The importance of specific and clear instructions for sample preparation when analysing organochlorine compounds in fish

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Introduction

Most organochlorine compounds (OCCs) used in agriculture and industry have been banned for decades in Sweden and many other countries and the release of dioxins (PCDD/DFs) have decreased. However, these substances are still found in the surrounding biota as a result of the environmental persistence and high lipophilicity of the compounds. The levels of most OCCs have declined in food since the 1970s but the decline of PCDD/DFs and some PCBs in fish from some areas of the Baltic Sea appear to have ceased in the 1990s[1]. The maximum level for PCDD/DFs in fish and fishery products, 4 pg WHO-PCDD/DF-TEQ/g set by the European Council, is expressed on fresh weight basis due to the extremely large difference in fat content between lean (e.g. cod 0.7 %) and fatty fish (e.g. eel 30 %)[2]. In Sweden, some herring products are sometimes consumed with the fish skin and therefore the Swedish National Food Administration has historically made OCC analyses on muscle tissue of herring including the skin. However, many products are sold without the fish skin. It is therefore of interest for both food authorities and industry to analyse different parts of the herring. Differences in concentrations of OCCs have been observed for herring samples prepared with or without the subcutaneous lipids and skin[3]. Results from a study on salmon have also indicated large differences in sum PCB depending on which part of the fish that was analysed. Due to differences in results, depending on sample preparation methods used before analysis, EU discussions on sample preparation procedures of fish are ongoing at present. Regarding removal of skin, it has been suggested that the subcutaneous lipids are carefully and completely scraped off from the skin and thereafter added to the muscle sample to be analysed. In this pilot study, this recently suggested sample preparation procedure is tested on herring and a comparison is made with our previous sampling procedure for analysis of OCCs in herring.

Methods and Materials

From 46 individual "Rügen herrings" caught in 2004 in the Baltic Proper, three sets of pooled samples were prepared. From each gutted fish, the head was removed and both of the fillets were skinned. The muscle tissue *without* subcutaneous lipids and skin from one of the fillets was used for three pooled samples (n=13, 16 and 17, respectively) and the corresponding fillet *with* the subcutaneous lipids (scraped off from the skin but without skin) was sampled and used for three corresponding pooled samples. The skin *without* subcutaneous lipids was used for another three pooled samples.

The pooled samples were analysed for ortho PCBs (PCB congeners 28, 31, 52, 66, 74, 101, 105, 110, 114, 118, 128, 138, 149, 153, 156, 157, 158, 167, 170 and 180) and organochlorine pesticides (HCB, α-HCH, β-HCH, γ-HCH), chlordane (oxychlordane, transnonachlor, α-chlordane, γ-chlordane) and DDTs (*o,p´*-DDT, *p,p´*-DDT, *p,p´*-DDD, *p,p´*-DDE). The analyses were performed by HRGC/ECD according to validated methods at the Swedish National Food Administration. The samples were thoroughly homogenised and extracted with mixtures of acetone/n-hexane and n-hexane/diethyl ether. After evaporation the lipid content was determined gravimetrically and the lipids were then removed by treatment with sulphuric acid. The PCBs were separated from most of the chlorinated pesticides by fractionation on a silica gel column. Finally, the samples were quantified on a GC equipped with dual capillary columns and dual electron capture detectors (ECD).

Results and Discussion

In Table 1, the sums of PCBs, DDTs, HCHs and chlordanes are shown for the pooled herring samples prepared with or without subcutaneous lipids. The concentrations of PCBs in muscle including or excluding subcutaneous lipids differ significantly from each other (mean levels 22.0 and 15.0 µg/kg, respectively; paired t test, $P<0.05$). It can be

seen that the sum of PCBs decrease when the subcutaneous lipids are removed from the fish muscle. The results presented in Table 1 are in accordance with earlier results that have shown a highly significant decrease (approximately 60 %) of PCB levels in herring muscle without skin and subcutaneous lipids compared to herring analysed with skin[3]. Also for dioxins and dioxin-like PCBs, a dramatic decrease (54 %) of dioxin and dioxin like PCBs was found when the skin and subcutaneous lipids were removed.

Table 1. Raw data with mean (min-max) of the herring fillet analysed with and without subcutaneous lipids. The skin was removed from the Rügen herring and the skin without subcutaneous lipids was analysed separately. The concentrations are given on fresh weight basis. See material and methods for further details.

	Herring
No of analyses; individuals	3; 46
Weight (g)	109 (85.7-141)
Fat content in muscle incl subcutaneous lipids (%)	11.2 (10.4-12.4)
Fat content in muscle excl subcutaneous lipids (%)	8.1 (7.0-9.1)
Fat content in skin excl subcutaneous lipids (%)	28.5 (22.4-38.7)
Sum PCB in muscle incl subcutaneous lipids (µg/kg fw)[a]	22.0 (19.0-27.4)
Sum PCB in muscle excl subcutaneous lipids (µg/kg fw) [a]	15.0 (12.3-18.9)
Sum PCB in skin excl subcutaneous lipids (µg/kg fw) [a]	61.7 (41.7-73.7)
Sum DDT in muscle incl subcutaneous lipids (µg/kg fw)[b]	14.7 (12.4-18.8)
Sum DDT in muscle excl subcutaneous lipids (µg/kg fw)[b]	10.3 (8.3-13.5)
Sum DDT in skin excl subcutaneous lipids (µg/kg fw) [b]	41.2 (27.5-50.4)
Sum HCH in muscle incl subcutaneous lipids (µg/kg fw)[c]	2.3 (2.2-2.5)
Sum HCH in muscle excl subcutaneous lipids (µg/kg fw)[c]	1.7 (1.5-1.9)
Sum HCH in skin excl subcutaneous lipids (µg/kg fw)[c]	6.5 (4.7-8.5)
Sum Chlordanes in muscle incl subcutaneous lipids (µg/kg fw)[d]	1.5 (1.2-2.0)
Sum Chlordanes in muscle excl subcutaneous lipids (µg/kg fw)[d]	1.0 (0.7-1.5)
Sum Chlordanes in skin excl subcutaneous lipids (µg/kg fw)[d]	4.3 (2.6-5.5)

[a] The sum of the PCB congeners 28, 52, 101, 105, 110, 118, 138, 149, 153, 170 and 180.

