PCDD/F AND DL-PCB ANALYSIS OF CHICKEN EGGS AND RELATED SAMPLES: EXPERIENCES WITH DIOXIN ANALYSIS DURING A DIOXIN CRISIS

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Introduction

Polychlorinated dibenzo-p-dioxins and –furans (PCDD/F) and their relatives, the dioxin-like PCBs (dl-PCBs) are even nowadays substances of high concern. Even with extensive regulations, there are still incidents on a regular basis with contamination of food and feeding stuff. A recent issue has been the use of technical fatty oils for animal feed in northern Germany. There, a producer of pre-mixtures for animal feed purpose used technical grade waste fat/oil contaminated with PCDD/F as a raw material for production of animal feed, mainly for chicken. The consequence was a widespread detectable contamination of several foodstuffs, mainly eggs, in Germany and neighbouring countries. The present paper shows the impact onto the findings of PCDD/F in egg samples around the routine surveillance analysis. Emphasis is posed upon pattern showing the contribution of the distinct PCDD contamination pattern to the total PCDD/F.

Materials and methods

All analysed samples were egg samples in terms of either being whole egg, egg yolk, or other egg products. The samples reflect projects from January 2010 to begin of April, 2011. The samples have been analysed for the 17 2,3,7,8-substituted PCDD/F and/or 12 dioxin-like PCB (dl-PCB, "WHO-PCB"; IUPAC# 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189). *All analytical data are reported in pg/g fat, all TEQ values are given as upperbound TEQ using WHO-TEF(1998)*. The analyses have been performed at the Eurofins GfA GmbH Dioxin/POPs competence centre, Hamburg. Egg samples were extracted after lyophilisation (if necessary, depending on their constitution). Extraction method was Soxhlet hot extraction with toluene or in case of egg yolk ultrasonic extraction. Clean-up consisted of multiple column chromatography, e.g. with silica and aluminium oxide columns and a state-of-the-art GC/HRMS determination method on Waters Autospec mass spectrometers using HRMS at mass resolution $r \geq 10.000$.

Quantification was performed by an isotope dilution method using ^{13}C-labeled quantification standards added before extraction. All analytes have been quantified by their proper ^{13}C-analogon with exception of 123789-HxCDD. The methods are based on the EC requirements for reference analyses[1]. For QA/QC, recovery rates have been monitored with a set of 8 ^{13}C-labelled standards – 4 for PCDD/F and 4 for dl-PCB – added before GC/HRMS injection. Recovery rates were accepted between 50-130%. Further QA/QC measures have been taken, e.g. batch blank preparation over the whole procedure as well as reference samples. Blank values have been below the quantification limits given. The limit of quantification was targeted to meet 1/5 to 1/10 of the appropriate regulatory levels[2]. Furthermore, the laboratory participated successfully and regularly in national and international laboratory comparison studies[3].

Results and discussion:

A total of 1364 egg samples have been analysed for PCDD/F whereof 224 were analysed between January and November 2010 and 1140 from December 2010 to begin of April 2011. PCB analysis took place for 1018 of them - not necessarily within the same samples. The shown results are – where necessary – displayed for matching pairs of samples. In order to work out the differences between 2010 in general and the beginning of the waste oil issue in December 2010, data have been split into two sets of samples where necessary for evaluation. One set covers the time before the crisis, from January to November, 2010 and the second set covers the crisis time between December, 2010 and begin of April, 2011. The main results are shown in figure 1.

The major part of the analysed samples are well below the EC legislative maximum limits of 3 pg TEQ (WHO1998) /g for PCDD/F only and 6 pg TEQ (WHO1998) / g fat for PCDD/F+dl-PCB in total. Exceedance of the maximum limits occurred only in 1.8 % of the cases for PCDD/F and 0.3 % for the dl-PCB. The average (median) levels are with 0.57 (0.25) pg PCDD-TEQ / g fat and 0.84 (0.39) pg PCDD+PCB-TEQ / g fat even

clearly below the average levels of the EFSA monitoring of dioxin levels in food and feed (EFSA 2010)[4] which are at around 1 pg TEQ / g fat for PCDD/F and 2 pg TEQ / g fat for PCDD/F+PCB.

