ANALYSIS OF DIOXIN-LIKE PCBs IN WILD AND FARMED SALMON AND FARMED TROUT FROM IRISH WATERS

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Introduction

Since the crisis in Belgium in 1999 there has been an increased awareness within the European Union (EU) of the threat posed by dioxins, furans and polychlorinated biphenyls (CBs) in the food chain. These persistent environmental pollutants are ubiquitous within nature and as a result of their lipophilic properties pose a potential risk to the consumer of contaminated food.

In 1998 the WHO identified 12 PCBs to be dioxin-like (DLPCBs - non-ortho: 77, 81, 126, 169 and mono-ortho: 105, 114, 118, 123, 156, 157, 167 and 189)[1]. The congeners with 4 or more non-ortho substituted chlorines exhibit strong dioxin-like characteristics and are considered to be amongst the most toxic.

In a study carried out by the Food Safety Authority of Ireland (FSAI) concentration levels of the non-ortho CBs 77, 81, 126 and 169 and the mono-ortho CBs 105, 114, 118, 156, 157, 167 and 189 were determined in "wild" salmon, farmed salmon and farmed trout from Irish waters and aquaculture sites.

With the application of toxic equivalency factors (TEFs), where the toxicity of these CBs has been assessed relative to the most toxic dioxin (2,3,7,8-TCDD), concentration data can be converted into toxic equivalent quantities (TEQs). TEQs data are considered to be an accurate representation of toxicity as they reflect specific toxicological processes. This paper summarises the resulting DLPCB data, examines possible CB metabolism/excretion and investigates single congener analysis as a tool for the estimation of CB-TEQs.

Methods and Materials

15 samples each of "wild" and farmed salmon *Salmo salar* and farmed trout *Oncorhychus mykiss* were obtained from waters and aquaculture sites around Ireland. Muscle tissue was sampled the sample homogenised prior to analysis. Before extraction ^{13}C-UL- labelled internal standards for the dioxin-like CBs of interest were added to the sample. Cleanup was performed on a carbon glass-fibre column and analysis carried out by HRGC-MS on a DB5 stationary phase. For each analyte 2 isotope masses were measured and quantification was carried out by isotope dilution.

Results and discussion

Concentrations of the non-ortho and mono-ortho CBs in muscle tissue of wild and farmed salmon and farmed trout are presented in table 1. CB 77 was found to be the most abundant non-ortho CB at concentrations between 2-3 times that of CB126 while CB118 was the most abundant mono-ortho CB present. Overall the combined concentration of the mono-ortho CBs was approximately one order of magnitude higher than the non-ortho CBs.

Table 1: TEFs[1] employed and concentration ranges of dioxin-like CBs in wild and farmed salmon and farmed trout (n=15 for each group) from Ireland (ng/kg wet wt.)

Congener	TEF	Wild salmon	Farmed salmon	Farmed trout
CB 81	0.0001	0.35-1.26	0.80-3.53	0.52-1.24
CB 77	0. 0005	4.51-18.6	30.8-121	11.3-29.7
CB 126	0.1000	3.52-8.80	12.7-36.5	4.41-11.2
CB 169	0.0100	0.56-2.33	2.13-4.63	0.55-1.57
CB 105	0.0001	247-541	774-1887	255-535
CB 114	0.0005	17.1-37.4	40.8-107	13.5-27.3
CB 118	0.0001	740-1559	2119-5121	695-1451
CB 156	0.0005	63.2-139	194-582	73.1-163
CB 157	0.0005	19.9-41.2	53.5-169	22.6-43.2
CB 167	0.00001	45.1-96.6	119-394	55.5-114
CB 189	0.0001	4.62-10.7	14.9-70.7	8.34-15.6

Investigation of CB metabolism.

The use of the relatively easily quantified CB153 for normalization purposes in such studies has been reported by a number of authors[2,3,4]. The ratio of the other CBs to CB153 may give an indication of the metabolic degradation/elimination capacity of a species towards specific congeners. The ratio of CB77 to CB153 was observed to be much lower in wild salmon than for any of the farmed species, while the ratio of all other dioxin like CBs was similar between the 3 species examined. This may indicate that oil used in the manufacture of food pellets for farmed species may originate from a source with a more elevated tetra CB77 level or it may suggest that wild salmon have some capacity to excrete/metabolise CB77 where accumulation occurs through natural feeding patterns.

To further investigate metabolism/excretion effects principal component analysis (PCA) was carried out on the ratio of CB153 to each of the DLPCBs and to CBs 28, 52, 101, 138 and 180. Logarithmic transformation of the data was required to ensure that a normally distributed dataset was obtained, this method of treating such nominal data being widely used in environmental applications. The direction and length of vectors for CBs 77, 189 and 126 seem to differ from those of the other CBs (see figure 1) and may suggest that some capacity for metabolism/excretion of these congeners exists in wild salmon.

