

THE ANALYSIS OF MINERAL FEED FOR DIOXINS AND DIOXIN-LIKE PCBs: RESULTS AND CHALLENGES

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Introduction

The analysis of mineral material used especially for animal feed has become more and more important over the last decade, at the latest since several dioxin crises. The findings of PCDD/Fs in kaolinite bring into focus the total feed/food chain including mineral compounds leading to the intake of PCDD/Fs in humans. Since the introduction of maximum contents for PCDD/Fs and dioxin-like PCBs (dl-PCBs) for food and feeding stuff some years ago by the European Union, a huge number of samples have been investigated according to the prescribed methods, resulting in an enormous pool of data. These data demonstrate the actual need for ongoing observation of minerals and show as well that the analysis of mineral samples is still a challenge – as could be seen e.g. from the analytical issues with kaolinitic clay¹. Especially with regard to the re-evaluation of the EU limit values in 2008² it is valuable to examine the results of the performed analyses in terms of found concentrations, contribution of PCDD/Fs and dl-PCBs to the results and encountered analytical difficulties.

Materials and Methods

The data presented here are a collection of results from the analysis of mineral samples at the Eurofins | GfA dioxin competence centre in Hamburg from 2006 and 2007. All data are presented as maximum TEQ using the 1997 WHO-TEF calculation scheme and are calculated with 12 % moisture. The analytical method for mineral feed used in our laboratory is based on the requirements for reference analyses as laid down in the EU Directive 2002/70/EC³. After preparation, the samples were extracted using hot Soxhlet extraction with a mixture of Toluene/Acetone (90:10 v/v). The sample clean-up consisted of a multi-step column chromatography with a mixed silica column, a basic alumina column for fractionation and a Florisil column (other columns used in case of additional need). After concentrating the resulting final extracts, the instrumental analysis was performed on Agilent 5890/6890 GCs equipped with non-polar 60 m DB5ms-type columns and polar SP2331 columns in case of confirmational analysis. Detection was done by HRMS/SIR on Waters AutoSpec mass spectrometers at a mass resolution of $R \geq 10000$. The quantification method has been isotope dilution with every analysed compound (exception: 123789-HxCDD) having its own ¹³C₁₂-labelled internal standard added to the sample before extraction. Recovery rates have been determined by calculation against a set of ¹³C-labelled standards added before GC injection.

This method is a well-established “classical” routine method for PCDD/F and PCB-analysis which has been modified according to the recommendations issued by the European Community Reference Laboratory in Freiburg, Germany in 2006 concerning the analysis of mineral feed samples⁴. According to this recommendation, no acid digestion of the samples has been performed. Also, the extraction was modified by adding a reasonable percentage of polar solvent to the classical toluene extraction (see above).

Results

A total of 507 analyses has been included into this study for PCDD/Fs and in parallel a total of 420 analyses for dl-PCBs, the major part of which has also been analysed for PCDD/Fs. The project data for these samples have been reviewed in order to get information about their chemical identity, that is, to identify the metal constituent of the sample. This could be achieved for about 58 % (dl-PCBs: 46 %) of the samples. These samples contained aluminium, calcium, copper, iron, magnesium, manganese and zinc. Aluminium is represented as its silicate form of kaolinite which has its own category here due to the significant amount of samples. The remaining samples could only be generally identified as mineral feed. Table 1 shows the analytical results and their distribution for PCDD/Fs and dl-PCBs.