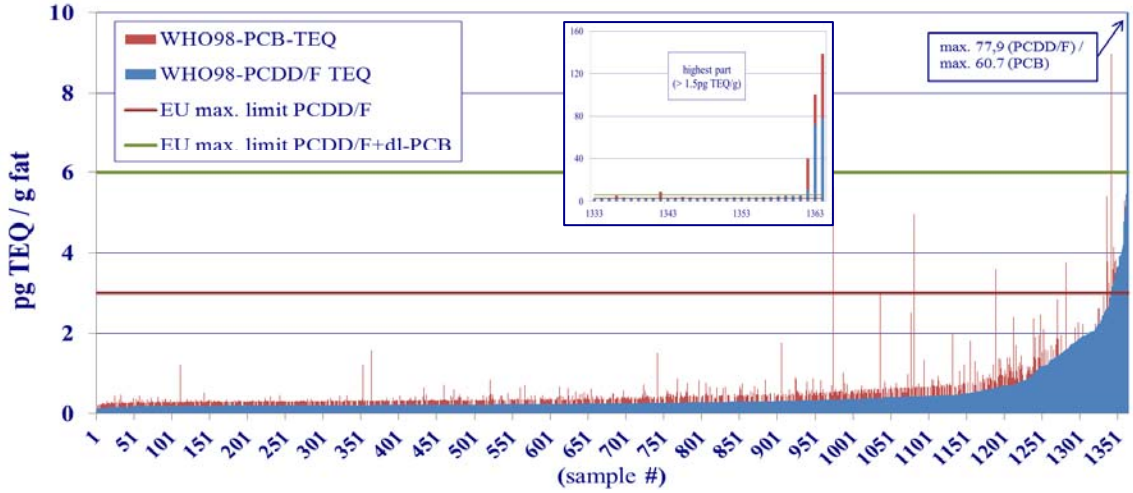

Figure 1: PCDD/F concentrations in egg samples from Jan., 2010 to Apr., 2011(inner diagram: max. values)

A closer look towards the results also shows a certain contribution of dl-PCB towards the total TEQ of the egg samples. There is no clear indication for a single common correlation between PCDD/F and dl-PCB though an overlap of different contamination sources is likely, considering the main relations (figure 2). Especially, there is no clear connection between the dl-PCB concentration in eggs and 2,3,7,8-TetraCDF or 2,3,4,7,8-PentaCDF as a marker for PCDF resulting from technical PCB mixtures. Only few samples – whereof the highest contaminated single samples – show characteristics of PCB-related pattern due to their high PCB-TEQ contribution.

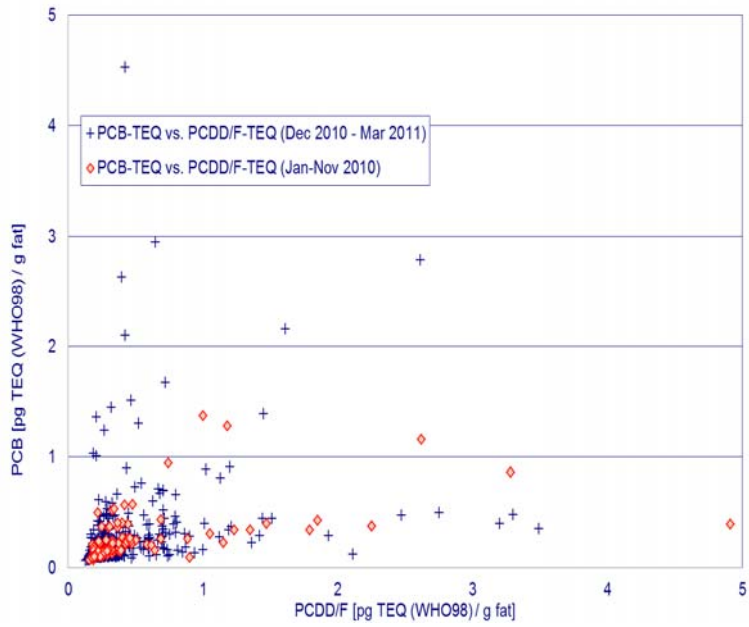

Figure 2: PCB-TEQ vs. PCDD/F-TEQ in egg samples (inner diagram: complete data showing max. values)

The PCDD/F pattern is shown for samples above half the EU maximum limit (> 1.5pg PCDD/F-TEQ/g fat) in figure 3, splitted into two parts showing the typical averaged pattern for egg samples before the crisis situation (January 2010 to November 2010) and during the crisis situation (December 2010 to April 2011). The averaged pattern has been calculated only from reasonably contaminated samples, in order to get as complete sets of single congener data as possible.

There is a clear distinction of patterns between the time intervals. The pattern for PCDD/F shows a peculiar subpattern for a certain set of samples analysed around begin of 2011. These samples could easily be identified via the characteristic ratio of PCDD/F-TEQ vs. 1,2,3,6,7,8-HexaCDD, being strongly correlated for these samples. From those samples the typical averaged pattern (figure 4) has been deduced. This pattern is clearly dominated by the presence of Hexa- and HeptaCDD, namely 1,2,3,6,7,8-HexaCDD which is also contributing dominantly towards the TEQ value.

