Organohalogen Compounds, Volume 60, Pages 13-16 (2003)

Comparison of Methods for Extraction of PCDD/F from Kaolinitic Clays and Copper Oxide in View of European Feedingstuff Limits

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Introduction

Within a chemical analysis scheme it is the primary purpose of the extraction step to achieve quantitative extraction yields of the target compounds. Besides this, the amount of interfering co-extracting compounds should be kept as low as possible. In general, crucial parameters for extractions are the kind of pretreatment, the water content, type of solvent and the detailed extraction conditions.

That drying of samples prior to extraction can represent a crucial step is well known from residue analysis of organo chlorine compounds. So, for sediments e.g. it was described that complete drying of samples can lead to problems due to a kind of aging effect of the sediment resulting in reduced extraction yields[1]. This phenomena was explained by rearrangements in the inter-lamella space of clay minerals and encapsulating of the target compounds in that space[2] where they are not easily available for extraction. Addition of water generally facilitates desorption of compounds by deactivation of the particle surface. Additionally, inclusion of a polar solvent at the extraction can completely wet particle surface and therefore remove target compounds more efficiently. As drying of samples is a widespread method in dioxin analysis, the influence of this step on extraction efficiency has to be considered. Besides extraction efficiency however, also any alterations in the compound composition such as changes in the isomer patterns have to be taken into account as part of the QA/QC protocol. It has to be excluded that the extraction method is capable to modify compound composition (e.g. by use of increased temperatures).

Both, extraction efficiency and possible alteration in compound composition are of particular importance in view of the current European regulations for food and feedingstuff control. The European Community (EC) has set maximum levels (limits) for dioxins in feedingstuffs which are valid since 1st July 2002[3]. Additionally, action levels were set about 25 – 40 % below the corresponding maximum levels[4]. Samples exceeding the action level should be investigated to identify an additional source of contamination. Samples exceeding the maximum levels should be excluded from the market. Furthermore European Commission Directives 2002/70/EC[5] and 2002/69/EC[6] for feedingstuffs and for foodstuffs, respectively, include general acceptance criteria for analytical results. However, at present criteria for extraction efficiency are not included. Therefore, this paper compares different extraction methods for two selected types of samples, clay minerals and metal oxides, both relevant in this context as feedingstuff supplements.

Methods and Materials

A ***clay mineral sample (triple-layer silicate)*** and some ***trace element supplements (copper oxide, zinc oxide)*** were chosen. Besides usual soxhlet extraction (24 h, toluene, appr. 5 * 20 cm glass tube) accelerated solvent extraction (Dionex ASE 200) was used for the clay sample. For the latter, 30 g of clay was taken after a 24 h wetting step with water (20 % weight[1], method “ASE+H2O”). ASE uses also toluene as solvent at 180 °C and 140 bar pressure, 5 min static time for two static cycles, a flush volume of 60 % of the extraction cell volume and a purge time of 120 seconds.

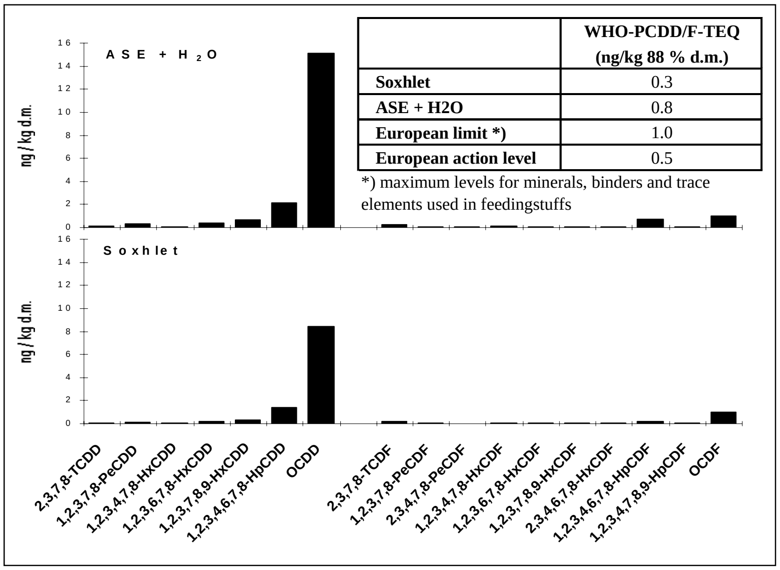
As a supporting investigation two ***soil*** samples from a round-robin test were taken to additionally compare soxhlet extraction (8 h in this case) with an extraction using a mixture of ethanol and toluene in a hot extraction device according to Twisselmann (8 h).

Three ***copper oxide*** samples of different origin and a ***zinc oxide*** sample was extracted by 24 h soxhlet extraction using toluene (30 g of sample). Again, supporting investigations have been done with copper oxide including toluene and ethanol/toluene extraction in the Twisselmann device and additionally a cold extraction at room temperature by means of shaking (30 min) and subsequent ultrasonic treatment (15 min). Cold extraction were executed as a single, triplicate and five-fold extraction for comparing purposes.