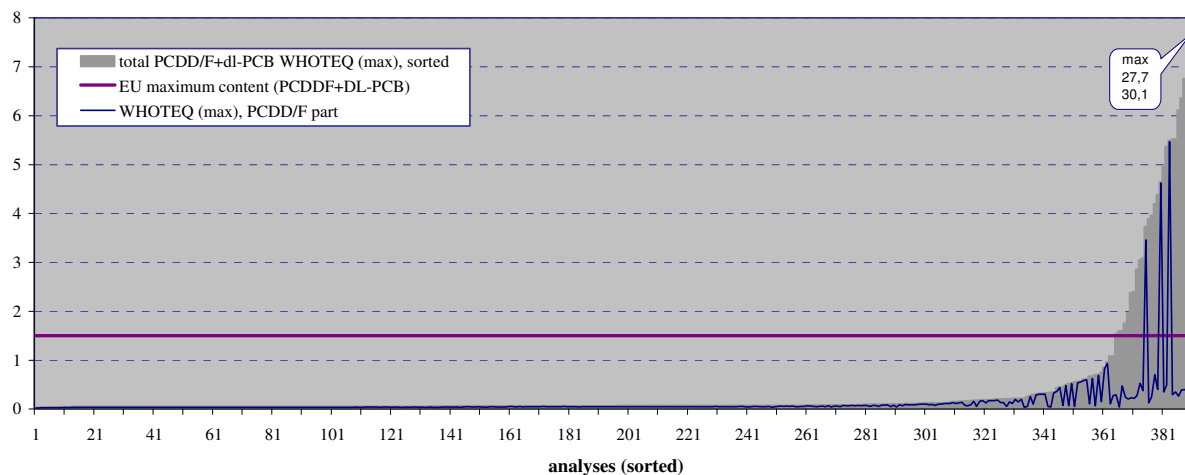
Table 1: Results of the analysis of mineral feeding stuffs (ng WHO-TEQ/kg calculated with 12 % moisture)

PCDD/F	n	average	median	max	min	% > EU action	
						thrsh.	maximum values
mineral (not assigned)	213	0.124	0.046	3.458	0.021	3.8%	1.9%
Calcium	13	0.058	0.036	0.197	0.023	---	---
Copper	87	0.089	0.046	0.545	0.025	1.1%	---
Iron	28	0.039	0.035	0.060	0.027	---	---
Kaolinite	53	0.527	0.214	7.148	0.039	26.4%	9.4%
Magnesium	11	0.152	0.091	0.596	0.034	9.1%	---
Manganese	36	0.530	0.037	5.528	0.033	13.9%	11.1%
Zinc	66	0.142	0.044	1.640	0.023	9.1%	4.5%
total	507	0.186	0.046	7.148	0.021	6.9%	3.2%
dl-PCB	n	average	median	max	min	% > EU action	
						thrsh.	maximum values
mineral (not assigned)	192	0.554	0.023	28.378	0.007	11.5%	9.9%
Calcium	15	0.057	0.018	0.598	0.012	6.7%	6.7%
Copper	81	0.559	0.024	6.651	0.011	12.3%	12.3%
Iron	27	0.020	0.018	0.030	0.014	---	---
Kaolinite	25	0.021	0.018	0.035	0.017	---	---
Magnesium	10	0.023	0.021	0.044	0.017	---	---
Manganese	29	0.021	0.018	0.055	0.017	---	---
Zinc	41	0.023	0.018	0.104	0.012	---	---
total	420	0.370	0.022	28.378	0.007	7.9%	7.1%

Compared with the EU action thresholds of 0.5 ng WHO-TEQ/kg for PCDD/Fs and 0.35 ng WHO-TEQ/kg for dl-PCBs⁵, the major part of the results is lower, though about 7 % of the PCDD/F-results are above the threshold (dl-PCBs: 8 %) and about 3 % of the PCDD/F-samples even exceed the EU maximum contents (dl-PCBs: 7%). Looking at the results of the single compound groups will reveal significant differences between them. Of special interest are the high results of dl-PCBs in copper-containing samples, whereas the PCDD/Fs are highest in kaolinite samples and manganese samples (manganese oxide).

