

## Levels of Polychlorinated Dibenzo(p)dioxins, Dibenzofurans and Dioxin-like PCBs in Irish Farmed Salmon

Rainer Gruemping<sup>1</sup>, Stephan Hamm<sup>1</sup>, Dieter Stegemann<sup>1</sup>, Armin Maulshagen<sup>1</sup>

<sup>1</sup>eurofins/GfA, Muenster

### Introduction

A recent survey published by Hites in the journal *Science* compared the level of organochlorine contaminants including PCBs and dioxins in farmed versus wild salmon collected from around the world<sup>1</sup>. Most organochlorine substances analysed in the study show a significantly higher concentration level in farmed than in wild salmon. While dioxin and PCB levels of wild fish mainly reflect the contamination level of the environment in which the fish is grown, the dioxin and PCB concentration in farmed fish may mainly be attributed to the fish feed used.

As a consequence to the Belgian "chicken scandal" in 1999, the EC Commission enacted maximum levels for dioxins and furans (PCDD/F) in food and feed valid from July 1<sup>st</sup>, 2002 throughout Europe (Council Directive 2003/57/EC and Council Regulation EC/2375/2001). For muscle meat of fish and fishery products a dioxin maximum level of 4 pg WHO-PCDD/F-TEQ/g fresh weight was set. The levels of dioxins reported in the study cited above are all below this limit value<sup>2</sup>. Latest until the end of 2004, the maximum levels will be reviewed in particular regarding the inclusion of dioxin-like PCBs.

In January 2004, the Irish Sea Fisheries Board (BIM) conducted the present study on the concentration of Polychlorinated Dibenzo(p)dioxins (PCDDs), Dibenzofurans (PCDFs) and dioxin-like PCBs (WHO-PCBs) in farmed salmon from two locations in Ireland. The present study should examine whether the PCDD/F and WHO-PCB levels of Irish farmed salmon correlate to the dioxin data for farmed Atlantic salmon from other countries in Northern Europe (e.g. Scotland, Faroe Islands and Norway) presented in the study by Hites.

In the Hites survey, raw salmon filets with skin on were tested. Since PCBs, dioxins and other organic pollutants are mainly bound to the fish fat, a reduction of fat content by removal of the skin was supposed to lower the amount of organic contaminants. Thus, the effect of skin removal on the dioxin and PCB levels was also examined in the present study. In addition, the influence of cooking the fish meat was investigated.

### Materials and Methods

Within the present study, 13 salmon were caught in January 2004 at two fish farms in different areas of Ireland. As specified by BIM, the six salmon from one of the fish farms (Area 1) had an average filet weight of 529 g and the seven salmon from the other location (Area 2) showed an average filet weight of 1.069 g. One part of each salmon filet was not further pretreated while from another part, the skin was removed. Furthermore, a part of each raw salmon meat was cooked. Thus, four different sample materials from each fish were provided for analysis.

The 52 samples of salmon filet were homogenised and the frozen homogenates were shipped to the GfA without interrupting the cooling chain. The provided homogenates were freeze-dried (Christ, Beta 1-8 Freeze-dryer) and further homogenised by means of grinding. A fat extraction of about 20 g of the dried and grinded sample material was done by means of Accelerated Solvent Extraction (ASE) using an ASE 300 instrument of Dionex Corp., Sunnyvale, CA, USA. The fat fraction finally was determined gravimetrically after evaporation of the solvents.

The analytical methodology is in compliance with the requirement for the HRGC/HRMS confirmatory analysis of food for PCDD/Fs and PCBs as laid down by the EU Directive 2002/69. Each analysis included the determination of the seventeen PCDD/F congeners with 2,3,7,8-chlorosubstitution and the 12 dioxin-like PCB congeners for which toxic equivalency factors (TEF) were established by a working group of the WHO<sup>3</sup>. For 16 native PCDD/F and each PCB congener to be quantified, the corresponding <sup>13</sup>C<sub>12</sub>-labelled compound was added to the fat extract as internal standard prior to the defatting and the subsequent chromatographic clean-up. The recoveries of the internal standards through the fat separation and all clean-up steps were determined by means of further <sup>13</sup>C-labelled internal PCDD and PCB standards added to the PCDD/F and the PCB fraction before GC/MS analysis. All the <sup>13</sup>C-labelled standards were from Cambridge Isotope Labs, Endover, USA. Both, the PCDD/F and PCB analyses were performed on a HP 5890 HRGC connected to a VG AutoSpec HRMS (mass resolution > 9000). A 60 m DB-5 MS capillary column was used for the gas chromatographic PCDD/F separation and a 25 m HT-5 column for the PCB analyses. The limit of quantification was in the range of 0.02 pg/g fat for 2,3,7,8-TCDD and of 0.4 pg/g fat for PCB 126. Further details of the analytical procedures are reported in references 4 and 5.

