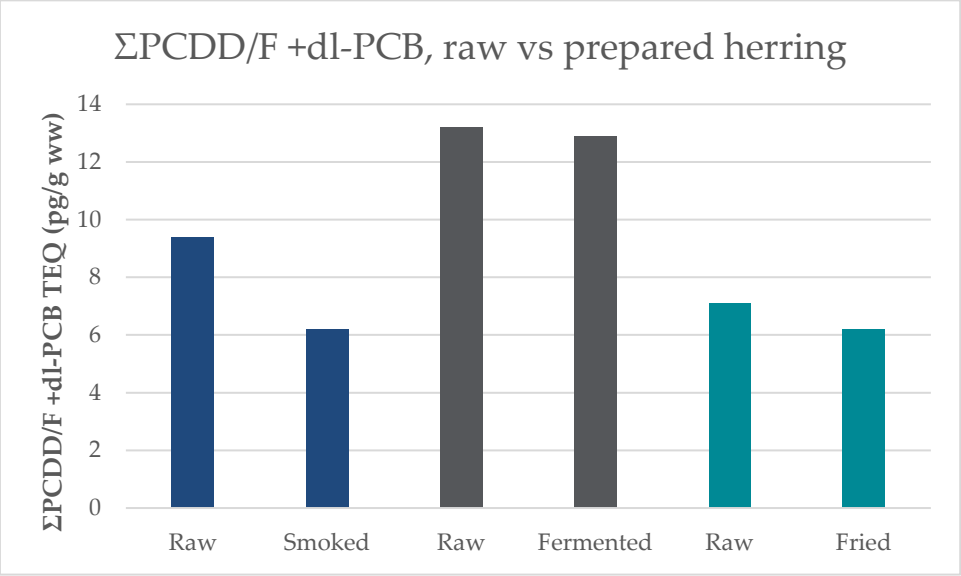

Figure 8 **Differences between measured ΣPCDD/F +dl-PCB content in cooked/prepared and raw herring. Each colour represents a batch, so comparisons are made colour-wise.**

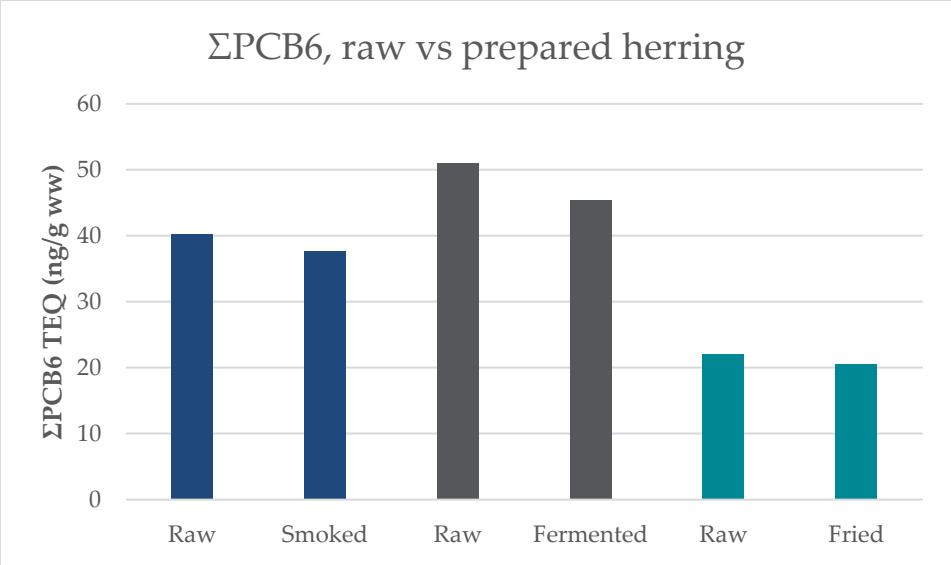

Figure 9 **Differences between measured ΣPCB6 content in cooked/prepared and raw herring. Each colour represents a batch, so comparisons are made colour-wise.**

Regarding fat content, the fried sample has a much higher value than the raw sample, likely due a combination of adding cooking fat in the frying process and evaporation of water when frying (reducing the wet weight). Possibly, the amount of fat reduced when herring was smoked. Fermentation show little difference.

Regarding ∑PCDD/F, the largest difference compared to the raw sample is when a sample is smoked. For ∑PCDD/F+dl-PCB, the relation between the raw and cooked sample is similar to ∑PCDD/F, not surprisingly since these substances are highly correlated. For ΣPCB6, no major difference is seen. Possibly, smoking herring leads to lower levels of dioxin-like compounds. The smoked herring has lower levels of fat, ΣPCDD/F, ΣPCDD/F +dl-PCB and ΣPCB6 than the raw sample from the same batch. ΣPCDD/F is 46% lower in the smoked sample, and ΣPCDD/F+dl-PCB is 34% lower in the smoked sample.

The differences that are seen should be interpreted cautiously, with the main reasons being; although samples are compared batch-wise, the prepared and raw samples consist of different individuals, with inherent differences with regards to dioxin like compounds and the laboratory analysis has some amount of uncertainty (about 25%) in the accuracy. Visible differences could therefore be due to measurement error in the laboratory and individual differences within a batch, and not differences in concentrations of the substances.

To investigate how different cooking procedures affect the content of PAH:s, PAH16 and PAH4 are compared between the raw and cooked sample. The outcome is seen in Figure 10 and Figure 11. The values are means of the lower and upper bound.

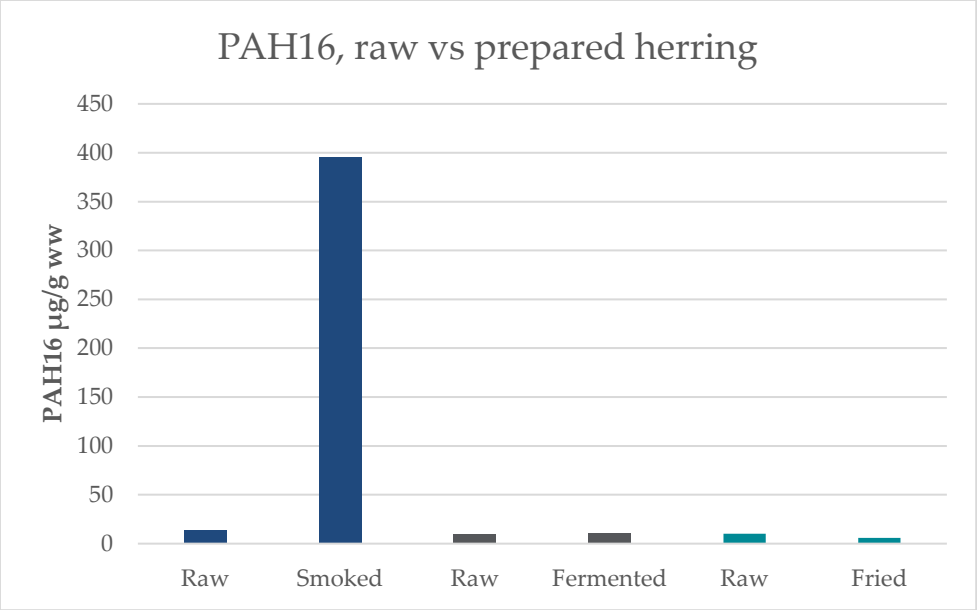

Figure 10 **Comparison of PAH16 for cooked/prepared herring compared to raw. Each colour represents a batch, so comparisons are made colour-wise.**

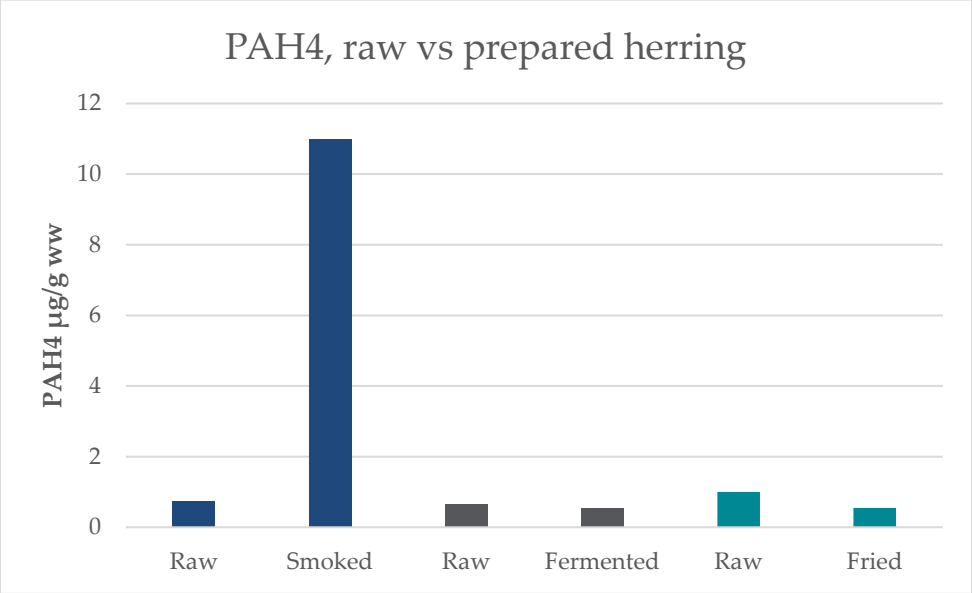

Figure 11 **Comparison of PAH4 for cooked/prepared herring compared to raw. Each colour represents a batch, so comparisons are made colour-wise.**

It is clear that by smoking herring, the amount of PAH:s increases. It is unreasonable that differences could be so large due to variation in the composite samples and measurement errors by the laboratory. As mentioned, the limit for PAH:s is only set for PAH4 and is 12,0 $\mu g/g$ (European Union, 2011). The value for PAH4 in the smoked sample is 11, which is below the limit.

Summary cooking methods

For salmon, the only cooking procedure (out of the tested procedures) that indicates a reduction of dioxin-like compounds is curing, although this indication is weak. Results are not at this point believed to apply to cured samples in general, but acts as an indication that possibly, curing can reduce content of dioxin-like compounds. For conclusions to be drawn, more samples are needed. In this data, both ∑PCDD/F and ∑PCDD/F+dl-PCB was on average reduced with 28% for the cured samples compared to the raw. For the cured samples, no reduction in fat content was observed.

