FOOD SAFETY MONITORING OF PCDD/Fs AND DLPCBs IN NORWEGIAN FARMED FISH FROM 2004 TO PRESENT

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Introduction
Norway has a substantial fish farming industry and the production of fish was almost 1 million tons in 2009. Farmed Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) are the principal species in the production. These are oily fish species which are susceptible to accumulation of persistent organic pollutants which are potentially hazardous to the consumers. These environmental contaminants include polychlorinated biphenyls (PCBs), dioxins polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), polybrominated diphenyl ethers (PBDEs), hexabromocyclodecane (HBCD), and organochlorine pesticides [1,2,3,4]. Since 2004, levels of PCD/Fs and DLPCBs in Norwegian farmed fish has been monitored in accordance with EU directive 96/23. The scope of the monitoring regime in directive 96/23 is coupled to the production volume of the industry. Since 2004 the production has increased by several hundred percent which is reflected in the number of samples analysed. During these years a total of 283 pooled samples of five fish have been analysed for PCD/Fs and DLPCBs.

Materials and methods
The sampling plan was designed to ensure statistical randomness over all seasons and regions, sampling was carried out by inspectors from the Norwegian Food Safety Authority (NSFA). According to current legislation, the minimum number of samples to be collected each year must be at least 1 per 100 tons produced fish according to EU directive 96/23. Two-thirds of the samples were sampled according to the regime associated with directive 96/23 group B parameters. The PCD/Fs and the DLPCBs belong in group B according to this directive. Farm sites from all regions with aquaculture activity, and at least 10% of the total number of sites were included in the sampling plan. Five fish were sampled from each farm, all from the same cage. The sample-IDs were coded so that NIFES staff was unaware of their origin. The samples received by the laboratory were frozen fillets with skin or frozen chops with skin and backbone, according to the Norwegian standard quality cut [5.] The fillets or filleted chops from each five-fish samples were skinned and homogenized to pooled samples with equal contribution from each fish. The advantage of pooled samples is that a large number of fish can be included in the surveillance. The cost of this procedure is the loss of the fish variance in the data. The observed standard deviations reflect the variability among the farms rather than between individual fish.

Analytical procedure: The method is an adaptation to the US-EPAs (Environmental Protection Agency) methods No. 1613 and 1668. The samples were analysed for the PCDDs, PCDFs, and DPCBs congeners which have been assigned a WHO-TEF_{1998} and are included in the current EU maximum limit [6]. A mixture of 27 different ^{13}C-labelled internal standards is mixed in with a homogenized freeze-dried sample corresponding to 3 g of fat. Hydromatrix® is mixed in before extraction to aid the solvent penetration. Extraction with hexane under elevated pressure and temperature was performed in an Accelerated Solvent Extractor (ASE 300®, Dionex, Sunnyvale, CA, USA). Fat and other matrix components are removed by oxidation in a separate layer of sulphuric acid on silica in the extraction column. Further clean-up is performed in a Power-Prep® instrument (FMS-USA) where successive chromatographic steps are carried out in three columns: "Multi-layered silica, basic alumina and activated charcoal. The mobile phase is changed successively from hexane, 2% dichloromethane (DCM) in hexane, 50% DCM in hexane, ethyl acetate and finally back-flush with toluene. The PCDD / PCDF and the non-orto PCBs are collected with the toluene fraction. The mono-orto PCBs are collected with the 50% DCM/hexane fraction. The two collected fractions are each evaporated to 10 ml in a TurboVap ® concentration Workstation (Zymark, USA). Two ^{13}C-labelled congeners are added to serve as "recovery standards" The samples are analysed in a HRGC/HRMS instrument (DFS or MAT 95, Thermo Finnigan).

The sums of the TEQ values are calculated for all 29 congeners, as an “upper bound” sum (UB-Sum WHO-$_{1998}$ TEQ ng / kg). Recovery data is calculated for each sample based on the recoveries of the internal standards relative to the two labelled recovery standards. There are individual LOQ values for each congener. The LOQ values for all of the congeners are in the range of 0.006 to 0.2 ng TEQ/ kg.

Results and discussion

Table 1 presents the findings of dioxin and dioxin-like PCB levels in different species of farmed fish from 2009, with the results split to cover each biological species assayed. Table 2 presents a summary of the dioxin and dioxin-like PCB concentrations in farmed Atlantic salmon sampled annually between 2004 and 2008.

The sum of PCDD/Fs in 2009 farmed fish ranged from 0.18 ng TEQ/kg to 0.57 ng TEQ/kg w.w. Both the mean dioxin levels and the concentration ranges are consistent with the values found annually since 2004. Thus there seems to be no clear trend in the dioxin levels measured in farmed Atlantic salmon between 2004 and 2009. The maximum PCDD/F concentration of 0.57 ng TEQ/kg w.w found in Atlantic salmon in 2009 was well below the EU's upper limit of 4.0 ng TEQ/kg w.w. The differences in the analytical levels between the biological species are consistent with their lipid content as would be expected since these compounds are lipophilic. Thus the levels are highest in Atlantic salmon, slightly lower in Rainbow trout and lowest in arctic char. Typical lipid contents in these species are around 15 % for fillets of salmon and rainbow trout and just around 10% for the char.