[b] The sum of *p,p´*-DDT, *p,p´*-DDD, *p,p´*-DDE.

[c] The sum of α-HCH, β-HCH, γ-HCH.

[d] The sum of oxychlordane, transnonchlor, α-chlordane.

In Table 2 the concentrations of PCBs found in skin, subcutaneous lipids and muscle of herring together with the distribution of muscle, skin and subcutaneous lipids in herring samples are presented. When comparing the contribution of PCBs from muscle, subcutaneous lipids and skin to the total content of PCB in herring samples it can be seen that the muscle and subcutaneous lipids contribute to the major part (62 and 32 %, respectively), Figure 1.

Table 2. The relative distribution (mean (min-max)) of muscle, skin and subcutaneous lipids in herring samples, together with fresh weight PCB concentrations (mean (min-max)) in muscle, skin, subcutaneous lipids and muscle with skin.

	Herring
No of analyses; individuals	3; 46
Weight (g)	109.2 (85.7-141)
Sum PCB in muscle with skin and subcutaneous lipids (μg/kg fw)[a]	22.9 (19.5-28.3)[b]
Muscle excl subcutaneous lipids in herring samples, % (w/w)	94 % (94.2-94.7)
Sum PCB in muscle excl subcutaneous lipids (μg/kg fw)[a]	15.0 (12.3-18.9)
Skin excl subcutaneous lipids in herring samples, % (w/w)	2%
Sum PCB in skin excl subcutaneous lipids (μg/kg fw)[a]	61.7 (41.7-73.7)
Subcutaneous lipids in herring samples, % (w/w)	3%
Sum PCB in subcutaneous lipids (μg/kg fw)[a]	219 (201-252)[b]

[a] The sum of the PCB congeners 28, 52, 101, 105, 110, 118, 138, 149, 153, 170 and 180.

[b] Estimated values.

The highest concentration of PCBs (219 μg/kg) was found in the subcutaneous lipids, Table 2. Also in the skin, the PCB levels were relatively high (61.7 μg/kg). However, it may not be necessary to remove the skin, since this part only contributes to a minor extent (4-10%) to the total PCB concentration. When using a paired t test, the concentration of PCBs in muscle with subcutaneous lipids does not differ significantly from the estimated concentration in herring sampled with skin (paired t test, $p<0.05$). It may therefore be possible to prepare herring samples by using the muscle with the skin. This will result in a less time-consuming sample preparation procedure compared to a procedure where the subcutaneous lipids are carefully and completely scraped off from the skin and thereafter added to the muscle sample to be analysed. For other fishes, however, the skin may be thicker and thereby contribute to a higher extent to the PCB concentration.

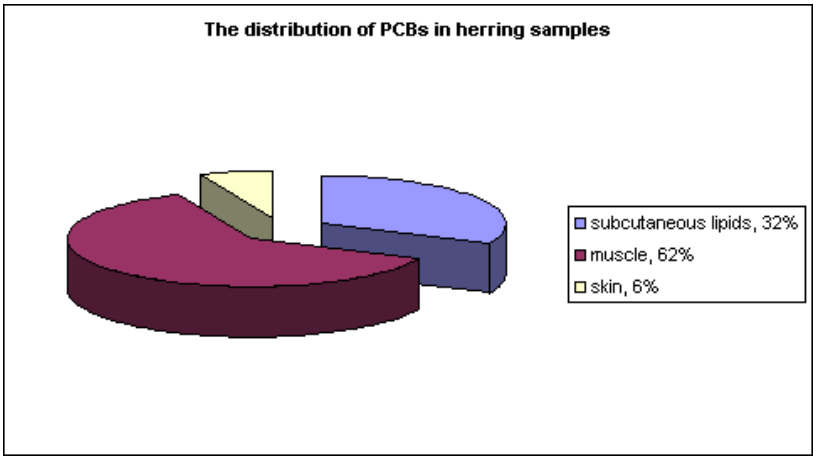

Figure 1. The distribution of PCBs in herring samples.

In addition to the results in the present pilot study, results from an earlier study show that there can be significant

differences in reported levels of sum PCB and organochlorine pesticides depending on which part of the fish that is analysed[3]. All these results address the need to have distinct regulations and to carefully describe the fish sample preparation procedure. The decrease of dioxin and dioxin like PCBs when the skin and subcutaneous lipids is removed might, for example, have significant impact on species allowed for intra trade and export. The specific and clear instructions for sample preparation is of importance in order to avoid misleading results and conclusions based on comparisons of non-representative values. Future studies could address the question how industrially removal of the skin will affect the OCC concentration in the final food product.

References

1. Bignert A., Asplund L. and Wilander A. (2004) Comments Concerning the National Swedish Contaminant Monitoring Programme in Marine Biota. Available at www.naturvardsverket.se

2. Council Regulation (EC) No 2375/2001 amending Commission Regulation (EC) No 466/2001

3. Aune M., Bjerselius R., Atuma S., Larsson L., Bergh A., Darnerud P.O., Andersson A., Arrhenius F., Bergek S., Tysklind M. and Glynn A. (2003) Organohalogen compounds 64, 378-381.