ASE WITH A CARBON TRAP COMBINED WITH AN IMMUNOASSAY AS A FAST AND RELIABLE SCREENING METHOD TO DETECT POLYCHLORINATED-*P*-DIOXINS AND FURANS FROM SOIL SAMPLES

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Introduction

There are a wide numbers of industrial sites polluted with dioxins at levels exceeding the guideline value of 250 pg/g[1] set by the Swedish environmental protection agency and thereby needs remediation. To ensure an efficient remediation of a polluted area, a thorough screening of the soil of the site is needed in order to locate hotspots. Such screenings are commonly performed with Soxhlet extraction combined with multi-step clean up followed by analysis by gas chromatography / high resolution mass spectrometer (GC/HRMS). This is a reliable but expensive, time and labor consuming method.

The use of an immunoassay such as Enzyme-linked immunosorbent assay (ELISA) opens up for much more rapid and cost effective screenings[2]. Although ELISA is not congene specific, it can give a good prediction of the total toxic equivalent (TEQ) value. However, there are a wide number of substances that can interfere with the ELISA and give a false dioxin signal and the extracts must therefore, just as in GC/HRMS analysis, undergo a thorough clean up prior the analysis[3]. The need for this multi-step clean reduces the benefits of the ELISA and calls for alternative efficient extraction and clean up methods. Accelerated solvent extraction (ASE) is an extraction method that has been shown to match the extraction efficiency of Soxhlet[4-6]. ASE has been used together with an integrated carbon trap to streamline the processing of biotic samples such as fish meal and fish oil [7].

The aim of this study was to examine if the ASE-carbon trap procedure could be adopted for use with soil samples and if it could be combined with ELISA detection to form an efficient dioxin screening method.

Materials and methods

In this study, nine soils with different levels and characteristics were analyzed. Three samples were from small-scale and industrial waste combustion sites in Uruguay, three were from different wood treatment sites in Sweden and two were from a Swedish chloralkali site. These samples were analyzed along with an artificial soil (10% Peat, 20% Kaolin and 70% Sand) which was not supposed to contain dioxins. The organic matter contents were in the range of 0.5%-27%. The samples were extracted with both Soxhlet and ASE and each extract were split into two for analysis with ELISA and GC/HRMS respectively.

Soxhlet combined with multi-step clean up

The Soxhlet extraction was done with toluene for 15h and each extract were spilt into two aliquots. One was spiked with internal standard (for analysis by GC/HRMS) and to the other where left untreated (for analysis by ELISA). Both fractions were cleaned up according to protocol described elsewhere[8]. The solvent for the GC/HRMS analysis were changed to tetradecane and [$^{13}C_{12}$]-labeled standards of 1,2,3,4-TCDD and 1,2,3,4,7,8,9-HpCDF were added to in order to assess recovery. For the unspiked fraction, the solvent were changed to Dimethylsulfoxide (DMSO) prior to the ELISA analysis.

ASE with carbon trap

The ASE was performed with an ASE 200® (Dionex, Sunnyvale, CA, USA) equipped with 33 ml stainless steel cells. Each cell was filled with a mixture of approximately 2g AX21-carbon and Celite in the ratio of 1:3 (w/w). The carbon mixture were washed with 15 ml dichloromethane/*n*-hexane/toluene (15:4:1), followed by 8 ml dichloromethane/*n*-hexane and 20 ml *n*-hexane. After washing the carbon, each cell was filled with approximately 1 g sample mixed with 3 g of Na_2SO_4 and toped up with Na_2SO_4 before the cell was sealed. The extraction procedure started with continuous pumping of solvent through the cell and simultaneous heating the pre-set temperature. The cells were extracted with 2 cycles of *n*-heptane followed by one cycle of heptane/Acetone (Temp: 100 ºC, Static: 5 min). During these cycles most pollutants are

washed out of the soil but the dioxins and furans are captured in the carbon mixture. In the next step, the cells were turned upside down and the dioxins are back flushed with four cycles of toluene (Temp:180 ºC, Static: 7 min). Every soil sample was extracted in duplicate. Internal standards where added to one of the two replicates (for analysis on GC/HRMS) prior ASE. Following extraction, the four toluene fractions were pooled, concentrated into tetradecane and passed trough a Pasteur pipette filled with 0.3g KOH-silica, 0.3g silica, 0.6g 40%-H_2SO_4-silica and 0.2g Na_2SO_4 using 8 ml och *n*-hexane to remove remaining residues. To the spiked samples, [$^{13}C_{12}$]-1,2,3,4-TCDD and [$^{13}C_{12}$]-1,2,3,4,7,8,9-HpCDF were added to assess recovery. Finally, the *n*-hexane was exchanged to tetradecane or DMSO prior to GC/HRMS and ELISA analysis, respectively.