For herring, there is some indication that smoking reduces both fat content and levels of dioxin-like compounds, with 46% lower ΣPCDD/F in the smoked sample, 34% lower ΣPCDD/F+dl-PCB in the smoked sample and 34% lower fat content. Since the sample size is so small (n=1), the lower levels of dioxin-like compounds in the smoked sample is not considered to apply to smoked herring in general, but more of an indication that smoking might reduce levels of dioxin-like compounds. For fermentation and frying, no large difference between the cooked and raw sample was seen.

When looking a PAH:s in both herring and salmon, there is strong evidence of an increase due to smoking. For herring, PAH16 increased from approximately 13,5 µg/g ww to approximately 395 µg/g ww, and for PAH4 from 0,75 µg/g ww to 11 µg/g ww. For salmon, a large increase was seen for two out of three smoked samples. The average PAH16 value for the smoked samples is 473 µg/g ww and 12.5 µg/g ww for the raw. The average PAH4 value for the smoked samples is 6,45 µg/g ww and 0,27 µg/g ww for the raw. Variations between the samples are large, and one of the samples is above the limit for sales, at 12 µg/g ww. A summary of the results from this section is presented in Table 6.

Table 6 **Summary of results from comparing levels of dioxins and PCB between cooked and raw samples of salmon and herring. The number of observations is denoted as "n" and the percentage changes are when compared to the corresponding raw sample. Substantial.(Yes/No) indicates if changes are large enough to be suspected not only to be a consequence of measurement error and inherent variation between the raw and cooked sample. Due to small sample sizes, no formal tests are made. Substantial differences act merely as an indication that the cooking procedure could influence the prevalence of dioxin like compounds.**

Species	Preparation	n	Delta-fat (%) Substantial. (Yes/No)	Delta-∑PCDD (%) Substantial. (Yes/No)	Delta-∑PCDD+dl-PCB (%) Substantial. (Yes/No)	Delta-∑PCB6 (%) Substantial. (Yes/No)	Delta-PAH4 (%) Substantial. (Yes/No)
Salmon	Cold-smoked	2	+8, No	+23, No	+5, No	+4, No	-5, No
Salmon	Cured	3	+14, No	-28, Yes	-38, Yes	-28, Yes	-19, No
Salmon	Hot-smoked	4	+26, No	+20, No	+22, No	+17, No	+960, Yes
Herring	Smoked	1	-34, No	-46, No	-34, No	-6,6, No	+1300, Yes
Herring	Fried	1	+147, Yes	-3, No	-13, No	-7, No	-45, No
Herring	Fermented	1	+0,6, No	+15, No	-2, No	-11, No	-15, No

Levels in different parts of the fish

In this statistical analysis of salmon and trout, the neck, middle and tail are compared with regards to fat content, concentrations of dioxin and PCB. It means that three laboratory analyses have been made for every individual. Also, for some individuals, a sample of neck, middle and tail is taken when the belly fat has not been removed, and when it has. It is therefore also of interest to see whether inclusion of the belly fat increases content of dioxin-like compounds and if there are any differences between salmon and trout. The statistical analysis is based on 17 individual salmon and 3 individual trout. In total, the analysis is based on 75 observations.

Data was analyzed using a linear mixed model. To take into account the correlation between observations that comes from the fact that an individual is analyzed multiple times, a "subject" random effect was included, hence the term "mixed model". Several models are fitted, one for each of the dependent variables; *Fat*, *∑PCDD/F*, *∑PCDD/F+dl-PCB* and *∑PCB6*. Explanatory variables are; if the belly fat is included or not (*Belly fat*), if the analyzed part is from the neck, middle or tail (*Part*) and if the fish is a salmon or trout (*Species*).

Formal tests of parameters are likelihood-ratio tests, and for the variable which codes from which body part the muscle sample is from, a post-hoc Tukey test is performed to correct for the family-wise error rate that occurs when comparing levels of a variable with more than two levels. P-values are not reported for intercepts (β_0).

The dependent variable and continuous explanatory variables are logarithmized for the interpretation of parameter estimates to be in terms of percentage changes.

It is assumed that salmon and trout have a similar biology with respect to how fat, dioxin and PCBs are distributed in the fish. This assumption leads to no interaction effects with "*Species*" being stated in the following models. Also, the number of trouts are very few, so estimated interactions including this variable would not be reliable.

In the section "Comparing middle part with fillet", comparisons are made between the middle part and its corresponding fillet.

Two observations from composite samples of salmon are from analysis of only subcutaneous fat. These are not included in the statistical analysis described above, but they are shown and discussed separately (section "Subcutaneous fat"). The same applies to one observation that is a farmed salmon from Norway. This farmed salmon is compared to the wild salmon (section "Farmed salmon vs salmon from the Baltic Sea").

Results

Results from estimation of models with different dependent variables are presented here. Each dependent variable is estimated and discussed separately.

Fat

The model is:

$$\ln(Fat) = \beta_0 + Subject_j + \beta_3 Bellyfat + \beta_{2k} Part + \beta_4 Species + \varepsilon$$

With $k = 1{,}2$ and $Subject \sim N(0, \tau^2)$ and $\varepsilon \sim N(0, \sigma^2)$

Results from estimation are seen in

Table 7. Reference categories are Belly fat=yes, Part=middle, Species=Salmon. They are included in the intercept when estimated.

Table 7 **Parameter estimates of model with Fat as dependent variable.**

	Estimate & significance
Intercept	2.27
Belly fat=no	-0.22***
Part=neck	0.092**
Part=tail	-0.30***
Species=Trout	-1.0**
Number of obs.	75
Number of groups	20
Variance: Subject(intercept)	0.22
Variance: Residual	0.018
***p<0.001, **p<0.01, *p<0.05	

The estimation indicates that fat content is in general higher if the belly fat is included in the sample of muscle tissue and that trout in general has a lower level of fat.

The interpretation of the magnitude of estimates is as follows: The percentage change in fat content when the level of a variable changes from the reference levels to another level is $100 * (e^{\hat{\beta}} - 1)$.

So, if the belly fat is not included in the sample material, the estimated decrease in fat content is $100 * (e^{-0.22} - 1) = 19.5\%$. The estimated difference between salmon and trout is 63%. This value should be interpreted cautiously, since there are only three trout in the sample. There could be something other than other than just difference in species that is not captured by the model that causes this significance. For *Subject*, no interpretation is made, since it is only included in the model to account for the correlation between observations from the same individual. Regarding *Part*, Tukey's multiple comparison is performed, with results seen in Table 8.

Table 8 **Results from Tukey's test of "Part" when modelling fat content.**

H_0	Estimate	Std.error	p-value
Neck-Middle=0	0.092	0.038	0.044
Tail-Middle=0	-0.30	0.039	<0.001
Tail-Neck=0	-0.39	0.039	<0.001

All differences are significant at the 5-% level, and by looking at the estimates, the test indicates that the fat content is highest in the neck, second highest in the middle, and lowest in the tail. The estimated differences are that the neck part on average have $100 * (e^{0.092} - 1) = 9.6\%$ higher fat content than the middle, and that the tail part on average have 25.7 % lower fat content than the middle.

PCDD/Fs and dioxin-like PCBs

Here, the joint measure of dioxins and dioxin-like PCBs is modelled. The following model is fitted:

$$\ln(y) = \beta_0 + Subject_j + \beta_3 \ln(Fat) + \beta_4 Bellyfat + \beta_{2k} Part + \beta_4 Species + \varepsilon$$

Where y is ∑PCDD/F+dl-PCB TEQ pg/g ww.

The full results table from the estimation is not included, the results are instead for simplicity explained in words:

Fat is significant with a positive sign in the estimation, and no other variable is significant. Important is that this model controls for differences in fat content, meaning that interpretation of estimates and significances for other parameters are based on holding *Fat* constant. Now, insignificant parameters are removed and parameters re-estimated. Parameter estimates of this this smaller model (with only *ln(Fat)* as explanatory variable) are the following:

Table 9 **Parameter estimates of model with ∑PCDD/F+dl-PCB as dependent variable.**

	Estimate & significance
Intercept	-0.26
ln(Fat)	0.84***
Number of obs.	75
Number of groups	20
Variance: subject(intercept)	0.13
Variance: Residual	0.027
***p<0.001, **p<0.01, *p<0.05	

Fat is highly significant, and since *Part* was not, this means that when holding fat content constant, there is no difference in ∑PCDD/F+dl-PCB between any parts of a salmon or trout. Since *Part* was significant in the model predicting changes in fat content, this estimation indicates that most likely, differences in ∑PCDD/F+dl-PCB between different parts of salmon and trout are related to differences in fat content between the parts. Since both ∑PCDD/F+dl-PCB and fat content are logarithmized, the estimate is interpreted in terms of percentage changes. It is estimated that a one percent increase in fat content (not one percentage) on average leads to 0.84% increase in ∑PCDD/F+dl-PCB. In other words, this is an estimate of how much we can expect ∑PCDD/F+dl-PCB to change for some percental change of fat content.

PCDD/Fs

Here, the following model is fitted:

$$\ln(dioxin) = \beta_0 + Subject_j + \beta_3 \ln(Fat) + \beta_4 Bellyfat + \beta_{2k} Part + \beta_4 Species + \varepsilon$$

One trout has ∑PCDD/F TEQ pg/g ww equal to zero for two of its three observations. Since the natural logarithm of zero is undefined, this trout is not included in the estimation.

Results from the estimation were similar as to when all dioxin-like compounds were the dependent variable. When controlling for differences in fat content, the only significant variables are *Fat* and *Species*. Results when re-estimating the model with only significant parameters are the following:

Table 10 **Parameter estimates of model with ∑PCDD/F as dependent variable.**

	Estimate & significance
Intercept	-1.63
Species=trout	0.83*
ln(Fat)	0.83***
Number of obs.	72
Number of groups	19
Variance: subject(intercept)	0.12
Variance: Residual	0.038
***p<0.001, **p<0.01, *p<0.05	

These results indicate that most likely, differences in dioxin between the neck, middle and tail of salmon and trout are related to differences in fat content between the parts. The estimate is 0.83, interpreted as that a one percent increase in fat content on average leads to 0.83% increase in ∑PCDD/F. Notable is that the estimate for ∑PCDD/F and ∑PCDD/F+dl-PCB are very similar.

Since only three trout are in data, *Species* is not interpreted here.