The DLPCB concentration in 2009 farmed salmon ranged from 0.16 to 1.07 ng TEQ/kg wet weight. Both mean concentrations and ranges of DLPCB levels in Atlantic salmon have been stable from 2004 until2009. As with dioxins, the levels of DLPCBs reflect the lipid content in the fillet of the different species: Atlantic salmon> rainbow trout > Arctic char. The levels of DLPCB have been consistently higher than the PCDD/PCDF levels in farmed salmonids.

The total concentration of dioxins and DLPCBs in farmed fish in 2009 ranged from 0.44 to 1.52 ng TEQ/kg w.w. The mean concentration ranged from 0.65 to 0.88 ng TEQ/kg w.w for the fish species examined which is about 10% of the EU's upper limit of 8.0 ng TEQ/kg w.w. No sample of farmed Atlantic salmon analysed in this project since 2004 has approached or exceeded the EU's legal limit.

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References

1.Hites, R.A., Foran, J.A., Carpenter, D.O., Hamilton, M.C., Knuth, B.A., Schwager, S.J., 2004. Global assessment of organic contaminants in farmed salmon. Science 303, 226-229.
2.Maule, A.G., Gannam, A.L., Davis, J.W., 2007. Chemical contaminants in fish feeds used in federal salmonid hatcheries in the USA. Chemosphere 67, 1308-1315.
3.Shaw, S.D., Berger, M.L., Brenner, D., Carpenter, D.O., Chia-Swee Hong, C.-S., Kannan, K., 2008. Polybrominated diphenyl ethers (PBDEs) in farmed and wild salmon marketed in the Northeastern United States. Chemosphere 71, 1422-1431.
4.van Leeuwen, S.P.J., van Velzen, M.J.M., Swart, C.P., van der Veen, I., Traag, W.A., de Boer, J., 2009. Halogenated Contaminants in Farmed Salmon, Trout, Tilapia, Pangasius, and Shrimp. Environ. Sci. Technol. 43, 4009-4015.
5.Standard Norge, 1994. NS-9401, (www.standard.no)
6.Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775-792.

Table 1. Levels of dioxins (PCD/F) and dioxins-like PCBs (ng TEQ/kg w. w.) in fillets of farmed fish sampled in 2009.

		Atlantic Halibut	Atlantic Salmon	Rainbow Trout	Arctic Char		EU Limit
Number of samples	**N**	**1**	**53**	**8**	**2**		
Sum DLPCB	**Mean**	**-**	**0.58**	**0.51**	**0.40**		
	Min	**-**	**0.16**	**0.29**	**0.26**		
	Max	**0.76**	**1.07**	**0.66**	**0.54**		
Sum PCDD	**Mean**	**-**	**0.14**	**0.15**	**0.15**		
	Min	**-**	**0.07**	**0.10**	**0.08**		
	Max	**0.07**	**0.35**	**0.21**	**0.22**		
Sum PCDF	**Mean**	**-**	**0.16**	**0.13**	**0.10**		
	Min	**-**	**0.08**	**0.11**	**0.09**		
	Max	**0.16**	**0.30**	**0.16**	**0.10**		
SUM PCDD-PCDF	**Mean**	**-**	**0.30**	**0.28**	**0.25**		
	Min	**-**	**0.18**	**0.22**	**0.18**		
	Max	**0.23**	**0.57**	**0.37**	**0.31**		**4.0**
SUM DLPCB + SUM Dioxins	**Mean**	**-**	**0.88**	**0.79**	**0.65**		
	Min	**-**	**0.44**	**0.56**	**0.57**		
	Max	**0.99**	**1.52**	**1.03**	**0.72**		**8.0**[1]

UB: All sums and averages are"upper bound" calculations.
LOQ: All LOQ values are related to the individual congeners, NIFES has not established a LOQ values for their sums.

[1] Since 2006.

Table 2. Levels of dioxins (PCD/F) and dioxins-like PCBs (ng TEQ-/kg w. w.) in fillets of farmed Atlantic salmon? between 2004 until 2008.					
Parameter	***Year***	***N (pooled samples)***	***Maximum value***	***Minimum value***	***Mean value***
Sum of PCDD/DFs	***2004***	***13***	***0.37***	***0.15***	***0.26***
	2005	***31***	***0.47***	***0.07***	***0.25***
	2006	***25***	***0.5***	***0.2***	***0.3***
	2007	***116***	***0.6***	***0.1***	***0.3***
	2008	***98***	***0.5***	***0.1***	***0.3***
Sum of DLPCBs	***2004***	***13***	***1.21***	***0.58***	***0.82***
	2005	***31***	***1.77***	***0.30***	***0.95***
	2006	***25***	***1.5***	***0.6***	***1.2***
	2007	***116***	***2***	***0.6***	***0.9***
	2008	***98***	***1.6***	***0.3***	***0.7***
Total sum of TEQ	***2004***	***13***	***1.6***	***0.5***	***1.1***
	2005	***31***	***2.2***	***0.4***	***1.2***
	2006	***25***	***2.0***	***0.7***	***1.5***
	2007	***116***	***2.6***	***0.7***	***1.3***
	2008	***98***	***2.1***	***0.4***	***1.0***