



Determination of dioxins in mineral feed: Recommendation for extraction procedures

Extraction procedures for determination of dioxins and dioxin-like PCBs in products of mineral origin vary widely. They comprise methods using different organic solvents of varying polarity, different techniques (e.g. Soxhlet or ASE), different approaches to moisten dried products before extraction and different approaches to digest mineral products with HCl before extraction, if applied at all. It has been shown that recoveries of internal standards and dioxin results can vary considerably depending on the extraction procedure. This can cause severe problems if an analytical result has to be compared with maximum levels as fixed for feedingstuffs. Therefore, harmonizing extraction procedures for mineral feeds is of high priority for the Commission.

As a starting point for a comprehensive study, sepiolite has been chosen. Sepiolite is a hydrated magnesium silicate which is destroyed only under strong acidic conditions. It has been shown that very drastic conditions, e.g. 12 n HCl under reflux overnight, are necessary to completely destroy the crystalline structure, leading to an amorphous material. When sepiolite is exposed to a soft hydrochloric acid, e.g. 0.3 n HCl overnight, its crystalline structure remains unchanged. Only the calcite and dolomite phases contained in some natural sepiolite products are affected. Even stirring with 3 n HCl for 1 h at room temperature does not significantly destroy the sepiolite's crystalline structure, although these conditions are much stronger than usual digestive conditions.

Some minerals show exothermal reactions when treated with HCl. Application of external heat or exothermal reactions producing high temperatures might raise the question of formation of dioxins from precursors, in particular if possible catalysts (such as copper) are present. The issue of dioxin formation as result of high temperature during extraction can be avoided (i) by use of an ice bath if exothermal reactions occur and (ii) by not using quite extreme digestion conditions such as boiling with concentrated acids under reflux.

These examples demonstrate the complexity of the issue of treatment with hydrochloric acid before extraction. The issue becomes even more complicated if other organic or inorganic acids are taken into consideration.

Moistening of dried sepiolite products is found to increase recoveries of internal standards. The increase of recoveries for internal standard has to be compared carefully with corresponding changes of results for native dioxins.

After harmonization of the extraction procedure for mineral feeds, the question has to be answered whether this procedure has to be applied to premixtures and compound feeds containing the mineral feed to a certain extent, as well.



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With this background, the Community Reference Laboratory (CRL) for Dioxins and PCBs in Feed and Food has performed a comprehensive study with internal contribution (parameters being tested at the CRL) and external support (harmonization of parameters in cooperation with National Reference Laboratories [NRLs] and expert laboratories in this field). On 12 October 2006, a workshop „Extraction method for dioxins in feed with high mineral content“ was organized by the CRL. The following results provide a basis for the final conclusion:

- Toluene or other non-polar solvents are not suitable for extraction.
- A mixture of polar and non-polar organic solvents is necessary for extraction, regardless whether ASE or Soxhlet extraction is applied.
- No significant difference between extraction with a mixture of toluene and a polar solvent and pretreatment with 0.3 n HCl was observed.
- HCl > 0.3 n does not reflect physiological conditions and thus bioavailability.
- Therefore, higher dioxin levels after extraction with HCl > 0.3 n are not considered relevant.

These results were summarized in the following **conclusions**:

- **Toluene mixed with a polar solvent (e.g. ethanol, acetone, isopropanol) is the best suitable extraction medium.**
- **For the mixture, a substantial fraction of each solvent is required.**
- **This recommendation, derived from the results of the sepiolite study, is extended to all mineral feeds, also including products containing mineral feeds (premixes and compound feeds).**

Participants of the workshop concluded that the following aspects should be addressed in the future work programmes:

- Other feed additives have to be studied, in particular trace elements, e.g. Mn and Cu:
 - First, different trace elements (eg Mn, Cu, Zn, Fe,) should be prechecked.
 - It might be important to include several products for a certain trace element because different chemical properties might have to be taken into account depending on the kind of product (and batch) associated with a certain trace element.
 - An important screening test to be performed would be the comparison of two methods: direct extraction with toluene/polar solvent and extraction with toluene/polar solvent after pretreatment with 0.3 n HCl.
- After selection of (a) suitable trace element(s), the „full sepiolite programme“ should be applied.



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- For sepiolite, Mn and Cu: Comparison of mixtures of toluene with different polar solvents for direct extraction (methanol, ethanol, acetone)
- For sepiolite, Mn and Cu: Comparison of mixtures of toluene with acetic acid in increasing proportions for the latter
- Tests with sepiolite for „bioavailability“, e.g. simulation of in vivo-conditions with stomach (gastric) juice, if possible
- Inclusion of compound feeds
- Inclusion of PCBs as parameters

Final conclusions for mineral feed (additives) will be drawn on the basis of more comprehensive data obtained by future work programmes in which these suggestions are to be tested.

These observations, conclusions and recommendations were formally adopted at the CRL/NRL workshop on 13 October 2006 in Freiburg.

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