Non-dioxin-like PCBs (PCB6)

Here, the following model is fitted:

$$\ln(\sum PCB6) = \beta_0 + Subject_j + \beta_3 \ln(Fat) + \beta_4 Bellyfat + \beta_{2k} Part + \beta_4 Species + \varepsilon$$

The two significant parameters are the ones corresponding to *Fat* and *Part*, with *Fat* having a positive estimate, so a model with *Fat* and *Part* is refitted, with results seen in Table 11.

Table 11 **Parameter estimates of model with ∑PCB6 as dependent variable.**

	Estimate & significance
Intercept	2.78
Part=neck	-0.00052**
Part=tail	-0.12***
Fat	0.56***
Number of obs.	75
Number of groups	20
Variance: subject(intercept)	0.13
Variance: Residual	0.012
***p<0.001, **p<0.01, *p<0.05	

The significant estimate for *Fat* is 0.56 and is interpreted as that a one percent increase in fat content on average leads to 0.56% increase in ∑PCB6. The significance of *Part* is investigated further with Tukey's test for multiple comparisons. Results are presented in Table 12.

Table 12 **Results from Tukey's test of "Part" when modelling ∑PCB6.**

H_0	**Estimate**	**Std.error**	**p-value**
Neck-Middle=0	-0.00052	0.038	0.99
Tail-Middle=0	-0.12	0.048	0.011
Tail-Neck=0	-0.12	0.056	0.036

Results indicate that ∑PCB6 differs between tail-middle and tail-neck in salmon and trout, but not between neck-middle, with tail having lower levels, even when controlling for changes in fat content. In other words, the tail has significantly lower levels of ∑PCB6, but there is no indication that ∑PCB6 differs between the neck and middle. It must be kept in mind that differences between neck and middle do exist, it is just that they are a consequence of differences in fat between the parts. Since, even when controlling for changes in fat content, there is a difference in concentrations of ∑PCB6 between middle and tail, when calculating differences in ∑PCB6 between middle and tail, it has to be done by calculating the change due to the difference in fat and then add an additional $100 * (e^{-0.12} - 1) = 11\%$ loss that is not a consequence of reduced fat.

Comparing middle part with fillet

Significant differences were found between the neck, middle and tail part, but when eating salmon, it is often the fillet that is consumed, and when analyzing salmon according to EU regulations, the middle part is used. It is therefore of interest to see if the middle part is representative of the fillet. There are five observations available. In Table 13, differences between the middle part and fillet are shown. Values are $middle - fillet$, so if a value is positive it means that the middle part has a higher value, and vice versa.

Table 13 **Comparison of middle part with corresponding fillet. Values are the difference between the middle part and fillet.**

	Middle - Fillet			
Obs. Number	Fat	∑PCDD/F	∑PCDD/F+dl-PCB	∑PCB6
1	2,23	-1,00	-0,30	4,86
2	4,36	0,60	1,20	6,75
3	3,65	0,00	0,10	1,25
4	3,83	1,00	2,00	6,42
5	2,43	0,80	3,30	8,50

Since it was concluded that levels of dioxin-like components in different parts are to a large extent determined by differences in fat content between the parts, the focus here is on differences in fat content between the middle part and the corresponding fillet. Notable is that the column for "Fat" only have positive values, meaning that all middle parts had a higher measured fat content than the fillet. The sample size is too small for Wilcoxon signed-rank test to be meaningful, and data is not considered normally distributed. Instead a cruder test is performed, explained below:

If the middle and fillet would have the same mean fat content, differences would be connected to measurement error, and the chance of obtaining a larger value for the middle than the fillet would be 50%. Now, we have five consecutive samples for which the middle has higher values. If the probability of observing a positive value is 50%, the probability of observing five consecutive positive values is $0{,}5^5 \approx 3.12\%$ which is quite unlikely, meaning that these data indicate that the fat content could be lower than in the middle. From a consumer perspective, this would, together with conclusions on relations between fat content and dioxin-like compounds, imply that by eating the fillet, the consumer will get a lower intake of dioxin-like substances than one would think based on lab results from the middle part. A crude estimate of the reduction based on this data is that the fillet has 28,6% lower fat content than the middle part. This is the mean reduction in fat. By using the previous estimates of the relation between changes in fat and dioxin-

like compounds, it is calculated that the content of dioxin-like compounds is 28,6*0,835=23,9% lower in the fillet. For ∑PCB6, differences were significant between the tail and middle when controlling for fat, but not for neck vs middle. An estimate of PCB6 reduction in the fillet is therefore not as clear but is based on these data likely somewhere between 28,6*0,56=16% and 28,6*0,56*1,11=18%. In the summarizing table for this section (Table 16 and its replicate on page 6), the average of these values (17) is used as the estimate.

By looking at the other substances, it is noted that most saw a reduction. For dioxins, one difference is negative, and there is one tie. For ∑PCDD/F+dl-PCB, only one difference is negative and for ∑PCB6, all differences are positive. Strengthening the argument about looking mainly at fat content is the results from the lab comparison in the section "Fitness of the results from different labs" indicating that the most correctly reported value is the fat percentage. For the comparison of middle and fillet, this implies that the differences that are most likely to correspond with the "true" difference is the one regarding fat content.

Subcutaneous fat

The two observations of composite samples of only subcutaneous fat are presented and compared to the corresponding sample of muscle tissue from middle parts with subcutaneous fat trimmed.

Table 14 **Comparison of fat content, dioxins, furans and PCBs in subcutaneous fat and muscle tissue.**

	Sample 1		**Sample 2**	
	Middle	Subcutaneous	Middle	Subcutaneous
Fat (%)	11,3	21,0	10,3	21,3
∑PCDD/F	3,8	8,3	3,3	12,0
∑PCDD/F+dl-PCB	12,1	22,3	9,8	32,0
∑PCB6	45	78	40	128

The levels of dioxin-like compounds are higher in the subcutaneous fat than in the middle part. For both samples, the fat content is about twice as high in the subcutaneous fat, and the content of dioxin-like compounds is about twice as high or more. ∑PCB6 is about 1,7 to 3,2 times higher in the subcutaneous fat. This is in correspondence with the lipophilic properties of dioxin-like compounds.

Farmed salmon vs wild salmon from the Baltic Sea

One sample is from a salmon farmed in Norway. Table 15 display descriptive statistics for the wild salmon and compares it to the values for the farmed. The wild salmon samples chosen for the comparison are the ones that are raw, with subcutaneous fat trimmed and with the middle part selected for analysis. The number of wild salmons is 66, and the minimum, maximum and mean values for each substance are reported for these salmon.

Table 15 **Comparison of fat, dioxins, furans and PCBs in wild salmon from the Gulf of Bothnia with a farmed salmon from Norway.**

	Wild(n=66)			**Farmed(n=1)**
	Min	Mean	Max	Value
Fat (%)	1.65	7.93	15.96	11.4
∑PCDD/F	0.44	1.79	4.50	0.2
∑PCDD/F+dl-PCB	1.22	5.90	14.7	0.5
∑PCB6	7.59	33.22	56.31	3.0

The fat content for the farmed salmon is above the mean fat content for the wild salmon while the ∑PCDD/F, ∑PCDD/F+dl-PCB, and ∑PCB6 are all lower than the minimum value for the wild salmon.

Summary of comparisons made between different parts

Differences were found between the different parts of trout and salmon. The estimated differences are that the neck part on average have 9,6% higher fat content than the middle, and that the tail part on average have 25.7 % lower fat content than the middle. Also, an indication of strong lipophilic properties of dioxin-like compounds was found. Differences with respect to dioxin-like compounds between parts of the fish was found to mostly be a consequence of differences in fat content between the parts. An estimate for the relation between fat content changes and changes in dioxin-like compounds was calculated. For a one percent increase (not one percentage) of fat content, the estimated increase of ∑PCDD/F is 0,83%, for ∑PCDD/F+dl-PCB the estimate is 0,84% and for ∑PCB6 the estimate is 0,56%.

Using these estimates, it is calculated that for ∑PCDD/F and ∑PCDD/F+dl-PCB (the estimates are so close that their average is used) the concentration is 8,0% higher in the neck compared to the middle section and 21,5% lower in the tail compared to the middle. For ∑PCB6 the estimate is that levels are 5,4% higher in the neck compared to the middle.

For the middle compared to the tail, it is considered that the difference was significant even when controlling for fat. The estimate is that the ∑PCB6 content is 16% lower in the tail compared to the middle.

If the belly fat is included in the sampled muscle tissue, it was estimated that the fat content was on average 19,5% higher. Using the estimates of the relation between dioxin, PCB and fat, it is estimated that levels of ∑PCDD/F and ∑PCDD/F+dl-PCB are 16,4% percent higher if the belly fat is included. For ∑PCB6, the estimate is an 11,0 % increase.

When the fillet was compared to the corresponding middle part from the same individual, all fillets had lower fat content measurement than the corresponding fillet, and together with the lipophilic properties of dioxin-like compounds, support was found for the levels of dioxin-like compounds being lower in the fillet, compared to the middle section. An estimate of the reduction is that the fillet has 28,6% lower fat content than the middle part. Since the sample size for the comparison of fillets and middle parts was small (n=5), results are more of indications than considered to apply in general. By using the estimates from the mixed model, it was estimated that the levels of dioxin-like compounds are 23,9% lower in the fillet, compared to the middle section. For ∑PCB6 the estimate is a difference of 17%.

A farmed salmon was compared to wild salmon from the Gulf of Bothnia. It was found that the farmed salmon have substantially lower dioxin and PCB content. Since only one farmed salmon was available, no estimate of how much lower the levels are for farmed salmon in general is made, but in the sample, the levels of ∑PCDD/F, ∑PCDD/F+dl-PCB and ∑PCB6 were all lower than the minimum value of 66 wild salmon.

Table 16 **Summary of differences between different parts of salmon and trout. The percentage changes are when compared to the middle part. The results for "neck", "tail" and "with belly-fat" are based on a much larger sample than "subcutaneous fat" and "fillet", so their estimated differences are believed to apply for the population of salmon and trout, whereas changes for "subcutaneous fat" and "fillet" are weaker indications which applies mainly to this specific sample.**

Part	Delta-fat (%)	Delta-∑PCDD/F (%)	Delta-∑PCDD/F+dl-PCB (%)	Delta-∑PCB6 (%)
Neck	+9,6	+8,0	+8,0	+5,4
Tail	-26	-22	-22	-16
Fillet	-29	-24	-24	-17
With belly-fat	+20	+16	+16	+11
Subcutaneous fat	+96	+190	+160	+150

Fitness of the results from different labs

There is no doubt that experimental results comprise measurement errors which are commonly human and machine-originated. On the other hand, some errors are systematic which are generally associated with persistent and run biases.