In parallel with the egg samples, samples of the original technical oil related to the feed contamination have been analysed. The higher contaminated oil samples with PCDD/F concentrations at around 50-200 pg PCDD/F-TEQ/g exposed a typical pattern of PCDD/F (see figure 5). This pattern clearly corresponds to the peculiar pattern identified for the egg samples shown above, where only the relative contributions of Hexa-, Hepta- and OctaCDD are different, probably due to different accumulation/metabolism within the chicken.

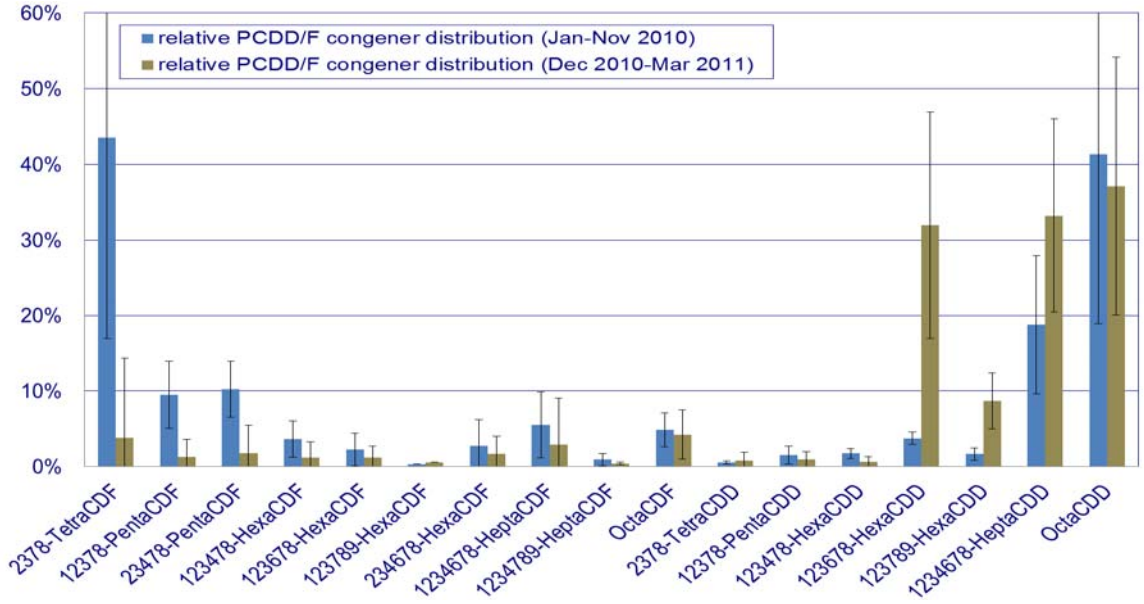

Figure 3: averaged PCDD/F pattern for egg samples before and during the dioxin crisis in 2010/2011. Pattern and standard deviation are given for samples above 1.5 pg PCDD/F-TEQ / g fat

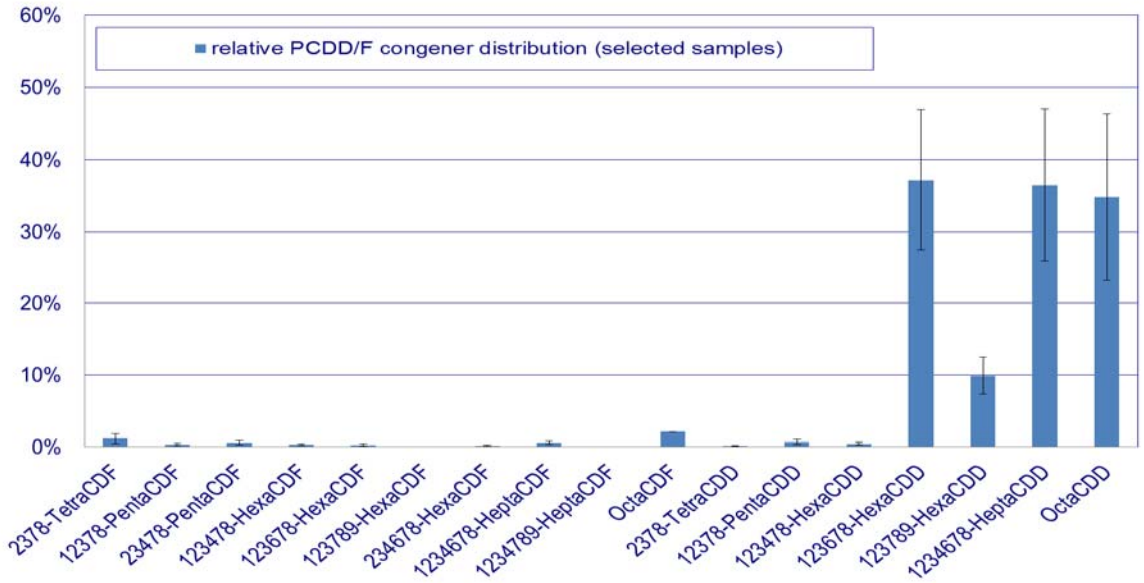

Figure 4: averaged PCDD/F pattern for selected 2011 egg samples showing the typical phenolic pattern. (selected samples above 1.5 pg PCDD/F-TEQ / g fat and high 123678-HxCDD:TEQ ratio)

