PCDDs/PCDFs and dioxin-like PCBs in Fish, Fish products and Fish Feed

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Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are unwanted by-products in a variety of industrial and thermal processes. Because of their many sources, PCDDs and PCDFs are ubiquitously distributed. Polychlorinated biphenyls (PCBs) were widely used in various technical processes (e.g. as transformer oil, capacitor oil, softening agent) and thus are ubiquitously distributed as well.
It has been proven that human dioxin and PCB exposure derives mainly from food, whereby food of animal origin is a major contributor. The level of these contaminants in food is directly related to the relevant levels in the feedingstuffs used.
On November 27, 2001, a directive[i] of the European Council stipulating dioxin limit levels in feeds – so far without taking into account dioxin-like PCBs – was adopted. According to this, all feeds and their initial products must be excluded from use in the feed and food chains if they exceed these limit levels, set out in Table 1. Comparable EU limit values were set up for foodstuff[ii] on November 29, 2001 (compare Table 2**Fehler! Verweisquelle konnte nicht gefunden werden.**).

Table 1: EU PCDD/PCDF limit values for feeding stuff

Products	**Maximum content**
	ng WHO-PCDD/F-TEQ/ kg relative to a feeding stuff with a moisture content of 12 %
Fish oil	**6**
Fish, other aquatic animals, their products (e.g. fish meal) and by-products with the exception of fish oil	**1,25**
Feeding stuff for fish Pet foods	**2,25**

Table 2 EU PCDD/PCDF limit values for foodstuff

Products	Maximum content
Muscle meat of fish and fishery products and products thereof	**4** pg WHO-PCDD/F-TEQ/ g fresh weight
Fish oil intended for human consumption	**2** pg WHO-PCDD/F-TEQ/ g fat

The limit values will be revised for the first time before 31 December 2004 on the basis of new data on the presence of PCDDs/PCDFs (17 congeners) and dioxin-like PCBs (12 congeners, in the following called "dioxin-like PCB"), especially with a view to including the dioxin-like PCBs in the levels to be stipulated. The dioxin-like PCBs can be compared with PCDDs/PCDFs as regards their importance and their toxicological effect on humans.
According to the EU regulation "Action levels" were set up as well to allow foresight measures.

Material and Methods

Origin of samples
All samples analyzed were received in 2001-2003 (fish, fish feed), in 2003 (fish oil) respectively in 2002/2003 (fish meal) within our international routine analytical service. Out of all samples received in our laboratory in these periods of time, the samples evaluated in this paper were chosen by random selection, thus there is no possibility to trace back the results to a certain sample or customer - confidentiality was fully maintained. For the same reason no single data but just a statistical evaluation is presented here.

Brief method description
Fresh fish aliquots equal to approximately 1.5 g of lipids (ranging approximately between 10 g and 200 g of fresh weight material) were homogenized with sodium sulphate and a column extraction by means of cyclohexane/dichloromethane (v:v, 1:1) followed. Fishmeal samples were soxhlet extracted for 20 h by means of n-hexane/aceton (v:v, 1:1). Fishoil samples were dissolved in hexane (crude oil was slightly warmed and homogenized before solving). Before extraction or solving, a mixture of ^{13}C-labelled internal standards (17 2.3.7.8 substituted PCDDs/PCDFs, 12 dioxin-like PCBs) was added to the sample. All ^{13}C-labelled internal standards were delivered by Cambridge Isotopes Laboratories (USA) or Wellington Laboratories (Canada). After solvent evaporation a gravimetric lipid determination was performed. A multicolumn clean-up including silica gel, different treated silica gel (H_2SO_4-SiO_2, $CsOH$-SiO_2), activated carbon and alumina oxide followed. $^{13}C_{12}$-1.2.3.4-TCDD and $^{13}C_6$-1.2.3.4.6.7.8- HeptaCDF were added to the final extract as recovery standards.
The measurement was performed by high-resolution gas chromatography /high resolution mass spectrometry (HRGC /HRMS) on a HP 5890 II GC coupled with a Micromass AutoSpec mass spectrometer (ionisation mode: Electron impact (EI), resolution: 10,000). A DB 5 column was used for gas chromatographic separation. Quantification was done by means of isotope dilution method using a five-point calibration.

TEQ data was calculated by using WHO-TEFs and by taking into account the whole detection limit for non-detected compounds (upperbound procedure).