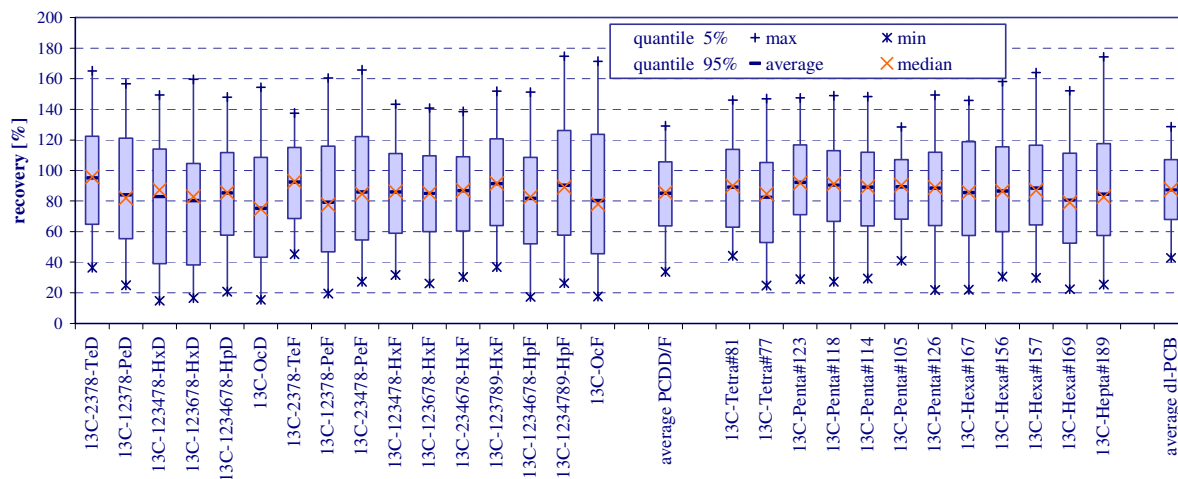
Figure 1 shows the totals of PCDD/F+dl-PCB-TEQ of samples analysed for both parameters, sorted after concentration and compared against the combined EU maximum content for the two groups. It also shows the relative contribution of PCDD/Fs and dl-PCBs to the TEQs, indicating that *either* the dioxins *or* the PCBs contribute the major part to the total level of contamination. This coincides with the different possible sources of both groups of chemicals.

Figure 1: Sorted total TEQ results and contribution of PCDD/Fs and dl-PCBs to the total TEQs of mineral feed (ng WHO-TEQ/kg, 12% moisture)



Where results for the content of dioxins or dioxin-like compounds demonstrate the need for constant investigation or source analysis, recovery rates for internal standards reflect (beside others) that the matrix-inherent analytical difficulties are still extant. Fortunately, the isotope dilution analysis as it is performed in our laboratory according to recent regulations and methods, is, compared to other techniques, more insensitive to many effects of sample preparation and clean-up. Figure 2 gives the recovery rates for the applied $^{13}\text{C}_{12}$ -labelled PCDD/Fs and dl-PCBs for the analysed mineral samples.

Figure 2: Recovery rates for $^{13}\text{C}_{12}$ -PCDD/Fs and $^{13}\text{C}_{12}$ -dl-PCBs quantification standards



The averages reach around 85 % for $^{13}\text{C}_{12}$ -PCDD/Fs and 87 % for $^{13}\text{C}_{12}$ -dl-PCBs with 88% ($^{13}\text{C}_{12}$ -PCDD/Fs) and 93 % ($^{13}\text{C}_{12}$ -dl-PCBs) of the recoveries lying in the range of 60-130 % which is a satisfying value for routine. Also, considering the different sample types, there are mainly satisfying recoveries for $^{13}\text{C}_{12}$ -PCDD/Fs as well as for $^{13}\text{C}_{12}$ -dl-PCBs, as can be seen from figure 3.

Nevertheless, there are still some cases which suggest that certain matrices give typical and selective patterns of recovery losses. Apart from the generally good analytical quality, some individual classes of compounds revealed sometimes poor recoveries for $^{13}\text{C}_{12}$ -PCDD/Fs, going down to about 20 %, in this case not covered by usual laboratory problems. There is a clear tendency of manganese and magnesium samples (especially oxides) to behave like that. These low recovery rates are fortunately not of great relevance for the TEQ values since they occur mainly for the high chlorinated compounds as can be seen in figure 4. No similar effect could be observed for $^{13}\text{C}_{12}$ -dl-PCBs.

Figure 3: Recovery rates of $^{13}\text{C}_{12}$ -labelled PCDD/Fs and dl-PCBs in dependence of the sample type

