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EXTRACTION OF DIOXIN-LIKE COMPOUNDS – ARTEFACTS IN THE FIRST INTERLABORATY COMPARISON OF BIOASSAYS AND CHEMICAL ANALYSIS

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Introduction

Bio-analysis of dioxin-like compounds is an important tool for a comprehensive analysis of environmental matrices (SCHWIRZER et al. 1998), foodstuffs (HOOGENBOOM 2002) and substrates of technical origin (SCHRAMM 2000). In addition, the molecular mechanism for dioxin-like compounds working in dioxin bioassays allows the comparison with data generated by chemical analysis in a concentration additive manner (SCHRAMM 2002). Uncertainties of both methodological approaches and their comparison were elaborated recently. In consequence the first inter-laboratory study on PCDD/F-determining bioassays was announced and executed (DIOXIN 2002). Preliminary conclusions have been drawn by the advocates and critics of bio-analytical methods for the determination of PCDD/F, WHO-PCB and related compounds. In the intercalibration study, fish and fly ash was used. The fly ash originated from former intercalibration studies of the executing body.

Materials and Methods

The extraction of the fly ash and the fish was done employing ASE technique and a mixture of hexane-acetone or toluene in GSF. This method was used successfully for other matrices including internal and external Reference Materials. The internal comparison of the fish and the fly ash results showed a satisfying agreement between chemical data and bioassay data . ASE was performed on an ASE200 instrument with 2 g sample mixed with diatomaceous earth with two static cycles of 10 min each using a pressure of 120 bar and a temperature of 120 degree Celsius. Fly ash B and C originated from a former intercalibration study (van Bavel 2002). The fly ash was acidified according to standard procedures with hydrochloric acid. The eluent was either toluene or a mixture of acetone:n-hexane 25:75(v/v). Labelled standards were added to the cartridge prior to extraction. The bioassay procedure is described in detail elsewhere (ENGWALL et al 2003). Chemical analysis??

Results

The dioxin bioassay intercalibration study 2002 showed that for the fish and the fly ash extract there was a good agreement between GSF chemical data and bioassay data, but not for the fly ash. The fly ash bioassay data was more than ten times lower in some laboratories, among them GSF. In the GSF laboratory, the micro-EROD bioassay was used instead of CALUX as bio-analytical method (ENGWALL 2003). However, also among the laboratories using the CALUX bioassay a very high variation of the results was observed, especially for the fly ash. This situation caused some confusion and ambiguous discussions from the advocates and critics of the application of bioassays for PCDD/F and PCB analysis.
A careful examination of the WHO-PCB-data, which were also included into the investigation, showed that these compounds were discriminated substantially in contrast to the non-WHO-PCB in case of the fly ash. This led us to the conclusion that in case of the fly ash an extraction problem could be the reason for the discrepancies.
Figure 1shows the results from the extraction of the fly ash employing ASE with acetone:n-hexane in comparison to toluene. The extraction with toluene resulted in values for the fly ash, which were in agreement with those reported by Engwall (ENGWALL 2003). In addition, **Figure 1** clearly shows that for cod liver there

was a very good agreement of the data – independently of the extraction – between chemical and bio-analytical determinations of I-TEQ.

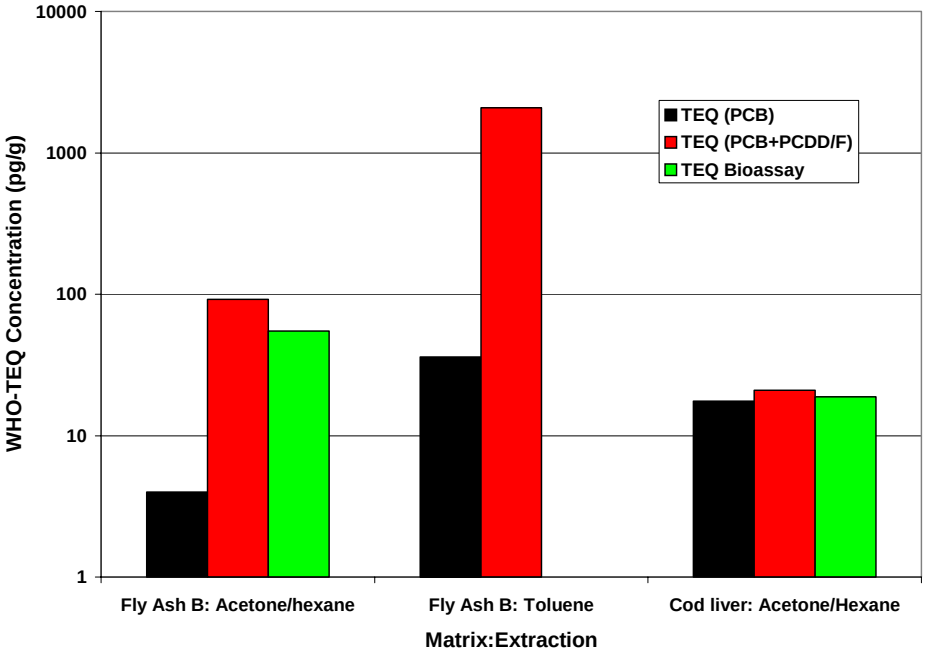

Figure 1: I-TEQ values for fly ash extracted either with acetone :n-hexane or toluene and codliver extracted with acetone:n-hexane in comparison with the micro-EROD bioassay.