Results and Discussion

Tables 3, 4 and 5 are presenting the evaluation of TEQ data from fish, fish oil, fish meal and fish feed based on

- PCDDs/PCDFs only (Table 3)
- dioxin-like PCBs only (Table 4)
- PCDDs/PCDFs and dioxin-like PCBs (Table 5)

In the tables, the number of samples analyzed (n), the 5-, 25-, 75- and 95-percentile as well as the median and mean are shown. All samples were analyzed for PCDDs/PCDFs, but not all of the samples were analysed for dioxin-like PCBs, leading to different number of samples (n) presented in the different tables.

Table 3: Evaluation of only PCDDs/PCDFs in fish, fish oil, fish meal and fish feed, data obtained in ERGO routine analysis

Matrix	Year of sample receipt	Unit	n	5 PCT	25 PCT	Median	Mean	75 PCT	95 PCT
Fish	2001 - 2003	pg WHO-TEQ/g, fresh weight	80	0,17	0,25	0,41	0,54	0,62	1,10
Fish oil	2003	ng WHO-TEQ/kg, related to a feeding stuff with a moisture content of 12%	178	0,16	0,24	0,91	1,91	2,90	6,30
Fish meal	2002 - 2003	"	64	0,11	0,32	0,53	0,73	0,82	1,47
Fish feed	2001 - 2003	"	56	0,48	0,73	0,92	1,21	1,55	2,71

PCT =Percentile

Table 4: Evaluation of only dioxin-like PCBs in fish, fish oil, fish meal and fish feed data, obtained in ERGO routine analysis

Matrix	Year of sample receipt	Unit	n	5 PCT	25 PCT	Median	Mean	75 PCT	95 PCT
Fish	2001 - 2003	pg WHO-TEQ/g, fresh weight	75	0,50	0,79	1,33	1,73	2,16	3,87
Fish oil	2003	ng WHO-TEQ/kg, related to a feeding stuff with a moisture content of 12%	62	2,11	4,23	7,05	7,14	8,50	14,90
Fish meal	2002 - 2003	"	42	0,20	0,70	1,26	1,48	1,85	3,96
Fish feed	2001 - 2003	"	29	1,37	1,90	2,60	2,85	3,58	4,85

PCT =Percentile

Table 5: Evaluation of both PCDDs/PCDFs and dioxin-like PCBs in fish, fish oil, fish meal and fish feed, data obtained in ERGO routine analysis

Matrix	Year of sample receipt	Unit	n	5 PCT	25 PCT	Median	Mean	75 PCT	95 PCT
Fish	2001 - 2003	pg WHO-TEQ/g, fresh weight	75	0,74	1,10	1,80	2,29	2,70	5,25
Fish oil	2003	ng WHO-TEQ/kg, related to a feeding stuff with a moisture content of 12%	62	3,13	6,30	9,90	10,55	13,00	22,80
Fish meal	2002 - 2003	"	42	0,30	1,00	1,85	2,10	2,66	5,38
Fish feed	2001 - 2003	"	29	1,96	2,70	3,80	4,15	4,50	7,85

PCT =Percentile

PCDD/PCDF concentrations of a certain number of samples of fish oil, fish meal and fish feed exceeded the EU limit values (as can be seen by comparison of 95 percentiles in Table 3), although the majority of samples showed values below these limits. Including the TEQ data of dioxin-like PCBs leads to a significant increase of the total WHO-TEQ (based on PCDDs/PCDFs and dioxin-like PCBs) for many samples.

When evaluating the results presented here, it has to be taken into account, that the data represent samples sent to a laboratory acting as international analytical service institution. Thus it cannot be excluded, that some of the batches (represented by the samples analysed) never were used as feeding stuff or food, due to the fact that they exceeded the limit values. On the other hand, we cannot exclude, that a few batches might have been treated by activated carbon aiming to reduce the PCDD/PCDF level (e.g. cleaning of fish oil). We excluded samples from this paper from which we definitively knew about such a procedure. Nevertheless we had some indications for a treatment of samples, which were not announced as being treated (in these few cases, the ratio between PCDD/PCDF (usually easy to remove) and mono-ortho-PCBs (usually difficult to remove) was unexpected low). Apart from these exceptional samples discussed, the results presented here, are surely representative for the majority of samples we analyzed.

References

[i] Council Directive 2001/102/EC, Official Journal of the European Communities, L6/45, 10.01.2002

[ii] Council Regulation (EC) No 2375/2001, Official Journal of the European Communities, L321/1, 06.12.2001