Clean-up procedure include acid/base silicagel and an alumina column[7] for the clay and metal oxide analyses, measurements were executed at a Varian GC 3400 using the DB-Dioxin column (30 m x 0.25 mm i.d., 0.15 µm film thickness) combined with a Finnigan MAT 90 HRMS. Additional investigation of soil and copper oxide samples used also a silicagel based cleanup and a florisil and Carbopack B step followed by HRGC/HRMS at a VG Autospec.

Results and Discussion

Figure 1 shows the results for the **clay sample**. With toluene as solvent, “ASE+H_2O” method resulted in a WHO-PCDD/F-TEQ level more than a factor of 2.5 higher in comparison to the usual soxhlet method showing that the latter method provides no sufficient extraction for this kind of samples. The results support the theory that deactivation and widening of silicate layers by addition of water makes those PCDD/Fs available for extraction initially encapsulated between the silicate layers. Considering the European maximum and action levels, it is obvious, that different extraction methods can lead to different results and thus can result in different evaluations: while the soxhlet results are below the European action level and thus representing a kind of “background level”, the “ASE+H_2O” results exceed the action level. Such different results and evaluations for an identical sample have occurred as the manufacturer of the described clay sample ordered analysis at different laboratories using different methods. Consequently, the manufacturer asked which is the “appropriate” method and evaluation for his product.



	WHO-PCDD/F-TEQ (ng/kg 88 % d.m.)
Soxhlet	0.3
ASE + H2O	0.8
European limit *)	1.0
European action level	0.5

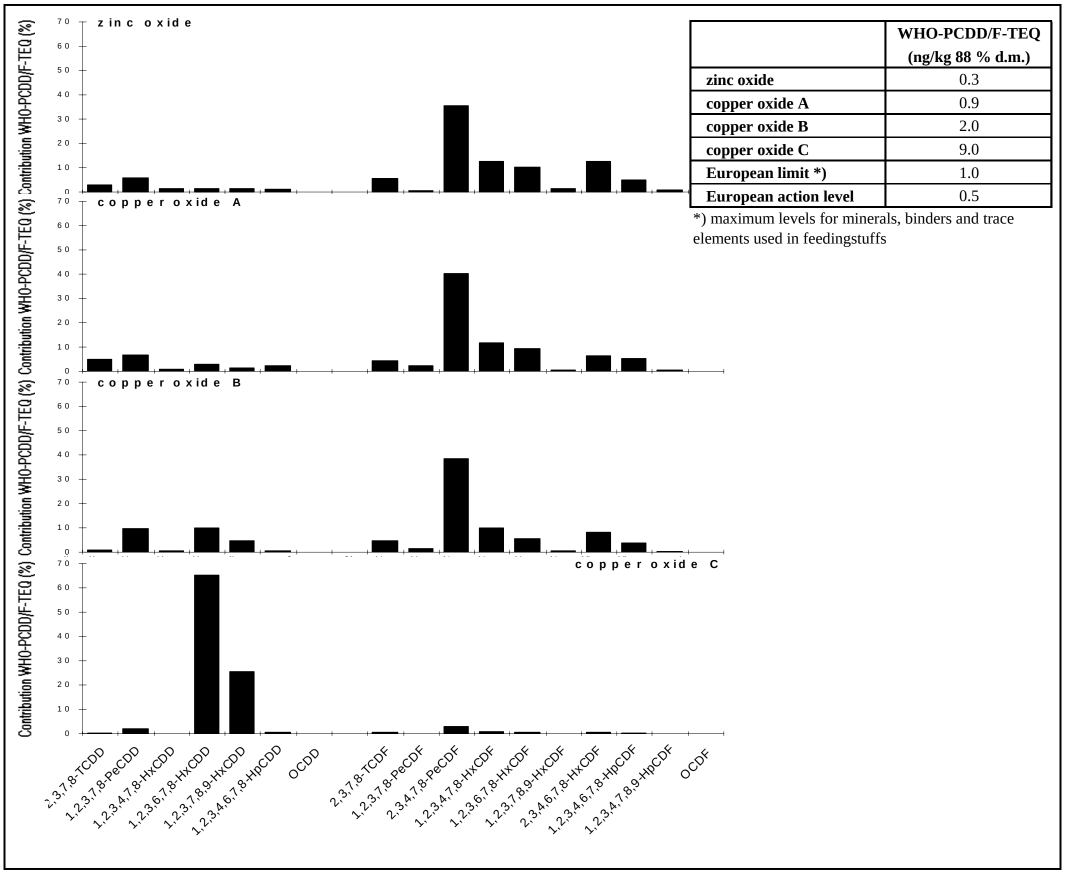
*) maximum levels for minerals, binders and trace elements used in feedingstuffs

Figure 1: PCDD/F levels in a clay sample: comparison of soxhlet and ASE+H_2O method.

