

## Modified Clean-up Procedure for PCDD/PCDF under Toxicological, Ecological and Economical Aspects

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### 1. Introduction

From 1984 to 1987 clean up procedures were developed for various types of relevant matrices. In our laboratory validated protocols have been developed, using a number of "building blocks" as clean-up steps for all types of matrices, from sewage sludge to human samples or chemical products [1]. During the last eight years we have successfully applied these procedures for several thousand dioxin analyses and a number of other dioxin laboratories have adopted and validated these procedures. There were two reasons for us to investigate the possibilities to modify the existing clean-up protocols: substitution of toxicological and ecological relevant solvents and minimizing the use of solvents and adsorbents with a simultaneous saving of time.

The following changes compared to previously described clean-up procedures are described:

- substitution of hexane with heptane and dichloromethane with ethyl acetate,
- treatment of samples with sulfuric acid at elevated temperatures and
- the introduction of a carbon column using a special activated coke.

The combination of these changes allow in most instances a considerable miniaturization of the clean up procedure under improved toxicological and ecological conditions.

The previously described "selective" separation of 2,3,7,8-TCDD from other PCDD/PCDF was reinvestigated with the modified Alumina B Super I "for Dioxin-Analysis". It could be shown that not only 2,3,7,8-TCDD but all 2,3,7,8-tetra- and pentaCDD/CDF can be selectively separated from the other PCDD/PCDF, allowing an unequivocal identification of these congeners in difficult to analyze samples. This procedure is described in detail elsewhere.

### 2. Experimental

The various "building blocks" described here were validated with over 100 fly-ash and stack gas samples from a municipal waste incinerator (1), over 50 soil samples (2), 15 stack gas samples from wood combustion (3) and several sewage sludge and compost samples. The only difference consists in the pre-treatment of the samples.

Fly-ash samples are spiked with <sup>13</sup>C-labeled standards and extracted with a mixture of toluene, ethoxyethanol and hydrochloric-acid for 18 h. The extract is then washed several times with water and dried over sodium sulfate.

Soil samples, stack gas samples, sewage sludge and compost samples are also spiked with <sup>13</sup>C-labeled standards and Soxhlet extracted with toluene for 18 h. The samples (1), (2) and (3) can be treated in the same way: In all three cases the samples are concentrated by rotary evaporation and solvent exchanged to n-hexane or n-heptane.

# ANA/BIOA

## *Heat treatment with sulfuric acid*

Sulfuric acid treatment as a clean-up step in dioxin analysis has to our knowledge first been mentioned by Firestone et al.[2]. Sulfuric acid is either used as such or adsorbed on silica or other inert adsorbents. We investigated the optimum conditions for an improved sample treatment with sulfuric acid at elevated temperatures removing most of the matrix. It was found that treatment at 70°C for 20 min. with sulfuric acid alone or sulfuric acid adsorbed on silica gave the best results as far as removal of matrix and prevention of selective losses of PCDD/PCDF are concerned.

Three different experimental approaches were tested:

- a) addition of 25 - 50 g of a mixture of 44% sulfuric acid and 56% silica (w/w) to a solution of the sample extract in heptane, removal of the solvent, and treatment of the dry residue at 70 °C for 20 min..
- b) addition of 25 - 50 g of a mixture of 44% sulfuric acid and 56% silica (w/w) to a solution of the sample extract in hexane (b.p. 70°C) and refluxing the mixture for 20 min.. The hexane solution is decanted and the residue washed twice with hexane or heptane.
- c) the sample extract in 2-3 ml of n-heptane is transferred to a 5 ml screw cap vial. After addition of 1-2 ml of sulfuric acid and vigorous mixing the vial is heated in a heating block at 70°C for 20 min. (there has never been observed a build up of pressure in the vial under these conditions). During the heating period the vial is shaken 3 to 4 times. After separation of the layers, the organic layer is removed and the sulfuric acid layer washed at least once with heptane.

It was found that the options b) and c) resulted in the most effective removal of matrix and especially of polycyclic aromatic hydrocarbons when present in high concentrations. Option c) is always used when relatively small amounts of organic matter is present in the extract. This is the case in all types of emission samples (but not if PUF is used as an adsorbent), various types of filter dust samples, soil samples etc.. Option b) is used whenever a considerable amount of organic matter is present in the extract, as is e.g. the case in sewage sludge and compost samples. For sewage sludge it might even be necessary to remove part of the matrix by treating the hexane solution first with cold sulfuric acid (100 ml) in a separatory funnel, avoiding any shaking.

The option a) has been used in our laboratory for more than 3 years, but has now been replaced by the options b) or c) due to their superior effect on matrix removal.

The treatment with the sulfuric acid at elevated temperatures was tested for up to two hours and temperatures up to 80°C. Under the conditions described no selective losses of PCDD/PCDF congeners occurred. With various mixtures of silica and oleum considerable losses due to matrix effects were observed and no reliable general procedure without such losses could be derived. We therefore can not recommend the use of oleum for clean up of PCDD/PCDF.

## *Substitution of solvents*

Since n-hexane is considered to be about 10-times more toxic than n-heptane it was studied whether n-hexane can be replaced by n-heptane in previously described clean-up procedures. In all "building blocks" tested hexane can indeed be replaced by n-heptane without any further alteration in the procedure. The only step where we use hexane today is in the sulfuric acid treatment under reflux due to the boiling point of n-hexane of about 70 °C.

We further studied the replacement of dichloromethane by other solvents in the clean up step that uses basic alumina. It was found that as long as at least 2 g of alumina are used,

dichloromethane can be replaced by ethyl acetate. The mixture of n-heptane/CH<sub>2</sub>Cl<sub>2</sub> (98:2) can be replaced by n-heptane/ethyl acetate (99:1), using half the volume and the mixture n-hexane/CH<sub>2</sub>Cl<sub>2</sub> (1:1) can be replaced by n-heptane/ethyl acetate (9:1), using also half the volume (see Figure 1).