To investigate the influence of lab bias (which would be accompanied with random and run bias), duplicate samples were sent to 2 different labs, Lab1 and Lab2. The amount of fat, dioxins, dioxin-like PCBs and non-dioxin-like PCBs in the duplicated samples were measured and reported by both labs. The experiment consists of measurement of 11 European whitefish (*sik*), 20 salmon and 3 herring.

Also, duplicates were analyzed by the same laboratory. Lab2 performed the same analysis twice for 48 samples. These consist of 15 European whitefish samples, 1 herring sample and 22 salmon samples. Most of the samples are composite, meaning that they contain muscle tissue from multiple individuals (herring samples are always composite samples in order to get enough sample material). This analysis of duplicates was performed to investigate how consistent the laboratory is. Most observations are not overlapping for the between-lab data and the within-lab data, meaning that when both duplicates are analyzed by Lab2, most of the observations are not in the samples for the between-lab comparison.

To test the prevalence of bias, it is tested if any of the labs systematically report higher or lower values than the other. This data does not support usage of tests based on normality, so the test used is Wilcoxons signed rank test, which is a nonparametric test with the following hypotheses:

$$Null\ hypothesis, H_0: Lab1\ and\ Lab2\ reports\ the\ same\ median$$

$$Alternative\ hypothesis, H_1:\ Lab1\ and\ Lab2\ do\ not\ report\ the\ same\ median$$

If the null hypothesis is rejected, it is concluded that there is a systematic bias, and in the case of rejection, it is investigated which of the labs that reports an on average higher/lower value. A 5% significance level is used for the test.

This section is therefore divided into two parts; one that investigates the between-lab variation and one that investigates the within-lab variation.

For both parts, complementary plots are shown. These plots show the values reported by Lab1 regressed on the values reported by Lab2 (or regression of sets of duplicates from the same lab). The reason for the regression is to visualize data and to obtain the R^2-value which is a measure of how close to each other the values are. A higher value indicates that the two labs reported more similar values (or that the same lab reported similar values for its replicates).

A comment is that, for the between- and within-lab comparisons, it was assumed that any bias present is not dependent on fish type.

Between-laboratory analysis

First, a plot (Figure 12) is displayed to get a sense of the performance of the labs.

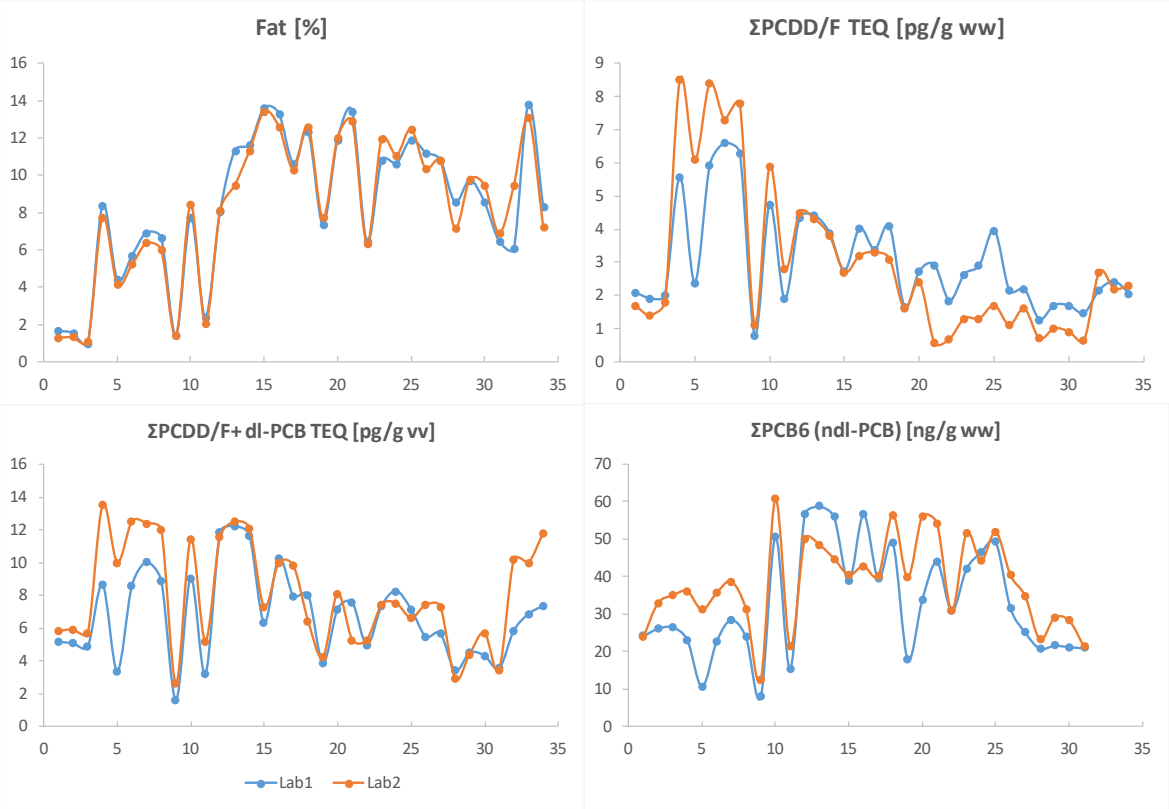

Figure 12 **The variations of four different parameters in 34 duplicated fish samples. The horizontal axis show the sample number. Samples 1-11 are European whitefish, 12-31 are salmon and 32-34 are herring. Due to the large concentrations of ndl-PCBs in the herrings, they are excluded from ΣPCB6 graph (bottom-right).**

There are relatively harmonic variations in the trends of the results from each lab, but gaps with varied ranges between the values reported by Lab 1 and Lab 2 are observed. None of the labs always reports higher/lower than the other, which introduces the need of a formal test. It is of interest to understand if these differences are due to random errors or if evidence of bias is distinguishable.

The results from Wilcoxon signed rank test for medians are displayed in Table 17.

Table 17 **Results from Wilcoxon signed rank test for equality of medians when comparing replicates from two labs.**

Substance	p-value
Fat	0,22
∑PCDD/F	0,29
∑PCDD/F+dl-PCB	0,00078
∑PCB6	0,013

Two of the tests have a p-value below the significance level of 0.05. It is the tests of ∑PCDD/F+dl-PCB and ∑PCB6. It is therefore concluded that at the 5%-level, there is a bias between Lab1 and Lab2 for ∑PCDD/F+dl-PCB and ∑PCB6, and no bias for fat content and ∑PCDD/F. After a closer analysis of the test (not shown here) it is noted that Lab1 on average reports a higher value than Lab2 for these substances.

Now, the regression plots with the R^2-value are presented. The regressions performed have the following structure:

$$Lab1 = \beta_0 + \beta_1 Lab2 + \varepsilon$$

Where *Lab1* is the value of a substance measured by Lab1, *Lab2* is the corresponding value for Lab2, and ε is the error term. The analyzed substances are presented in the following order: ∑PCDD/F, ∑PCDD/F+dl-PCB, Fat and ∑PCB6. The black line is the line of best fit (regression line from OLS) and the blue line is the "optimal" line (intercept=0 and slope=1). If the labs on average reports the same value, the blue and black line would overlap. Minor differences between the blue and black line can be due to randomness.

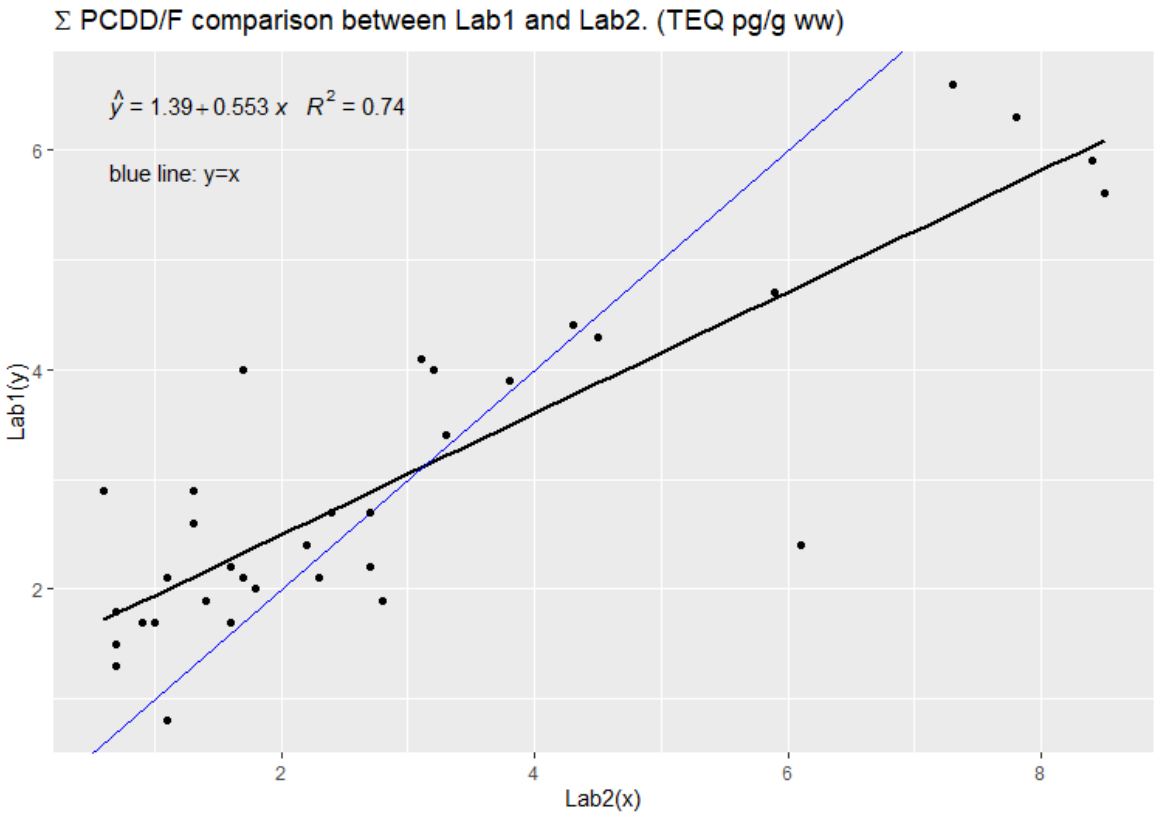

Figure 13 **Comparison of Lab 1 and Lab 2 with respect to ∑PCDD/F TEQ pg/g ww.**

It appears to be some structure indicating that for smaller values, Lab1 reports a higher value, but for larger values it reports a smaller value. The same pattern is, although not as obvious, seen in Figure 12. The main inference is anyhow based on the formal test, showing no difference in medians. The reason for ∑PCDD/F not being significant in the test could be that there is a bias, but the bias differs depending on if the value is large or small.