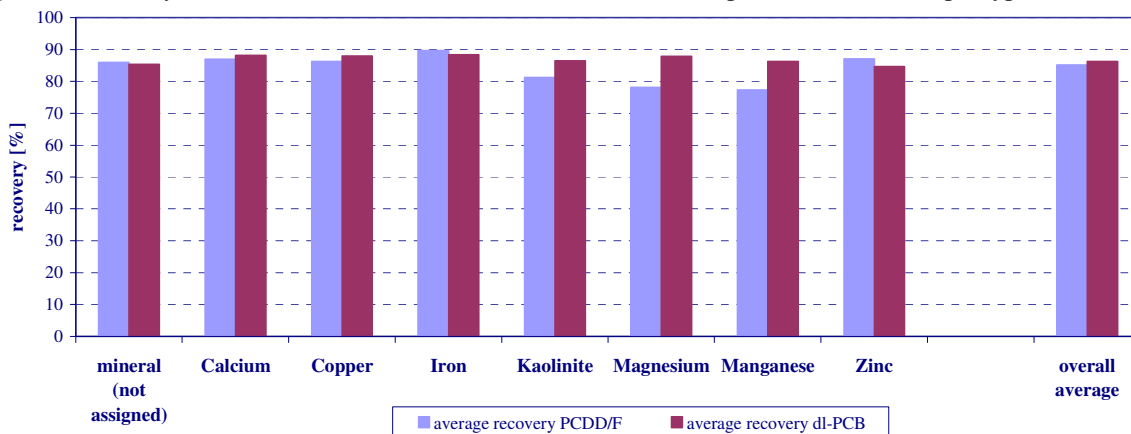
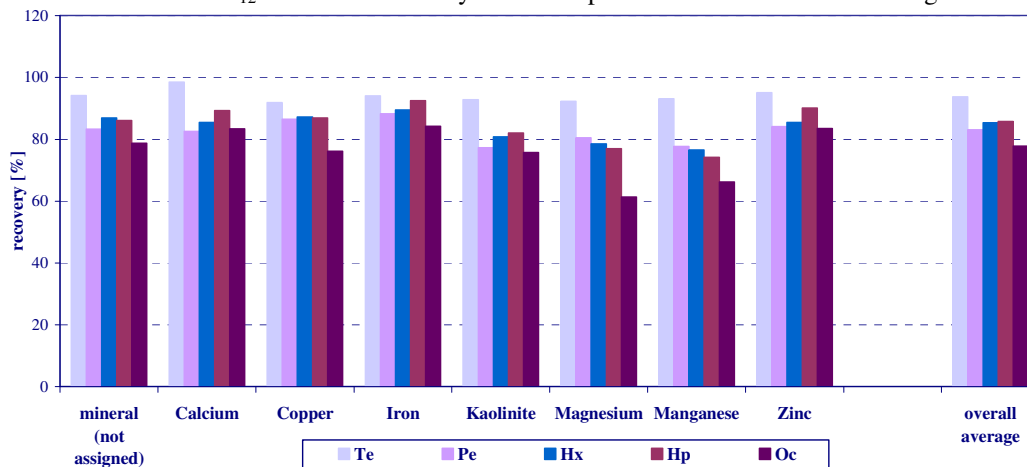


Figure 4: Distribution of $^{13}\text{C}_{12}$ -PCDD/Fs-recovery rates in dependence of the chlorination degree



Discussion

The chemical identity of a sample is hard to determine for a dioxin laboratory as can be seen from the high number of samples generally labelled as *mineral feed*. The identified samples are not necessarily representative for the whole range and distribution of mineral compounds used as feeding stuff but having this statistical reasonable number of data sets at our disposal allows us to draw some important conclusions. First, it has been possible to identify compounds bearing higher contamination risks. The given data sets point out that there is a necessity for a closer discussion especially of copper salts with regard to their relatively high dl-PCB contents of 0.56 ng WHO-TEQ/kg on average with a maximum value of 6.7 ng WHO-TEQ/kg. For the PCDD/Fs, kaolinite is still critical as could be expected, whereas, most interestingly, some manganese and zinc minerals exhibit higher findings with maxima of 5.5 and 1.6 ng WHO-TEQ/kg. On the other hand, minerals containing iron, calcium and magnesium do not show exceptionally high results. Generally, the significant number of results above the EU action thresholds clearly indicates the constant need of investigation.

The second conclusion is that there are still specific analytical problems to be discussed. From the viewpoint of analytical performance, there is a clear need for further work on the extraction/recoveries of the oxides of manganese and magnesium. A recent step in this direction has been done with the recommendation of the CRL Freiburg to extract metal oxide-containing feeding stuffs with pure toluene instead of solvent mixtures⁶.

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