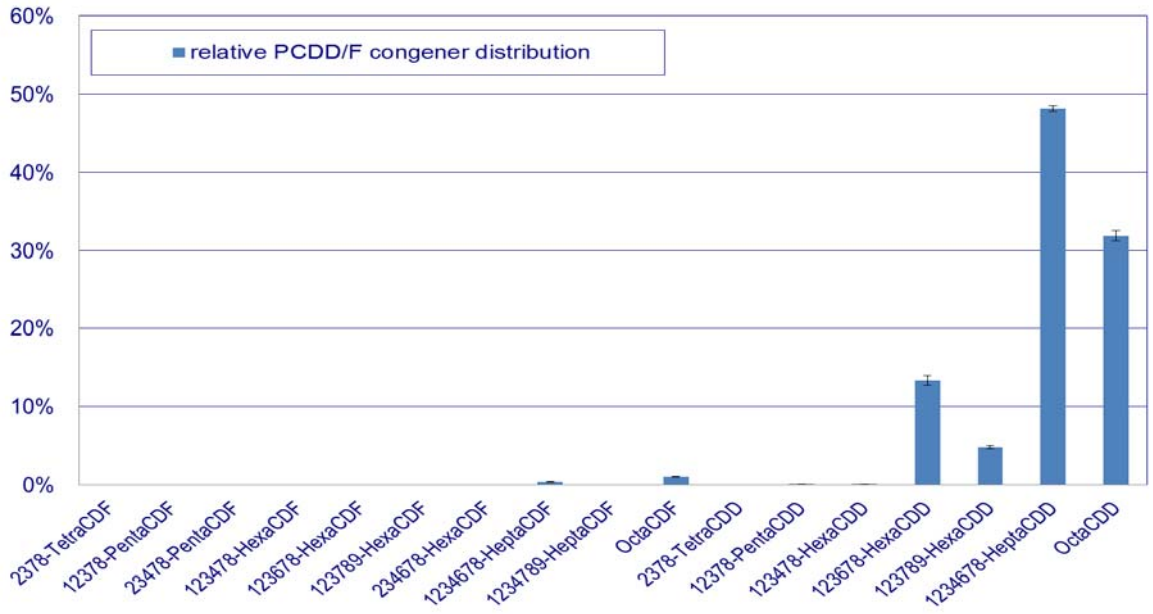

Figure 5: PCDD/F pattern for contaminated technical oils (n=5), related to the recent animal feed incident

One of the most important typical PCDD/F pattern is the pentachlorophenol pattern which is dominated by OCDD[5] formed by condensation of 2 PCP molecules. On the assumption of a mixture of Tetra- and Pentachlorophenols, the range of condensation products would cover the Hexa- through OctaCDD thus shifting the PCDD pattern maximum towards HeptaCDD, dependent on the exact chlorophenol ratio in the mixture. The most important detail about this chlorophenol influence from the point of view of the 2,3,7,8-substituted congeners is the pattern maximum formed by Hexa- and HeptaCDD and the dominance of 1,2,3,6,7,8-HexaCDD over the other two HexaCDD congeners with typical ratios of e.g. 1,2,3,6,7,8-HexaCDD:1,2,3,4,7,8-HexaCDD at 10:1 or higher.
Since we observe such a pattern within the above mentioned (figure 4) group of higher contaminated egg samples from begin 2011 this is a possible explanation for the PCDD pattern in these samples. Further evidence is present in the HRGC/HRMS chromatographic traces of the non-2,3,7,8-PCDD/F. Notably, the congener patterns of HeptaCDF and HexaCDD point toward the possible influence of chlorophenolic compounds, e.g. by showing a relatively high 1,2,3,4,6,8,9-HeptaCDF[6]. In combination this makes it likely to have the origin for the high concentrations of PCDD/F in the technical oils as well as in the chicken eggs in an original presence of chlorophenols. The presented data has yet to be refined in order to clarify the patterns and dependencies between the original oil and the PCDD/F distribution in chicken eggs, especially regarding the influence of 2,3,7,8-TetraCDD. Furthermore, there is an indication for further effects possibly from chicken metabolism in the PCDD/F pattern of analyses of chicken meat from the same period of time.

References:

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