#### *Carbon column*

Samples which are not sufficiently clean for GC-MS analysis after the described sulfuric acid treatment and a subsequent chromatography on Alumina B Super 1 for Dioxin Analysis (ICN Biomedicals, Wesel) are subjected to a clean up procedure on a specific type of activated carbon which allows good adsorption and effective desorption in a relatively small volume of toluene. The carbon used is carbonized lignite (Braunkohlekoks, produced by Rheinbraun Brennstoff GmbH, Köln). The carbon column is prepared as follows:

A glass column (length 10 cm, ID 10 mm) with mounting ends on both sides, is consecutively filled with a plug of quartz-wool, 500 mg of a mixture of silica/activated carbon 10:1 and another plug of quartz-wool. Carbon and silica have to be mixed thoroughly. The carbon column is pre-washed with a few ml of n-heptane.

The n-hexane or n-heptane fraction obtained after the heat treatment with sulfuric acid is placed directly on the carbon column. The carbon column is then washed with 10 ml of ethyl acetate. This removes the ortho-substituted PCB, sulfur, fat and fatty acids and similar interferences from the carbon column. The PCDD/PCDF and non-ortho substituted PCB are finally eluted from the carbon column by backflushing the column with 5 ml hot toluene. With a specifically designed column heater 6 samples can be run in parallel.

#### *The Micro Alumina Column*

For those samples for which a sulfuric acid treatment according to method c) (see above) is sufficient or which have been subjected to the carbon column, it was found, that the clean up step on Alumina B Super 1 can be considerably miniaturized. A Pasteur-pipette filled with 0,8 g of Alumina B Super 1 for Dioxin Analysis (Micro Alumina Column) is sufficient. The alumina is pre-washed with n-heptane. The n-heptane fraction is concentrated to 1-2 ml and transferred to the micro alumina column. The column is eluted with 2 ml n-heptane/ethyl acetate 99:1 and 5 ml n-heptane ethyl acetate 9:1 (or alternatively with 2 ml of heptane/dichloromethane 87:13 and subsequently with 5 ml heptane/dichloromethane 1:1). The 9:1 fraction (or 1:1 fraction respectively) contains the dioxins, the furans and the coplanar PCBs.

For all the fly ash, stack gas and soil samples analyzed the sulfuric acid treatment according to method c) and chromatography on the Micro Alumina Column was sufficient for analysis both by low and high resolution MS.

### **3. Results and Discussion**

The modified clean-up procedures described are presented schematically in Figure 1. Depending on the sample matrix two different approaches are used. With samples containing only relatively small amounts of toluene extractable material, like fly ash, stack gas and soil samples, the clean up of the extract can be carried out with as little as 20 ml of non-chlorinated solvent, which allows for an extremely economic clean-up, taking also toxicological and ecological aspects into account. The recovery rate was in every case more than 70%.

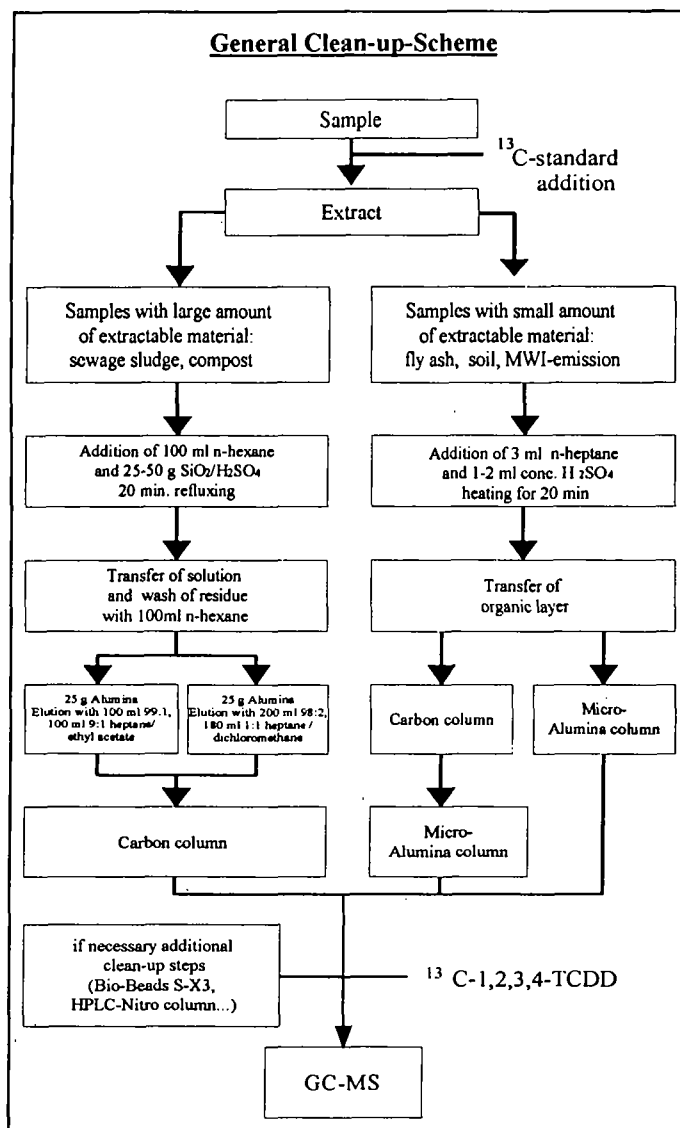


Fig. 1

#### 4. References

- [1] VDI-Berichte 634, 61-89 (1987).
- [2] J. of the AOAC 54 (6), 1293 (1971).