An inhouse reference material was analysed within this project for verifying the accuracy of the PCDD/F and PCB analysis. No PCDD/F or PCB blanks exceeded

the limit of quantification. The recoveries of the internal  $^{13}\text{C}_{12}$ -labelled PCDD/F and PCB standards proved to be in the range of 80 to 120 % for all measurements of this study, demonstrating well the appropriateness of the applied methods for the analysis of Dioxins/Furans and PCBs in fish. Expanded measurement uncertainties were calculated for the results of individual PCDD/F and PCB congeners as well as for the WHO TEQs. The uncertainties were calculated on the basis of the "Guide to the expression of Uncertainty in Measurement (GUM)" and the EURACHEM/CITAC Guide "Quantifying Uncertainty in Analytical Measurement (QUAM)"<sup>6, 7</sup>. The expanded uncertainties calculated here are based on a coverage factor of 2 and give a level of confidence of approximately 95 %. Expanded uncertainties within this project were in the range of 15 to 18 % for the individual PCDD/F and PCB congeners and in the range of 8 to 13 % for the TEQ values.

### Results and Discussion

The WHO-TEQ values determined for the six salmon from the Irish fish farm in Area 1 (Fish 1 to Fish 6) are presented in Table 1 and the data for the seven salmon from the fish farm in Area 2 (Fish 7 to Fish 13) are shown in Table 2. As can be seen in both tables, the four different sample materials of each fish are listed separately. According to the requirements of the European legislation, the WHO-TEQ values refer to the fresh weight of the fish meat and the non-quantified congeners were included by taking the full limit of quantification (upperbound concentrations). Minimum and maximum WHO-TEQ values as well as the median of each data set are also shown in both tables.

Table 1: Fresh weight-based WHO-TEQ values of filet meat samples from six Irish farmed salmon caught at the fish farm in Area 1

Sample	Fat content [%]	Dioxins WHO-TEQ [pg/g <sub>fw</sub> ]	PCB WHO-TEQ [pg/g <sub>fw</sub> ]	Dioxins and PCBs Total WHO-TEQ [pg/g <sub>fw</sub> ]
<b>uncooked, skin on</b>				
Fish 1	13,9	0,216	0,910	1,126
Fish 2	13,7	0,234	0,969	1,203
Fish 3	13,2	0,236	0,908	1,144
Fish 4	11,4	0,213	0,817	1,030
Fish 5	14,3	0,217	0,904	1,121
Fish 6	13,3	0,231	0,882	1,113
Minimum (1 - 6)	11,4	0,213	0,817	1,030
Maximum (1 - 6)	14,3	0,236	0,969	1,203
Median (1 - 6)	13,5	0,224	0,906	1,124
<b>uncooked, skin off</b>				
Fish 1	10,8	0,172	0,702	0,874
Fish 2	10,6	0,171	0,737	0,908
Fish 3	8,3	0,148	0,560	0,708
Fish 4	8,0	0,135	0,556	0,691
Fish 5	11,1	0,167	0,703	0,870
Fish 6	8,8	0,155	0,570	0,725
Minimum (1 - 6)	8,0	0,135	0,556	0,691
Maximum (1 - 6)	11,1	0,172	0,737	0,908
Median (1 - 6)	9,7	0,161	0,636	0,798
<b>cooked, skin on</b>				
Fish 1	15,1	0,234	0,969	1,203
Fish 2	14,5	0,245	0,951	1,196
Fish 3	13,7	0,252	0,936	1,188
Fish 4	14,6	0,260	1,040	1,300
Fish 5	15,4	0,227	0,951	1,178
Fish 6	16,7	0,318	1,130	1,448
Minimum (1 - 6)	13,7	0,227	0,936	1,178
Maximum (1 - 6)	16,7	0,318	1,130	1,448
Median (1 - 6)	14,8	0,249	0,960	1,200
<b>cooked, skin off</b>				
Fish 1	12,7	0,200	0,812	1,012
Fish 2	9,7	0,156	0,644	0,800
Fish 3	10,1	0,176	0,678	0,854
Fish 4	9,7	0,171	0,685	0,856
Fish 5	12,1	0,174	0,730	0,904
Fish 6	10,2	0,182	0,670	0,852
Minimum (1 - 6)	9,7	0,156	0,644	0,800
Maximum (1 - 6)	12,7	0,200	0,812	1,012
Median (1 - 6)	10,2	0,175	0,682	0,855
<b>Statistics on all salmon samples from area 1</b>				
Minimum (1 - 6)	8,0	0,135	0,556	0,691
Maximum (1 - 6)	16,7	0,318	1,130	1,448
Median (1 - 6)	12,4	0,207	0,815	1,021