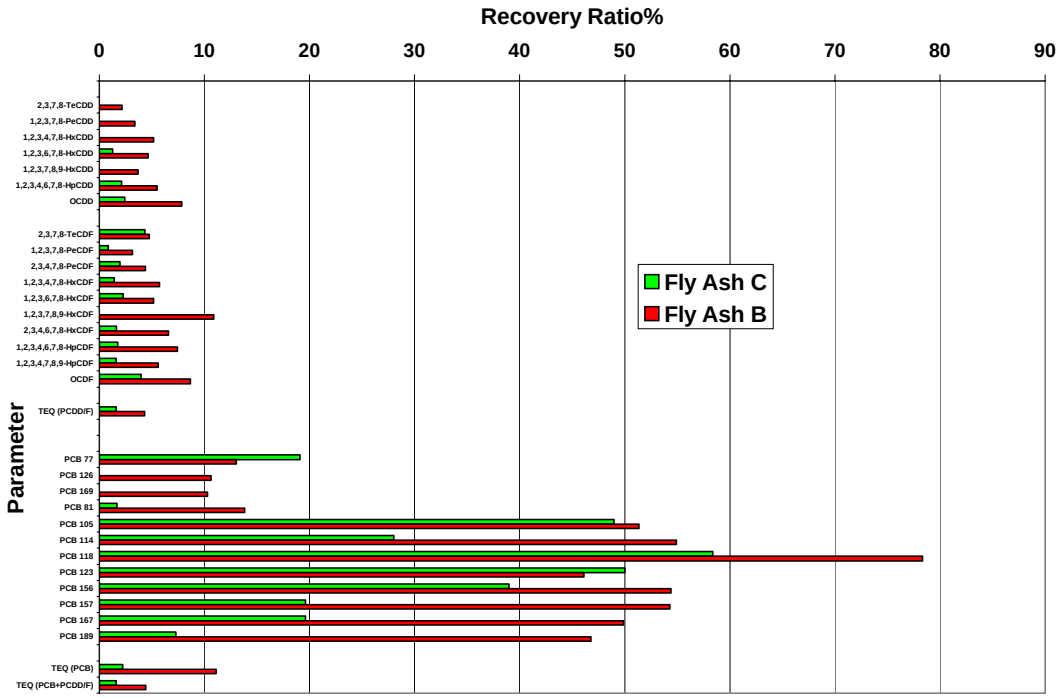

Figure 2: Efficiency of acetone:n-hexane extract compared to toluene extraction of the fly ash. Percentage ratios of extracted amounts of PCDD/F- and PCB congeners and TEQ values using acetone:n-hexane in comparison to what was extracted using toluene.

Figure 2 confirms the findings of the bioassay and the TEQ-values on a congener specific basis. The ratios between the concentrations in fly ash B and C in comparison to the extraction of toluene are very low (5-10%), especially for the planar and toxic congeners. The ratio is slightly different despite of the large difference of the TEQ values of both fly ashes which is about a factor of ten. Fly ash B, which had a TEQ value of about 2000 pg/g was used for the inter-laboratory-comparison. The extraction with toluene resulted in values closer to the values reported in the inter-calibration study for chemical analysis for both fly ashes (van Bavel 2002)

Discussion and Conclusion

The fly ash B contained elevated amounts of carbon. Therefore, a second type of extraction was proposed and performed using an aromatic solvent (toluene) as a competitive agent for the adsorption to aromatic sites in the matrix. Further it seems reasonable to achieve higher recoveries in case of higher concentrations because then the active sites are more saturated than in case of low concentrations.
Chemical analysis of these toluene extracts resulted in much higher concentrations of PCDD/F and WHO-PCB which are close to that of the inter-laboratory comparison of the bio-analytical results.

In conclusion the critics on the comparison of the DIOXIN 2003 inter-laboratory were misled by the artefacts of extraction in case of the fly ash sample and attributed the disagreements erroneously to the bio-analytical procedure. A part of the variation of the inter-laboratory comparison was caused due to a non-harmonized extraction scheme. In case of active carbon sites in fly ash even ASE – known as a harsh method for extraction - could not achieve satisfying recoveries. The use of an aromatic solvent which competes on the sites of adsorption seemed to overcome the unacceptable recoveries.

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