The complete population of wild salmon is also clearly distinguished from both farmed species whereas no clear separation is observed for either of the farmed samples, this may possibly relate to similarities in diet and/or metabolic activity for the farmed species. It was observed from the PCA that "clustering" of a number of farmed samples was evident; these samples generally originated from the same aquaculture site so similarities may be related to feeds employed at each specific location.

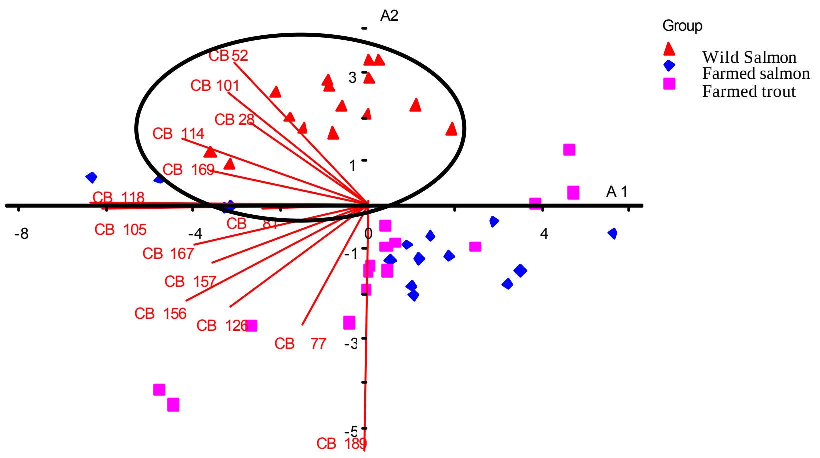

Figure 1: Principal component biplot of the ratio of CBs to CB153. (Separation of wild salmon from other groups is indicated by ellipse)

TCDD equivalents

Numerous studies have used toxic equivalency factors (TEFs)[1,5] in estimating the toxicity of CBs in fish. Multiplication of CB concentration data with its respective TEF allows the CB to be expressed in terms of a TEQ relative to dioxin 2,3,7,8-TCDD toxicity. From concentration data (table 1) it was determined that CB126 contributes 69-73% of the total CB related TEQ with CB118 the second highest contributor. The combined CB related TEQ for CBs 126, 118, 105 and 156 account for the majority (approx 95%) of the estimated toxicity. deBoer[2] reported that 85-90% of the CB-TEQ in fish from Dutch waters was as a result of the combination of CBs 126, 118, 156 and 170; this study shows similarity to that report. The contribution of the majority of the other CBs is much less pronounced but the overall toxicological significance of these CBs is yet to be fully investigated and current data are limited. For the purposes of this study TEFs described by the WHO[1] were employed.

Single congener analysis for the estimation of TCDD equivalents.

Williams[6] reported a correlation between CB153 concentration and the TEQ attributable to CBs alone. The value of such a predictive tool is dependant on the accurate determination of the CB involved and uncertainty increases where concentrations are low. This study investigated the predictive power of single congener analysis for a number of CBs for the estimation of the CB-TEQ and it was observed that various CBs might prove useful as indicators of the CB-TEQ.

CBs 118 and 156 occur at much more elevated levels than the majority of other dioxin like CBs and generally CB153 is found at easily quantifiable levels in marine biota and can routinely be analysed to a high degree of accuracy without the aid of high-resolution equipment.

It was observed that CB118 which itself contributes between 12-14% of the CB-TEQ shows a high correlation with the total CB-TEQ (r=0.57,0.82 and 0.74 for farmed trout, farmed salmon and wild salmon respectively). CB156 also contributes to a degree to the overall CB-TEQ and showed a good correlation (r=0.57, 0.81 and 0.83 for farmed trout, farmed salmon and wild salmon respectively) to the total CB-TEQ. The use of CB153 as a predictive tool may be possible for both wild and farmed salmon but little correlation was observed for farmed trout (r=0.70, 0.72 and 0.04 respectively). It is unresolved as to why this is observed for farmed trout only as no difficulties were encountered during analysis of these samples. The relationship observed for both CB118 and 156 was at least equal to that observed for CB153 and showed good correlation even at the low concentration levels recorded in these species. PCA seems to suggest little metabolism/excretion of either CB156 or 118 and therefore these may be suitable for the estimation of the total CB-TEQ. It should be noted that few data are available in the literature on the metabolism/excretion of these congeners in salmonids and therefore their use as a predictive tool is still unclear.

Conclusions

Wild salmon and farmed salmon and trout were found to contain relatively low levels of dioxin like CBs, the levels found were similar to those observed in other studies.

Possible metabolism of some CB congeners was observed, especially in wild species. A clear separation was observed between wild and farmed species, based on the ratios of CBs. No distinction was clearly visible between farmed species as a result of samples analysed.

Single congener determination of CBs 118, 156 and 153 was shown to provide a useful tool for the prediction of CB-TEQs for the species examined.

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