The ELISA-analysis

The ELISA analysis of the extracts was preformed according to previously described protocols[2]. Microtiter plates were coated with 100µl III-Bovine Serum Albumin (BSA) coating antigen per well with a concentration of 0.2 µg/ml overnight. The antibody 7598 was diluted in 1/5000 in Phosphate buffered saline (PBS) with 0.2% BSA. Goat anti-rabbit IgG conjugated to horseradish peroxidase was diluted 1/3000 in PBST (PBS with 0.05% Tween 20). The absorbance of the reaction mixtures was read in dual wavelength mode (450-650 nm) using a Spectramax microplate reader. Each dilution of sample extract and standard were analyzed in triplicates.

To generate standard curves 2,3,7-trichloro-8-methyl-dibenso-*p*-dioxin(TMDD) were used. The calibration curves of TMDD and 2,3,7,8-Tetrachloro-dibenso-*p*-dioxin (TCDD) are almost identical but TMDD is less toxic[9]. The standard curves were generated by plotting absorbance vs. the logarithm of TMDD concentration. The curves were fitted in to a four-parameter logistic equation.

$$y = \{(A-D)/[1 + (x/C)^B]\} + D$$

A is the maximum absorbance at zero analyte, *B* is the curve slope at the inflection point, *C* is the concentration of analyte giving 50% inhibition (IC_{50}), and *D* is the minimum absorbance at infinite concentration. The cross-reactivity (CR) for TMDD/2,3,7,8-TCDD is 1.3[10]. Hence, similar standard curves are obtained, but TMDD is less toxic and therefore preferred.

GC/HRMS:

The GC-HRMS analysis were preformed using a Micromass Ultima GC high-resolution MS system operating in selected ion recording mode with electron ionization and a resolution of 8000 or greater. Quantification was preformed according to the isotope dilution method technique.

Results and discussion

ASE with carbon trap vs. Soxhlet with multi step clean up

The recovery of the $^{13}C_{12}$-labeled congeners added prior the extractions were generally in the range of 35-130 % (Figure 1). No $^{13}C_{12}$ –labeled congeners were found in the *n*-heptane nor *n*-heptane/acetone fractions, indicating that the dioxins were quantitatively captured by and released from the carbon trap.

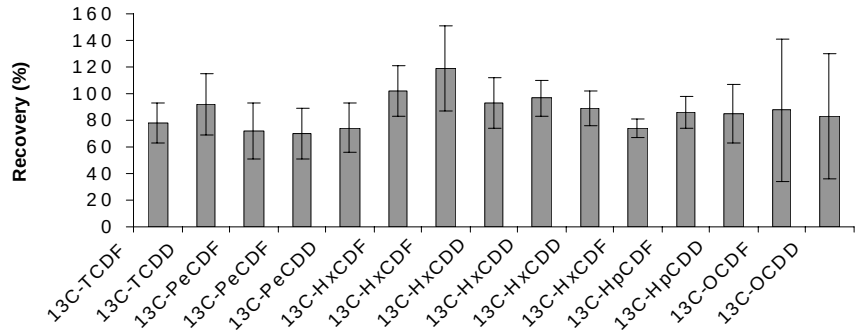

Figure 1, Mean value for the recovery of the 2,3,7,8-[$^{13}C_{12}$]-labeled congeners in the toluene fractions in all of the samples

The dioxin levels in soil samples ranged from ~1 pg/g (artificial soil) to ~37000 pg/g total WHO-TEQ (Chlor 2) according to GC/HRMS analysis. ELISA failed for some low level samples. The detected levels ranged between ~100 pg TMDD-eq./g (Comb. 3) and ~ 30000 pg TMDD-eq./g (Chlor 2).