The R^2-value is interpreted as: 74% of the variance in the measurements from Lab1 can be explained by the measurements from Lab2.

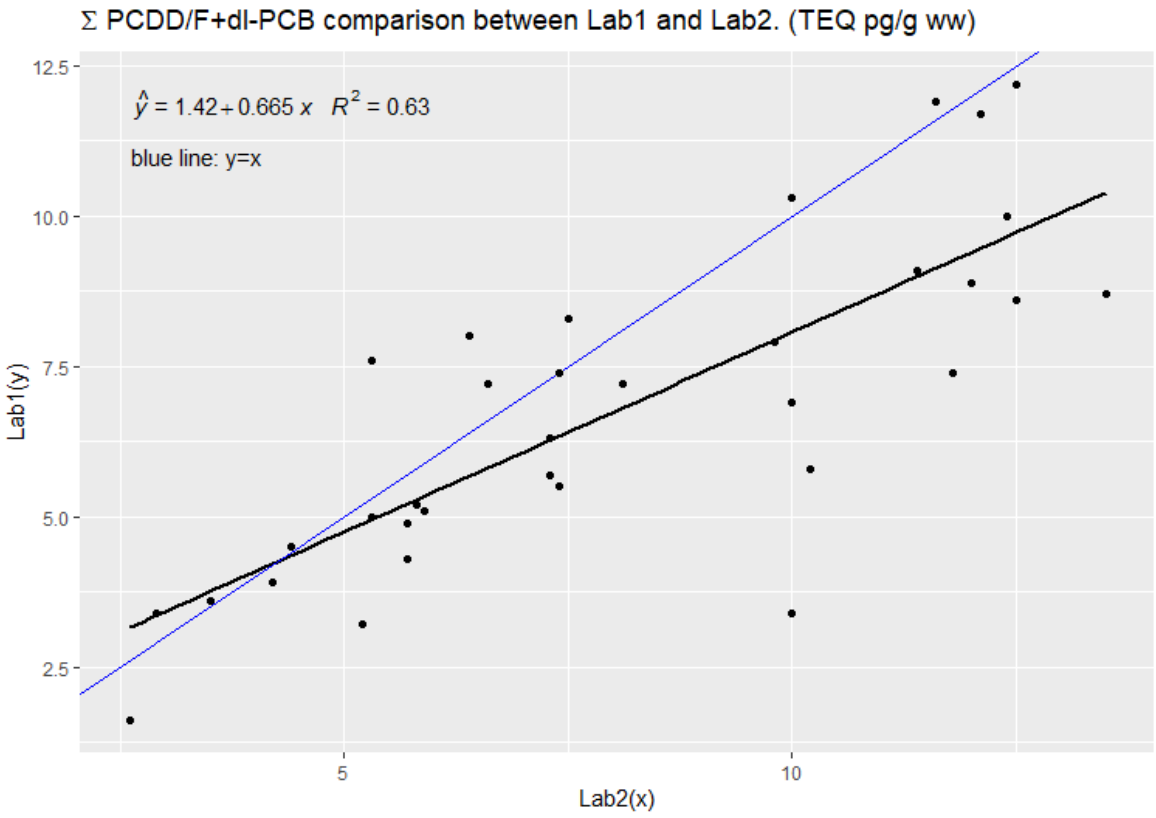

Figure 14 **Comparison of Lab1 and Lab2 with respect to ∑PCDD/F+dl-PCB TEQ pg/g ww.**

The R^2-value for ∑PCDD/F+dl-PCB is slightly lower than for ∑PCDD/F. The interpretation is that 63% of the variance in the measurements from Lab1 can be explained by the measurements from Lab2. This quite low R^2-value is reflected by the dots being far away from the regression line.

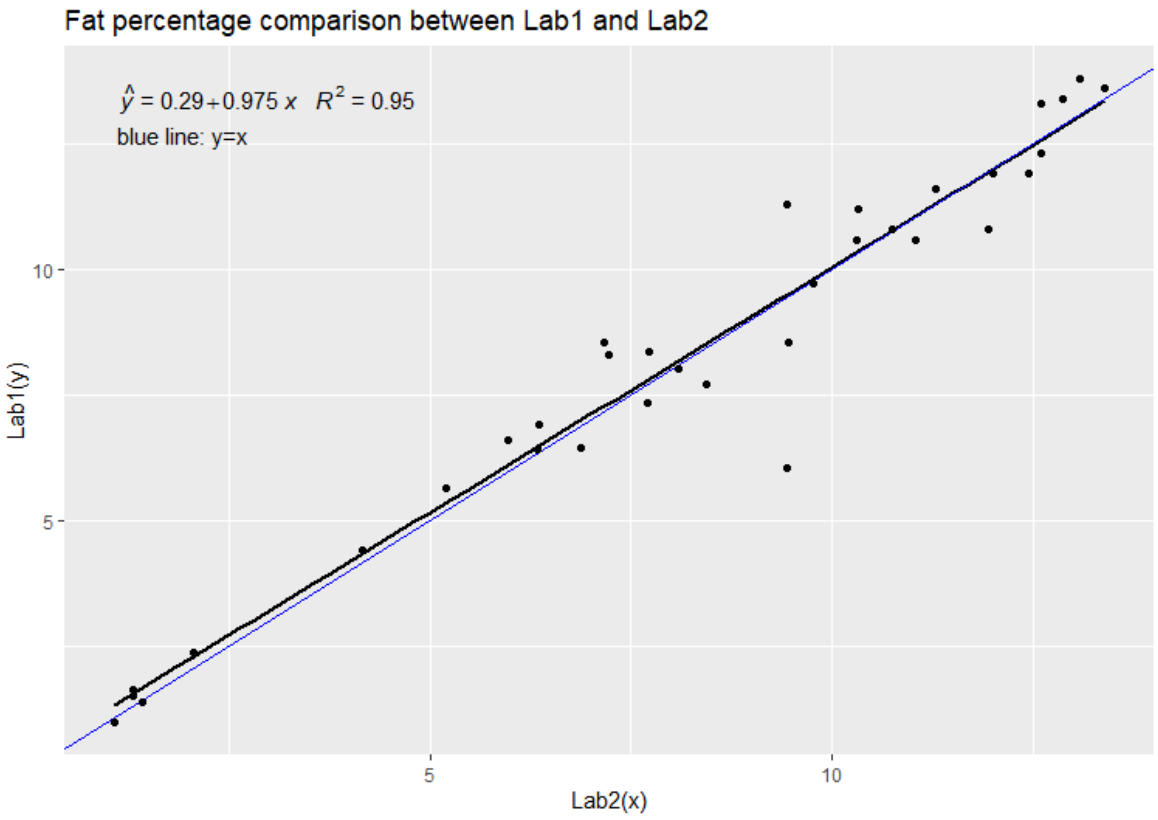

Figure 15 **Comparison of Lab1 and Lab2 with respect to fat content (%).**

The reported values are similar, seen by the points lying close to the line (equivalent to a high R^2-value). The impression is that for fat content, the laboratories deliver very similar values. As much as 93% of the variance in Lab1 measurements are explained by Lab2 measurements.

For the regression of ∑PCB6, the three samples from herring are excluded since they are very far off from the other observations. They have the values seen in Table 18.

Table 18 **Values for herring samples not included in the regression of ∑PCB6 comparison between Lab1 and Lab2.**

Obs. number	∑PCB6 ng/g ww Lab1	∑PCB6 ng/g ww Lab2
1	142	150
2	154	126
3	214	230

Notable is the magnitude of these values. Also, the values are quite similar.

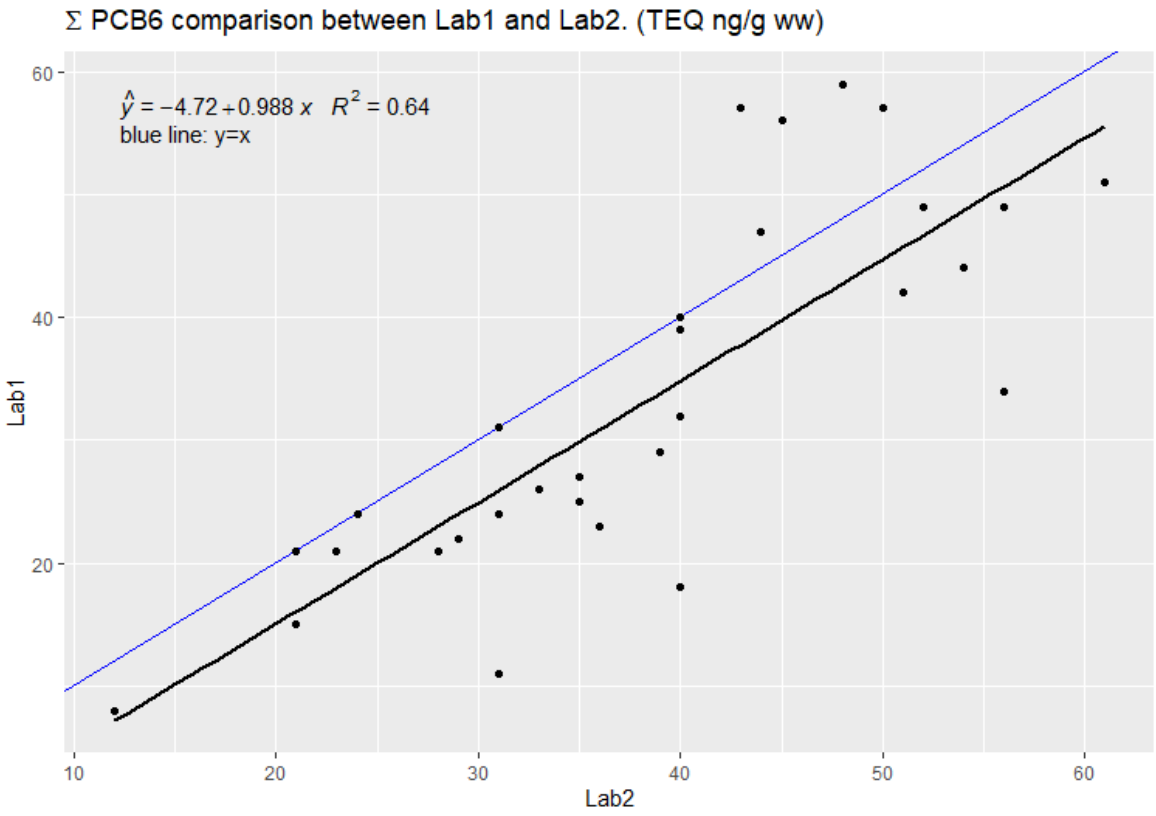

Figure 16 **Comparison of Lab1 and Lab2 with respect to ∑PCB6 TEQ ng/g ww.**

Large variations are seen, being noted by the quite low R^2-value.