Table 2: Fresh weight-based WHO-TEQ values of filet meat samples from seven Irish farmed salmon caught at the fish farm in Area 2

Sample	Fat content [%]	Dioxins WHO-TEQ [pg/g <sub>fw</sub> ]	PCB WHO-TEQ [pg/g <sub>fw</sub> ]	Dioxins and PCBs Total WHO-TEQ [pg/g <sub>fw</sub> ]
<b>uncooked, skin on</b>				
Fish 7	11,6	0,511	1,600	2,111
Fish 8	9,6	0,388	1,250	1,638
Fish 9	8,8	0,619	1,880	2,499
Fish 10	12,7	0,557	1,600	2,157
Fish 11	11,9	0,816	2,420	3,236
Fish 12	13,6	0,562	1,530	2,092
Fish 13	9,7	0,574	1,690	2,264
Minimum (7- 13)	8,8	0,388	1,250	1,638
Maximum (7 - 13)	13,6	0,816	2,420	3,236
Median (7 - 13)	11,6	0,562	1,600	2,157
<b>uncooked, skin off</b>				
Fish 7	7,4	0,317	0,968	1,285
Fish 8	6,3	0,245	0,811	1,056
Fish 9	9,5	0,380	1,200	1,580
Fish 10	8,0	0,330	0,995	1,325
Fish 11	9,1	0,330	1,000	1,330
Fish 12	9,1	0,360	1,020	1,380
Fish 13	5,5	0,299	0,928	1,227
Minimum (7- 13)	5,5	0,245	0,811	1,056
Maximum (7 - 13)	9,5	0,380	1,200	1,580
Median (7 - 13)	8,0	0,330	0,995	1,325
<b>cooked, skin on</b>				
Fish 7	13,2	0,575	1,830	2,405
Fish 8	12,4	0,511	1,650	2,161
Fish 9	14,9	0,632	1,960	2,592
Fish 10	12,1	0,513	1,520	2,033
Fish 11	12,5	0,794	2,410	3,204
Fish 12	14,0	0,584	1,630	2,214
Fish 13	8,8	0,507	1,490	1,997
Minimum (7- 13)	8,8	0,507	1,490	1,997
Maximum (7 - 13)	14,9	0,794	2,410	3,204
Median (7 - 13)	12,5	0,575	1,650	2,214
<b>cooked, skin off</b>				
Fish 7	8,2	0,349	1,140	1,489
Fish 8	7,1	0,288	0,925	1,213
Fish 9	11,5	0,453	1,490	1,943
Fish 10	8,8	0,369	1,120	1,489
Fish 11	5,7	0,349	1,080	1,429
Fish 12	8,7	0,364	1,000	1,364
Fish 13	5,4	0,285	0,853	1,138
Minimum (7- 13)	5,4	0,285	0,853	1,138
Maximum (7 - 13)	11,5	0,453	1,490	1,943
Median (7 - 13)	8,2	0,349	1,080	1,429
<b>Statistics on all salmon samples from area 2</b>				
Minimum (7- 13)	5,4	0,245	0,811	1,056
Maximum (7 - 13)	14,9	0,816	2,420	3,236
Median (7 - 13)	9,3	0,421	1,370	1,791

The dioxin/furan-assigned WHO-TEQs of the six salmon filets from Area 1 were between 0.135 and 0.318 pg WHO-TEQ/g. The median of this set of samples is 0.207 pg WHO-TEQ/g. The TEQs resulting from the 12 PCB congeners range between 0.556 and 1.13 pg WHO-TEQ/g with a median of 0.815 pg WHO-TEQ/g. Concerning the median values, the PCB contribution to the total TEQs is 80 %.