The total WHO-TEQ from GC/HRMS, as well as TMDD-equivalents from ELISA compared well for the two sample preparation procedures (Figure 2a and 2b).

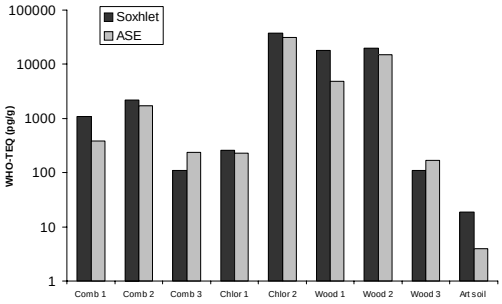

Figure 2a, GC/HRMS results from both sample preparations methods

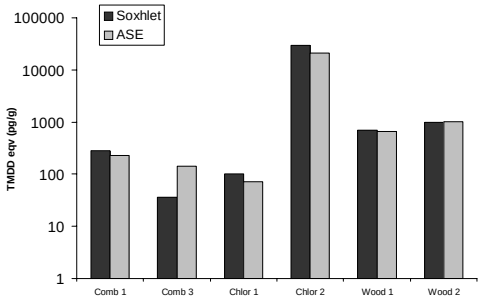

Figure 2b, ELISA results from both sample preparations methods

Thus, combined extraction/clean up with ASE/carbon trap yield acceptable recoveries and accurate WHO-TEQ data, as compared to the Soxhlet procedure, and therefore has a good potential for dioxin screening.

ELISA vs.GC/HRMS

To examine if ELSIA could serve as a reliable screening tool to detect the dioxins in the samples a comparison of the results from GC/HRMS (pg WHO-TEQ /g) and ELISA (pg TMDD-eq./g) was made (Figure 3a and 3b).

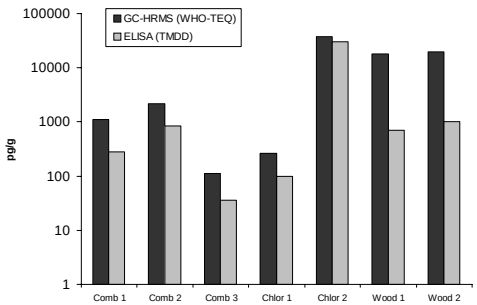

Figure 2a, GC/HRMS results from both sample preparations methods

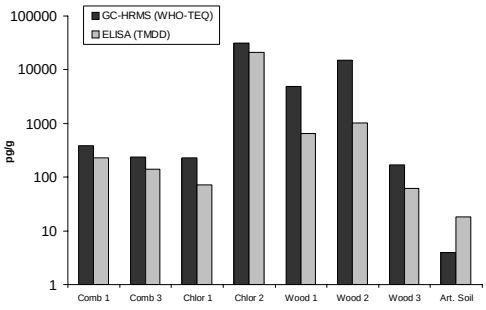

Figure 2b, ELISA results from both sample preparations methods

The results shows that the TMDD-equivalents level of ELISA overall were somewhat lower than the WHO-TEQ of GC/MS analysis. There are several plausible explanations for this. There might be losses in clean up, which are not compensated for the ELISA procedure. The underestimation may also be due to differences between the cross reactivities (CR) of the various congeners in ELISA[10] and their corresponding WHO-TEF´s[11]. For instance, soils polluted with highly chlorinated congeners will be underestimated with by ELISA since the CR for these are much lower than the established TEF´s. The congener profile for each sample is therefore of importance when evaluating ELISA results. Therefore, site specific corrections factors recommended when performing ELISA on soil samples[12]. Another possibility would be to use a general safety factor based on empirical data from parallel ELISA and GC/HRMS analysis of samples from a wide range of polluted sites

Conclusions

Our results imply that extraction and clean up using ASE with an intergraded carbon trap and ELISA immunoassay could be used for screening of dioxins in soil samples. The ASE method offers significant time; labor and cost savings as compared to Soxhlet with multi-step clean up. The results also suggest that ELSIA could serve as a reliable detection method. The use of GC/HRMS for the confirmation of selected samples is however strongly recommended. Also, the development of an internal standard for ELISA is foreseen as it would improve the performance. This might however prove difficult since the internal standard must have similar properties as 2,3,7,8-TCDD but not interfere with the immunoassay.

Acknowledgements

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