From these four plots, two main observations are made. First, that for ∑PCDD/F there is a possible bias, but it might not be discovered in the Wilcoxon test since the direction of the bias appears to be dependent on the size of the value. Secondly, that fat content is the substance measured most exactly.

Comments concerning previous fat content analyses by Lab1

Before the analysis of these samples, measurements of fat content performed by Lab1 were noted to be unlikely in relation to the reported content of dioxin-like compounds and to where the fish was caught, here meaning that the correlation between fat content and dioxin-like compounds was weaker than usual and that specimen from that area usually have lower fat content. Lab1 was informed about this and after they re-analyzed with another method, they confirmed the poor quality of the measurements. The method resulting in the unlikely values is called "Internal" and the re-analysis was done using the more conventional SBR-method. Since this was discovered, Lab1 only use the SBR-method, and the reported fat percentages are now more similar to the ones reported from Lab2.

This means that results based on older data containing fat content analyses from Lab1 should be treated with caution.

Table 19 presents the data leading to the doubts regarding the correctness of fat content analysis from Lab1. All samples are composite samples of European whitefish.

Table 19 **Comparison of fat content measures using Internal- and SBR-method from Lab1, with Lab2 (method unknown). Values are fat content (%).**

sample	Lab1 Internal	Lab1 SBR	Lab2
1	9,24	8,36	7,70
2	13,8	4,41	4,16
3	7,76	5,66	5,20
4	11,9	6,92	6,36
5	9,15	6,61	6,00
6	1,91	1,49	1,42
7	6,79	7,53	8,43
8	11,9	2,38	2,05

For many of the samples, the reported fat content is similar for the SBR (Lab1) and Lab2, but substantially higher when the "Internal" method was used. Only sample number 8 has a higher value for SBR than "Internal".

Within-laboratory analysis

Here, the duplicates analyzed by Lab2 are investigated to see how consistent the laboratory is. The procedure is the same as for the comparison between laboratories, the only difference being that both analyses are from Lab2.

For this data, eight of the observations are triplicates. In order to plot data, perform the test and the regression, there need to be only duplicates. The solution has been to randomly sample out one of the triplicates and to not use this observation in the regression. In order not to disregard useful information, the removed observations are presented descriptively and discussed separately. Also, two observations are of subcutaneous fat. These have high values that are not representative for data, and are therefore excluded from the regression, but are as for the removed triplets, shown and discussed separately.

First, the results from Wilcoxon signed rank tests are displayed (Table 20), then, the regression plots are displayed.

Table 20 **Results from Wilcoxon signed rank test for equality of medians when comparing duplicates from the same lab (Lab2).**

Substance	p-value
Fat	0,19
∑PCDD/F	0,99
∑PCDD/F+dl-PCB	0,25
∑PCB6	0,0035

Here, only the test for ∑PCB6 rejects the null hypothesis, so it is concluded that at the 5%-level, there is a bias between one set of duplicates and the other for ∑PCB6, but not for the other substances. By observing the test more closely (not shown here) it is concluded that the average for one set of duplicates is 13% higher than for the original samples.

Next, the regression plots are shown, with substances shown in the same order as for the between-lab section. The set of duplicates that was analyzed in the first round of analysis are denoted as "original", and the samples analyzed in the second round are denoted as "replicate". Regarding the triplets, eight of the "replicates" had been analyzed twice.

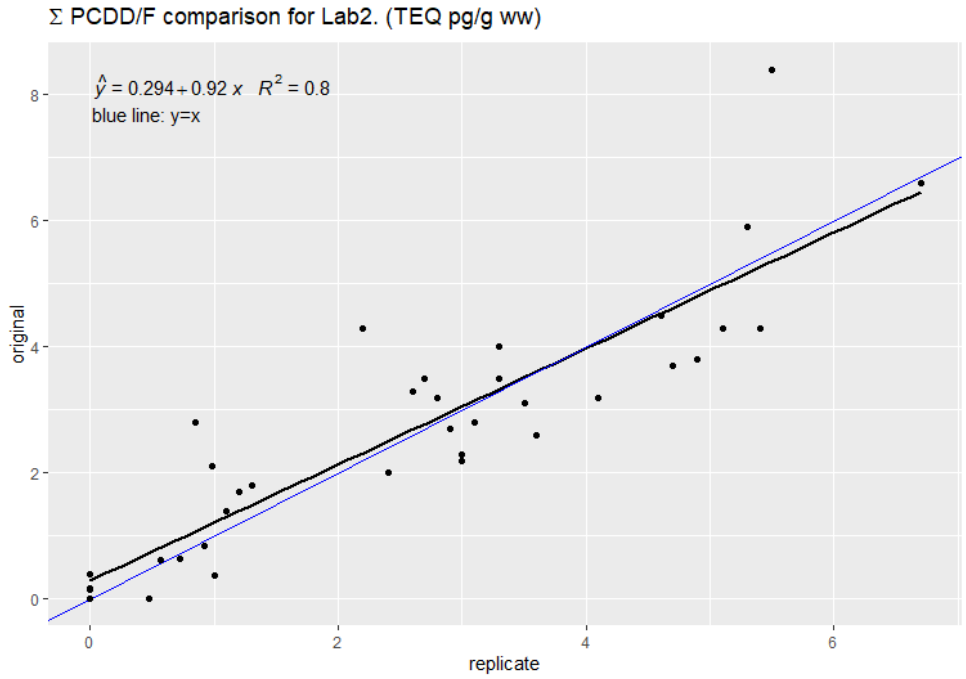

Figure 17 Comparison of Lab2 duplicate measurements of ∑PCDD/F TEQ pg/g ww.

The R^2-value is higher than for the comparison between laboratories, and the blue and black line almost overlap, which is good. This is an indication that Lab2 perform quite consistently with regards to dioxin.

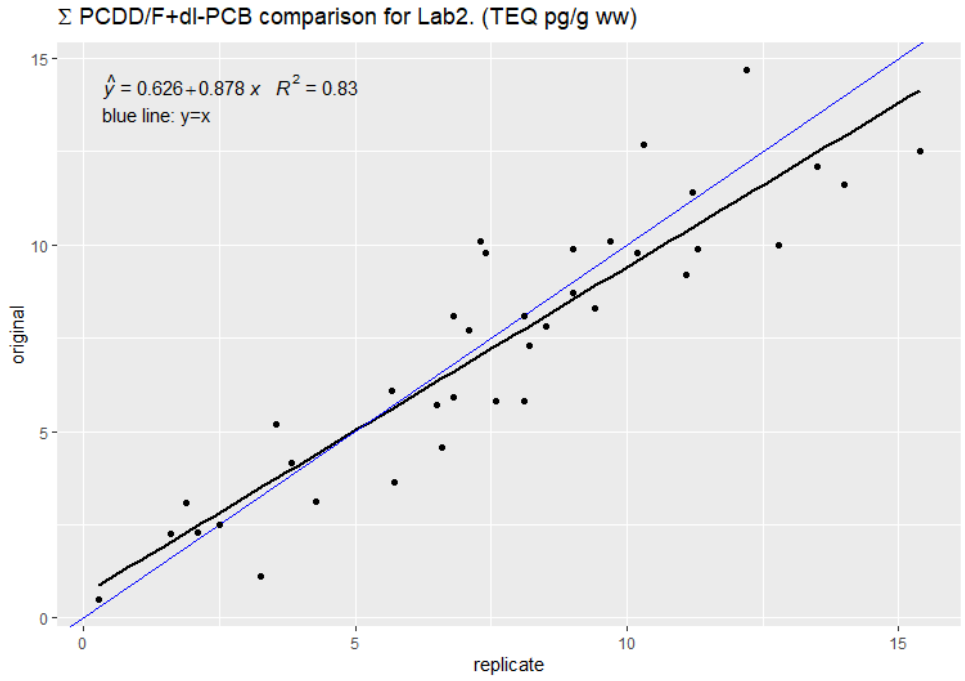

Figure 18 **Comparison of Lab2 duplicate measurements of ∑PCDD/F+-dl-PCB TEQ pg/g ww.**

The R^2-value for the within-lab measurement of ∑PCDD/F+dl-PCB is higher than for the corresponding comparison between-lab, and the blue and black line are close to overlapping, indicating that for ∑PCDD/F +dl-PCB, the measurements are more consistent within Lab2 than between Lab1 and Lab2.

Figure 19 **Comparison of Lab2 duplicate measurements of fat content (%).**

The R^2-value and the closeness of the blue and black line are very similar compared to the corresponding between laboratory comparison. This result gives an indication that there is no major difference in the consistency of the fat content analysis when the replicates are analyzed by the same lab, or when two labs analyze the same sample.