A further indication for an incomplete soxhlet extraction with toluene are shown by the results of **soil** analyses at CVUA, Freiburg. Extraction with ethanol/toluene resulted in higher levels for nearly all congeners in comparison to the toluene method. It should be noticed at this point, that also ASE extraction resulted in significant higher extraction efficiencies in this case[8].

The ethanol/toluene mixture has also proved in a certification study for **milk powder** to result in a more effective extraction of lipids in comparison to non polar solvents[9]. Here, ethanol/toluene helps to solve the particular problem to extract milk fat quantitatively from homogenized dried milk products.

A completely different problem could be observed with different extraction methods of trace elements (**copper oxide, zinc oxide**). Figure 2 shows the results for the trace element supplements and the contribution of individual congeners to the WHO-PCDD/F-TEQ for each sample (“relative pattern”). While the zinc oxide was clearly below the corresponding European limit and action level, higher PCDD/F levels could be detected for three samples of copper oxide with sample B and C significantly exceeding the maximum limit. Looking at the “relative pattern” sample C showed an increased contribution of two hexa-chlorinated dioxins to the TEQ, while all the other oxides showed quite comparable patterns with a dominant contribution of 2,3,4,7,8-PeCDF.



	WHO-PCDD/F-TEQ (ng/kg 88 % d.m.)
zinc oxide	0.3
copper oxide A	0.9
copper oxide B	2.0
copper oxide C	9.0
European limit *)	1.0
European action level	0.5

*) maximum levels for minerals, binders and trace elements used in feedingstuffs

Figure 2: PCDD/F levels in trace element supplements: zinc and copper oxide of different origin

Internal QA/QC protocol of the analyzing laboratory requires additional measures in such cases (e.g. pattern deviations) in view of a reliable evaluation of the product and in order to meet EC dioxin strategy intention to identify further sources of dioxin input into the food and feedingstuff chain. Being aware of the catalytic properties of copper considering dioxin formation, the samples were analyzed for chlorinated phenols and benzenes, and significant amounts of chlorinated phenols in copper oxide C could be detected. Therefore, dioxin formation from phenolic precursors during copper oxide processing could be a reason for the different pattern of sample C. Furthermore, even an additional formation during analytical dioxin extraction under usual extraction temperatures could not be excluded. Therefore, further detailed studies have been initiated prior to a final evaluation of the corresponding copper oxide sample. As part of these studies, another copper oxide sample was re-analyzed to test a possible dioxin formation during the extraction. However, extraction with ethanol/toluene in the “Twisselmann” hot extraction device and extraction with toluene showed comparable results (6.3 ng WHO-PCDDD/F-TEQ/kg with ethanol/toluene extraction and 6.8 ng WHO-PCDDD/F-TEQ/kg with toluene extraction). During the extraction, the matrix is permanently extracted with hot solvents. Therefore, in addition, an extraction at room temperature with toluene using an ultrasonic device and shaking was performed. The results of extraction at room temperature confirmed the results of hot extraction. Therefore, the chlorophenols in copper oxide did not cause formation of dioxins.

Conclusions

Results have shown that extraction efficiency can be a critical parameter in dioxin analysis in some matrices, with significant influence on the product evaluation according to regulations. Thus, extraction efficiency should be regarded as a important parameter. In view of a correct product evaluation additional QA/QC measures should be included if exceptional qualities are found in samples (e.g. unusual patterns or levels).

Acknowledgement

We like to thank those who reliably did the analysis: at CVUA Freiburg, Mrs Renate Tritschler and Mr. Helmut Winterhalter and at Oekometric Ms. Regine Becker and Mr. Mohammed Rachidi.

References

1. Malisch, R.(1981): Ph.D.-Thesis. University of Muenster, Germany.
2. Adams, R. S. Jr.(1973): Residue Rev. 47, 1-47
3. European Commission (2001): Council Directive 2001/102/EC. Official Journal of the European Commission, L 6, 45-49
4. European Commission (2002): Commission Recommendation 2002/201/EC. Official Journal of the European Commission, L 67, 69-73
5. European Commission (2002): Council Directive 2002/70/EC. Official Journal of the European Commission, L 209, 15-21
6. European Commission (2002): Council Directive 2002/69/EC. Official Journal of the European Commission, L 209, 5-14
7. Horstmann M., Kaune A., McLachlan M.S., Reissinger M., Hutzinger O. (1992): Chemosphere, 25, 1463-1468
8. Umweltbundesamt (1999): Bund/Laender Arbeitsgruppe DIOXINE UAG „Referenz-messprogramm“, Ringversuch „Dioxine im Boden“. Final Report

9 European Commission, BCR information “Reference Materials” (1996): „The Certification of the contents of five PCDD and six PCDF in milk powder“, CRM 607