The dioxin and PCB levels of the seven salmons from the Irish fish farm in Area 2 tend to be somewhat higher. The median for the PCDD/Fs assigned WHO-TEQs is 0.421 pg/WHO-TEQ/g and the median for the PCB concentration of this set of samples is 1.37 pg WHO-TEQ/g. Thus, the contribution of the median PCB concentration to the median total TEQ value is 76 %. Different fish feed may be a reason for the differences in the dioxin and PCB levels between the salmon samples from the two fish farms.

The main constituent within the 17 PCDD/F congeners was 2,3,7,8-TetraCDF. Due to the high TEFs, the congeners 2,3,4,7,8-PentaCDF, 2,3,7,8-TetraCDD and 1,2,3,7,8-PentaCDD also contribute significantly to the PCDD/F-TEQ. From the dioxin-like PCBs, the congeners 118, 105, 156 and 167 showed the highest concentrations. However, the PCB-TEQ is dominated by PCB 126 due to its TEF of 0.1.

The separate listing of the values from the samples including the skin (skin on) on the one hand and without skin (skin off) on the other hand in both tables illustrates that by taking away the skin, a significant amount of dioxins/furans and PCBs is removed. Taking the median values into consideration, the total TEQs of all the 12 samples with skin on from Area 1 are by a factor of 1.41 higher than the total TEQs of the 12 samples without skin. The total TEQs for the 14 samples from Area 2 including the skin material are 1.64 times higher than the TEQs of the 14 samples with skin off. The clear influence of the skin removal on the resulting fat contents and thus on the dioxin and PCB levels is evident. The median value of the fat contents of the salmon meat with skin on is 1.42 (Area 1) respectively 1.49 (Area 2) times higher than the fat contents of the skinned samples.

Cooking the salmon meat has no significant influence on the PCDD/F and PCB levels. The range of values is only slightly higher in the cooked sample material which correlates with slightly elevated fat contents maybe due to water reduction during the cooking procedure.

The dioxin WHO-TEQ values of all the 52 analysed farmed salmon samples were significantly below the maximum level laid down by the EU in 2002. Even the highest PCDD/F concentration found in the uncooked meat of Fish 11 with skin on was only about 20 % of the limit set for muscle meat of fish (4 pg WHO-PCDD/F-TEQ/g). Furthermore, the total TEQs including both, the dioxin and the PCB-TEQ of this and all the other samples were below this maximum level.

In 2002 the Food Safety Authority of Ireland (FSAI) already conducted a national survey on dioxins and dioxin-like PCBs in wild and farmed salmon<sup>8</sup>. The dioxin levels found in Irish wild salmon were on average less than 10 % of the EU limit mentioned above and the dioxin levels found in Irish farmed salmon were on average less than 25 % of this limit. The dioxin-like PCB levels were higher than the dioxin levels. An average value for the total WHO-TEQ values in Irish farmed salmon of 4 pg WHO-TEQ/g wet weight was stated there. Thus, the concentrations of PCDD/Fs and dioxin-like PCBs in the farmed salmons from both locations investigated here tend to be consistently lower than the values reported for Irish farmed salmon by the FSAI in 2002.

In the paper by Hites the dioxin data are presented in graph form as mean values from different locations distinguishing farmed from wild salmon. Although no exact data are presented, it can be seen that the total TEQ values of farmed salmon samples vary from about 0.7 pg to 3 pg WHO-TEQ/g fresh weight (mean values of individual locations). When comparing the total TEQ data of the salmons with skin on from the current study with the range of values found by Hites, it can be seen that the total TEQs of the salmons from the Irish fish farm in Area 1 are in the lower range of values, while the total TEQs of the samples from the fish farm in Area 2 are in the higher range of values found for farmed salmon from around the world.

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