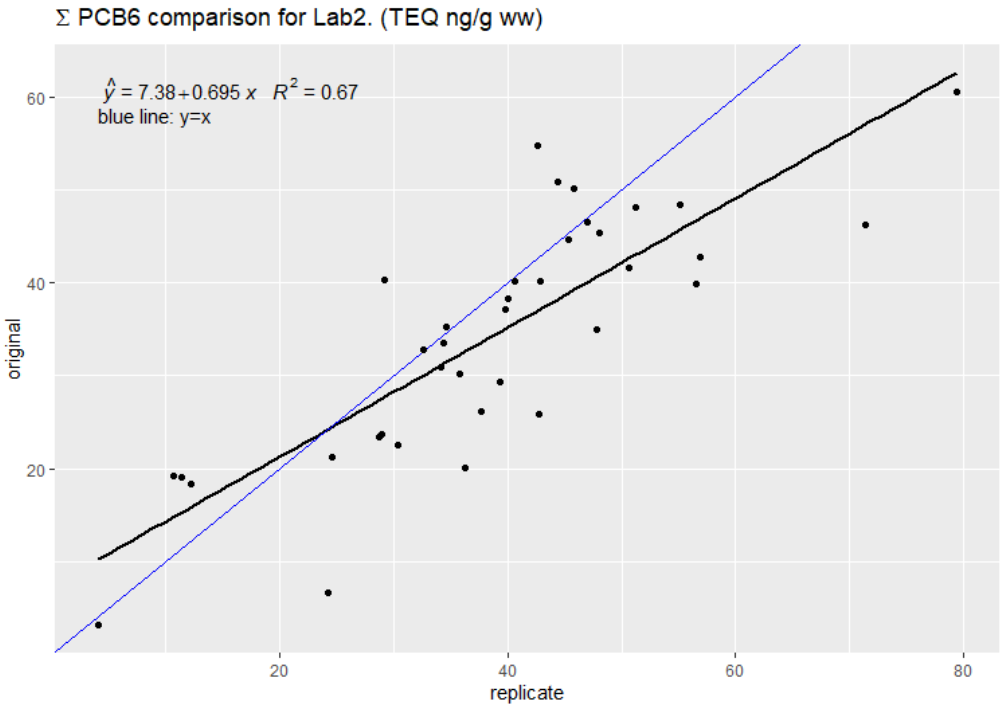

Figure 20 **Comparison of Lab2 duplicate measurements of ∑PCB6 TEQ ng/g ww.**

Of the compared substances, this is the one that show most inconsistency between the "original" and "replicate" samples. Also, the R^2-value is quite low, very similar to when Lab1 and Lab2 was compared, therefore giving no indication of increased accuracy with regards to ∑PCB6 when the same lab analyses both the original and replicate. It should anyhow be mentioned that in Figure 20, the values are somewhat condensed to the middle of the figure. Samples with a larger range of ∑PCB6 are likely to have resulted in a higher R^2-value and blue and black lines closer to overlapping. Compare with for example Figure 18, in which there is a wider range of values.

Overall, the impression from the plots is that the results of dioxin-like compounds are more similar when duplicates are analyzed by the same lab then when two labs analyze one set of duplicates each (seen by higher R^2-values and closeness of the "optimal" line and the regression line).

Triplets and subcutaneous fat

Here, the eight measurements that were not included in the regressions are discussed briefly. For the eight samples that are triplets, in order to get an indication if differences in consistency is large with regards to different substances, the coefficient of variation (CV)

is calculated for each of the eight triplets, and then the mean CV is calculated for each substance. So, for each substance, the mean of eight different CV-measures is displayed.

The CV is a measure of the variability in relative terms. Lower indicates a higher consistency. For a random variable Z, It is defined as:

$$CV(Z) = \frac{\sqrt{Var(Z)}}{mean(Z)}$$

Expressed in words, it makes comparisons of variability more meaningful when the scales of the variables are different, as is the case for this data. Table 21 displays the average coefficient of variation for the eight triplicates.

Table 21 Mean coefficient of variation for triplets analysed by Lab2.

Substance	**Mean CV**
∑PCDD/F	0,23
∑PCDD/F+dl-PCB	0,22
Fat	0,046
∑PCB6	0,091

Noticeable is that, in this sample, although the sample size is small, fat has the lowest mean CV, strengthening what was found in the fat content comparison for duplicates, namely that the analysis of fat content shows little variability.

Regarding the subcutaneous fat, the measurements are displayed in Table 22. Both samples are from a pooled sample of salmon. Note that the "original" samples are also found in Table 14 when subcutaneous fat is compared to muscle tissue samples.

Table 22 Table of measurements on duplicates of subcutaneous fat samples.

Substance/ID	**Sample1 (T11-15)**		**Sample2 (N11-13)**	
	Original	Replicate	Original	Replicate
∑PCDD/F	8,30	11,0	12,0	6,90
∑PCDD/F+dl-PCB	22,3	40,0	32,0	22,9
Fat	21,0	21,2	21,3	24,9
∑PCB6	78,0	169	128	85,0

As mentioned previously, the measurements of dioxin and PCB in the subcutaneous fat are very high, indicating the lipophilic properties. Also, there is considerable variability between measurements. For example, the measured ∑PCDD/F+dl-PCB for the "original" sample1 is 22,3 TEQ pg/g ww while the "replicate" is 40,0 TEQ pg/g ww, which is almost twice as high. As found previously, the smallest variability seems to be when fat is measured.

Summary of between and within-lab comparison

When comparing the values of duplicates analyzed by the two labs, bias was found for ∑PCDD/F+dl-PCB and ∑PCB6. It was also found that when fat is analyzed, the values for the duplicates are more similar as compared to when dioxin-like compounds and non-dioxin-like PCBs were analyzed.

When the same lab analyzed duplicates, the reported values of dioxin-like compounds were closer to each other than when two labs were involved. This indicates that there is more consistency within a lab than between labs. This pattern was not seen for ∑PCB, for which only 64% of the variation in the original samples could be explain by the replicates, and an indication of systematic differences was found. The average for the one set of duplicates (the "replicates") was 13% higher than for the other set (the "originals"). For fat, the consistency was high both between and within labs. The R^2-value was 93- and 95% respectively, which is high.

In the comparison of labs, it was concluded that Lab2 on average reports a higher value for ∑PCDD+dl-PCB and ∑PCB6. This investigation is however only based on a few time points. It cannot from this data be concluded if this bias is consistent over time. It might be the case that one or both labs update equipment, protocols, changes staff, or some other change that could affect the results from their analysis. No suggestion is therefore made to compensate for any bias in a control fishing program. To little is known about how systematic differences between labs behave over time.

For this data, when observing ∑PCDD/F+dl-PCB, the average of Lab2 was 8,1 TEQ pg/g ww and the average for Lab1 was 6,1 TEQ pg/g ww. The average for Lab2 is therefore 19% higher than for Lab1. Since fishermen are allowed to subtract measurement error (25% as reported by the labs) from an analyzed batch before it gets prohibited for sale, it is of interest to account for lab-bias as well. This investigation indicates that such error might be present but is not able to give a reliable estimate of how many percent to add to the now used 25%, since, as mentioned, it is unclear if the on average higher values reported by Lab2 are consistent over time.

On the other hand, what is suggested is that when performing statistical analyses on data involving dioxins and PCBs, is to pay attention to if more than one lab have analyzed data. If it is the case, results from this report indicates that this should be taken into account. Otherwise, there is a possibility that erroneous conclusions about e.g. time trends are made. The results from the within and between lab analysis are, for an overview, summarized in Table 23.

Table 23 **Summary of results from Wilcoxon signed rank test of replicates from Lab1 and Lab2. A p-value smaller than 0,05 is significant. In case of significance, it is concluded that at the 5%-level, a bias exists.**

	Fat	**∑PCDD**	**∑PCDD+dl-PCB**	**∑PCB6**
	Bias. (Yes/No) (p-val)	Bias. (Yes/No) (p-val)	Bias. (Yes/No) (p-val)	Bias. (Yes/No) (p-val)
Lab1 vs Lab2	No 0,22	No 0,29	Yes 0,00078	Yes 0,013
Lab2 vs Lab2	No 0,19	No 0,99	No 0,25	Yes 0,0035

Discussion

Previous investigations of the variations in amount of POPs in fish before and after cooking have led to controversial results. While many reports have expressed a direct and positive correlation between cooking and considerable reduction of PCDD/Fs and PCBs in different types of meat and fish, other studies claim no difference, and in some cases even an increase of these compounds after cooking (Zabik & Zabik, 1999; Del Gobbo, et al., 2008; Perellò, et al., 2009; Rawn, et al., 2013). Although the mechanisms during which dioxins and PCBs are removed are not clearly understood, there are hypotheses suggesting that surface area, temperature, evaporation, dilution in the cooking oil or a combination of these factors may account for the reduction of PCBs and dioxins before and after cooking (Bayen, et al., 2005; Domingo, 2010).

In this context, our findings have demonstrated that the cooking/preparation method could have an influence on the level of dioxins and dioxin-like PCBs in both herring and salmon. Smoking herring was found to potentially lower levels of dioxin-like compounds. For fermentation and frying, no indication of reduction was found. Regarding salmon, the preparation method that was found to potentially reduce levels of dioxin-like compounds is curing. As mentioned, mechanisms behind reduction of dioxin and PCB from cooking are largely unknown, so it cannot here be answered why the cured samples would show lower levels, and because of the low number of observations, the reduction is not concluded to apply for cured salmon in general. It acts mostly as an early indication that could be a question for further research. For the other cooking procedures, no indication of reduced levels was found. If instead looking at PAH:s for herring and salmon, a stronger result is that smoking increases the levels of PAH:s. Differences were large between the smoked and raw samples, but not for other preparation techniques compared to their corresponding raw samples. For salmon, where samples were both hot-smoked

and cold-smoked, the high PAH-values were in the hot-smoked samples, but also within these samples, large variations were seen. That there are large variations between hot/cold-smoking, but also for the same procedure is in line with results in (Hokkanen, et al., 2018), in which it was found that the level of PAH:s varies substantially with hot/cold smoking, distance from smoke source and smoking time.

Among different cooking methods, it is reported that frying the fish has the most impact on PCB removal (up to 50%) in fish (Domingo, 2010). This is not in accordance with results in this report where only a minor reduction with regards to ΣPCDD/F and ΣPCDD/F+dl-PCB was seen when frying herring. Although, the small sample size (n=1) does not give a good setting to draw a general conclusion. What can be said is that uncertainties in conclusions with regards to differences between cooked and raw samples are a consequence of small sample sizes and the relatively large error margin associated with the chemical analysis of the investigated substances.

A sample from farmed salmon was compared to wild salmon. Results indicated, as expected, that farmed salmon has lower levels of dioxin-like compounds (and non-dioxin-like PCBs). Only one sample of farmed salmon was analysed, but since farmed salmon are likely to be very similar with respect to content of dioxin and PCB due to standardized and quality-controlled feed, and since differences between the farmed and wild salmon were so large, the results are deemed credible. By looking at results in (Nøstbakken, et al., 2015), in which little variability and a decreasing time trend is seen for ∑PCDD/F+dl-PCB in Norwegian farmed salmon, it is reasonable to assume that one farmed salmon is quite representative of the population.

It is well-investigated that all PCB congeners are, more or less, fat-soluble and that fat content has a direct correlation with PCBs (Zabik & Zabik, 1999). Our results indicate a strong association between the amount of the fat and dioxins, dioxin-like PCBs and non-dioxin-like PCBs in salmon. In (Bayen, et al., 2005) it is declared that the amount of PCBs loss after losing fat during cooking was proportional with the ratio of 1.22:1. In a similar manner, (Aune, et al., 2003) stated that there is an undeniable correlation between the lipids and dioxins and PCBs in herring. They mentioned that, since the major part of fat accumulates in the subcutaneous fat, removal of skin and attached fat led to reduction of dioxins and dioxin like PCBs.

Other research has stated that removal of skin with subcutaneous lipids could remove dioxins and dioxin-like PCBs up to 54% in herring. It was shown that the skin alone does not contain a noticeable amount of PCBs and that the main proportion of PCBs are stored in the subcutaneous fat (Törnkvist, et al., 2005). This is in accordance with what we found with our salmon samples. It was not shown explicitly that the majority of PCBs are stored in the subcutaneous fat, but a strong indication emerged that lower levels of dioxins and PCBs are found in cuts with lower fat content.

Despite some findings that the middle part of a salmon is where the highest concentration of PCBs are (Bayen, et al., 2005), we found that the neck part contains the highest quantity of fat, and in the middle the fat content experienced a slight decrease, and towards the tail a further decrease. Furthermore, when modelling ∑PCDD/F and ∑PCDD/F+dl-PCB in

different parts of the fish, it was concluded that the main difference in these substances between different parts are due to changes in fat. When controlling for changes in fat content, only the fat content had a significant (positive estimate) effect on ∑PCDD/F and ∑PCDD/F+dl-PCB, not from which part of the fish the sample is taken. This is in line with well-known lipophilic properties of dioxin-like compounds. For ∑PCB6, a significant difference was found between the middle and tail when controlling for changes in fat. Reasons for this are to this point not known.

Consequently, the peak amounts of dioxins, dioxin-like PCBs and non-dioxin-like PCBs are believed to be in the neck of the salmon samples, with descending levels towards the tail. The findings can approve what was noted in (Persson, et al., 2007), about a significant difference between the fat content in the middle and the tail of salmon and also verifies what (Aune, et al., 2003) have described in their research. They discovered that the amount of non-dioxin-like PCBs in their salmon samples (n=2) was maximum in the neck (anterior) with about 300 ng/g ww. That value was reduced by about 17% (about 250 ng/g ww) and 78% (about 65 ng/g ww) in the middle and the tail respectively.

The comparison of the results from two different labs could explicitly highlight that we can expect significant variations in the results derived from duplicated fish samples. This can be due to the different errors in calibration, efficiency of their methods in connection with different type of fish tissues, number of replicates in each measurement and other instrumental and human errors (Grubbs, 1969; Taverniers, et al., 2004). Our statistical tests revealed that random error is not the sole cause of difference, but a systematic error is an unneglectable factor. For ∑PCDD/F and ∑PCDD/F+dl-PCB, an indication of bias between Lab1 and Lab2 was found. The bias correlates with the laboratory and method biases together with the matrix variation effect which are controllable but unavoidable (Taverniers, et al., 2004; Hutcheon, et al., 2010). Detection, tracking and neutralizing each of these errors in the results would take a large amount of time and resources due to the need for large number of replicates. Nevertheless, there can be occasions when it might be impossible to plan an experimental design with numerous replicas and therefore, adopting other methods to estimate the source of variations would be inevitable (Moseley, 2013).

For the duplicates analyzed by the same lab, the results were more consistent for ∑PCDD/F and ∑PCDD/F+dl-PCB as compared when they were analysed by Lab1 and Lab2 separately. Regarding fat content, measurements of the duplicates were more similar than for the other substances. There seem to be no difference in the exactness within a lab or between a lab with respect to analysis of fat content. Both regressions displaying fat content comparisons were very similar. Surprisingly, for ∑PCB6, results were not more similar when one lab analysed duplicates than when two labs did. A bias for ∑PCB6 was found for the within lab comparison as well as for the between lab comparison.

It should be kept in mind that the units of measurement differ between the substances: fat is measured in percent, ∑PCDD/F and ∑PCDD/F+dl-PCB are measured in TEQ pg/g ww and ∑PCB6 is measured in ng/g ww. This could be one of the reasons for the fat analysis being the most consistent one. The amount of fat in a fish is much larger than the amount

of dioxins and PCBs, and the chemical analysis is most likely more exact for a substance that are abundant in the sample. Also, fat and dioxins/PCBs have a completely different chemical structure, and it could be the case that lipids are easier to analyse more accurately.

Reasons for bias are unknown, but it can act as a justification of the uncertainties connected to analysis of dioxins and PCB. At this point, a suggestion to account for a lab-bias when deciding if a batch of fish should be prohibited or allowed for sale is avoided, since too little is known about how the bias behaves over time. If more research is made, and if consistent systematic differences are found between labs, there is good reason to account for it.

In conclusion, to interpret the results of the lab measurements of PCBs and PCDD/Fs with overwhelmingly small concentrations, we should consider a reasonable margin for the bias as the laboratories incorporate the errors from different sources. These includes, but are not limited to, the systematic errors of observing different calibration protocols, using different measurement devices with different meaningful precisions, utilizing different sample preparation for different type of fish and so on. However, verifying the results from one lab by testing the same samples by another lab has stressed the domain of the uncertainty of the results and limits our freedom to accept the results of a single test as the legitimate benchmark for our indisputable judgement. Even for the same lab, differences between test results of replicates can be large.

Finally, with regards to the results from comparisons of different parts of fish and comparison between and within laboratories, the belief of fat content being a strong proxy for dioxin and PCB content in fatty fish is strengthened. The laboratories report more exactly for fat content than for dioxins and PCB, making comparisons based on small samples more exact when looking at differences in fat content, and with respect to levels of dioxin-like compounds in different parts of salmon and trout, they were here shown to be almost exclusively related to the fat content in the different parts.

References

Aune, M., Rickard, B., Atuma, S. & Darnerud, P. O., 2003. Large Differences in Dioxin and PCB Levels in Herring and Salmon Depending on Tissue Analysed. *Organohalogen Compounds, Vol. 64,* pp. 378-81.

Bayen, S., Barlow, P., Lee, H. K. & Obbard, J. P., 2005. Effect of cooking on the loss of persistent organic pollutants from salmon. *Journal of Toxicology and Environmental Health, Part A, 68(4),* pp. 253-265.

Bignert, A., Danielsson, S., Faxneld, S. & Nyberg, E., 2016. *Comments concerning the national Swedish contaminant monitoring programme in marine biota,* Stockholm: 5;2016, Swedish Museum of Natural History.

Del Gobbo, L. et al., 2008. Cooking decreases observed perfluorinated compound concentrations in fish. *Journal of agricultural and food chemistry, 56(16),* pp. 7551-7559.

Domingo, L. J., 2010. Influence of cooking processes on the concentrations of toxic metals and various organic environmental pollutants in food: A review of the published literature. *Critical reviews in food science and nutrition, 51(1),* pp. 29-37.

European Union, 2011. Commission Regulation (EU) No 1259/2011 of 2 December 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for dioxins, dioxin-like PCBs and non dioxin-like PCBs in foodstuffs. *Off J Eur Union, 320,* pp. 18-23.

European Union, 2011. Commission Regulation (EU) No 835/2011 of 19 August 2011 amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs. *Official Journal of the European Union,* pp. 4-8.

Grubbs, F. E., 1969. Procedures for Detecting Outlying Observations in Samples. *Technometrics, 11(1),* pp. 1-21.

Hokkanen, M. et al., 2018. Critical Effects of Smoking Parameters on the Levels of Polycyclic Aromatic Hydrocarbons in Traditionally Smoked Fish and Meat Products in Finland. *Journal of Chemistry,* pp. Article ID 2160958, 14 pages.

Hutcheon, J. A., Chiolero, A. & Hanley, J. A., 2010. Random measurement error and regression dilution bias. *BMJ: British Medical Journal. BMJ, 340(7761),* p. 1402–1406.

Kelly, B. C. et al., 2007. Food web–specific biomagnification of persistent organic pollutants. *science, 317(5835),* pp. 236-239.

Moseley, H. N., 2013. Error Analysis and Propagation in Metabolomics Data Analysis. *Computational and structural biotechnology journal, 4(5),* p. e201301006.

Nøstbakken, O. J. et al., 2015. Contaminant levels in Norwegian farmed Atlantic salmon (Salmo salar) in the 13-year period from 1999 to 2011. *Environment International, Volume 74,* pp. 274-280.

Perellò, G. et al., 2009. Concentrations of polybrominated diphenyl ethers, hexachlorobenzene and polycyclic aromatic hydrocarbons in various foodstuffs before and after cooking. *Food and chemical toxicology, 47(4),* pp. 709-715.

Persson, M. E., Larsson, P., Holmqvist, N. & Stenroth, P., 2007. Large variation in lipid content, ΣPCB and δ13C within individual Atlantic salmon (Salmo salar). *Environmental Pollution, 145(1),* pp. 131-137.

Rawn, D. F. et al., 2013. Impacts of cooking technique on polychlorinated biphenyl and polychlorinated dioxins/furan concentrations in fish and fish products with intake estimates. *Journal of agricultural and food chemistry, 61(4),* pp. 989-997.

Taverniers, I., De Loose, M. & Van Bockstaele , E., 2004. Trends in quality in the analytical laboratory. I. Traceability and measurement uncertainty of analytical results. *TrAC Trends in Analytical Chemistry, 23(7,* pp. 480-490.

Törnkvist, A. et al., 2005. The importance of specific and clear instructions for sample preparation when analysing organochlorine compounds in fish. *Organohalogen Compounds (67),* pp. 1450-1453.

Zabik, M. E. & Zabik, M. J., 1999. Polychlorinated Biphenyls, Polybrominated Biphenyls, and Dioxin Reduction During Processing/Cooking Food. *Impact of Processing on Food Safety. Springer, Boston, MA.,* pp. 213-231.

Appendix A

Figure A1 To the left: raw herring. To the right: fried herring.

Figure A2 Raw salmon neck (above) and cold smoked salmon neck (below).

IVL Swedish Environmental Research Institute Ltd.
P.O. Box 210 60 // S-100 31 Stockholm // Sweden
Phone +46-(0)10-7886500 // Fax +46-(0)10-7886590 // www.ivl.se