Immunochemical determination of dioxins in soil samples

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Introduction

Trace analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs or 'dioxins') in complex matrices (soil, sediment etc.) requires extensive clean-up and very sensitive detection methods. So far, gas chromatography coupled with high resolution mass spectrometry (GC-HRMS) after multi-step column clean-up has been the most prevalent choice of method for determination of these analytes. However, it is time to recognize alternative methods to supplement the knowledge about the presence of dioxins in the environment. Research on immunochemical methods for dioxin detection has been in progress for many years and there have been several successful attempts to develop such bioanalytical based techniques, reviewed by Harrison and Eduljee.[1] The main advantage of immunoassays is the possibility to analyze many samples simultaneously under simple experimental conditions, facilitating rapid and cost-effective screening of a large sample load. A highly sensitive polyclonal antibody-based enzyme-linked immunosorbent assay (ELISA) has been developed using 2,3,7-trichloro-8-methyl-dibenzo-*p*-dioxin (TMDD) as surrogate standard.[2,3] It was validated for human milk[4] and further optimized for application to soil and biota[5] and sediment and serum samples.[6] In this study, dioxin levels in soil samples were determined with the optimized ELISA and the results were compared to GC-HRMS data on split extracts. An effort to predict ELISA performance on flue gas and herring samples was also made.

Materials and Methods

Aliquots from extracts of nine soil samples of different origin were analyzed both with GC-HRMS and ELISA. Four of the soil samples were collected at small-scale and industrial waste combustion sites in Uruguay. Three of the samples were from wood impregnation sites in Sweden, and two were from a chlor-alkali site, also in Sweden. The samples were Soxhlet extracted with toluene for 15 hours prior to division of the extracts. Before subsequent clean-up, one aliquot of each extract was spiked with internal standard (a mix of ^{13}C-labeled PCDD/Fs) facilitating GC-HRMS analysis, while another aliquot was left unaffected by isotopically labeled compounds to avoid interferences in the ELISA.

The clean-up of spiked and non-spiked aliquots were done in parallel using four columns: 1) A multi-layer silica column packed with KOH-silica, activated silica, and 40% sulphuric acid-silica, eluted with *n*-hexane. 2) A carbon column (AX-21/Celite), eluted with dichloromethane/*n*-hexane (1:1, v/v) after which the column was turned up-side down and eluted with toluene. 3) An alumina oxide column, for the toluene carbon fraction, eluted with *n*-hexane and dichloromethane/*n*-hexane (1:1, v/v). 4) A miniaturized multi-layer silica column, for the alumina dichloromethane/*n*-hexane fraction, eluted with *n*-hexane. Thereafter, the solvent of spiked extract was changed to tetradecane and the solvent of non-spiked extracts was changed to DMSO.

The ELISA analysis was carried out in accordance with previously described protocols.[5,6] Coating of the microtiter plates was done with 100 µl lll-BSA coating antigen per well at a concentration of 0.2 µg/mL. The antibody 7598 was diluted 1/5000 in PBS with 0.2% BSA. Goat anti-rabbit IgG conjugated to horseradish peroxidase was diluted 1/3000 in PBST. The absorbance was read in a dual wavelength mode (450-650 nm) using a Spectramax microplate reader (Molecular Devices, Sunnyvale, CA). All sample extracts and standards were analyzed in at least triplicates. Method blanks and an artificial soil were analysed for quality control purposes.

Standard curves were generated by plotting absorbance against the logarithm of TMDD concentration. The curves were fitted to a four-parameter logistic equation:

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

Where *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 concentration at infinite concentration. The calibration curves of 2,3,7,8-TCDD and TMDD are almost identical but TMDD is less toxic.[5]

GC-HRMS analysis of samples from Uruguay and the chlor-alkali site was performed using a HP 5890 GC (60 m SP2330 or 30 m DB-5ms capillary column) coupled to a high resolution VG AutoSpec (Fisons Instruments). Samples from wood impregnation sites were analysed on a HP 5890 GC (60 m DB-5 capillary column) coupled to a high resolution VG 70-S (Fisons Instruments). The selected ion-monitoring mode and a resolution of 8,000 or greater were used.

Results and Discussion

A representative TMDD-standard curve together with parameters defining the curve is shown in Figure 1. The limit of detection (28 ± 6 pg/mL) is defined as the concentration giving 80% of the maximum response.

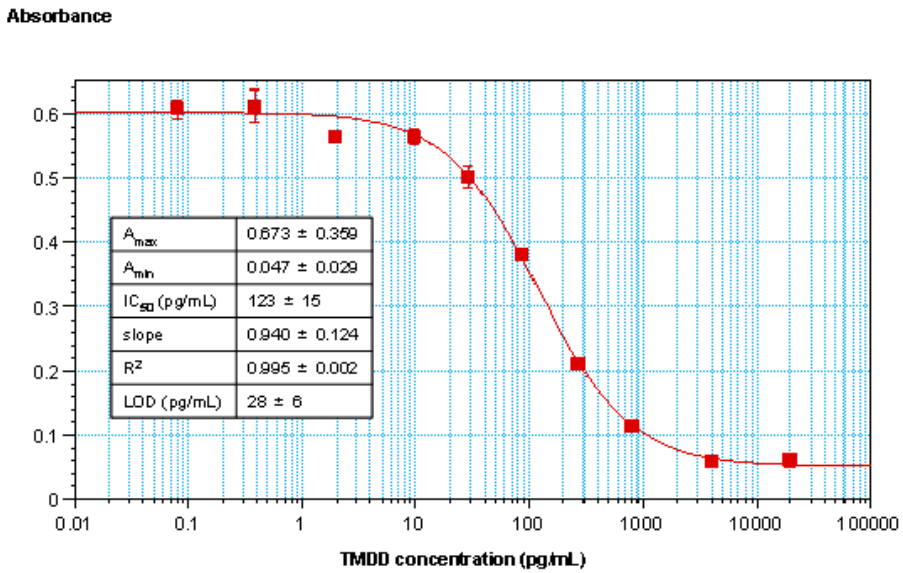

Figure 1. A typical TMDD-standard curve and features of the ELISA obtained from the four parameter equation used to fit the standard curve. The parameters correspond to the average of seven calibration curves run in three different days. Each curve was based on measurements of three-well replicates.

The correlation between ELISA and GC-HRMS results for the PCDD/F content in the nine soil samples under investigation is shown in Figure 2. The ELISA data represent the mean value of at least triplicate analysis of each sample extract. The relative standard deviation of replicate analysis ranged between 6 and 24%. As shown in Figure 2, for most of the samples, ELISA slightly underestimates the PCDD/F concentration. However, a false positive response was observed for the least contaminated sample, possibly due to high levels of compounds with significant cross-reactivity (CR), for instance 2,3,7,8-tetrabromodibenzo-*p*-dioxin (98% CR) and 2,3,7,8-tetrabromodibenzofuran (67% CR).

The general tendency to underestimate the PCDD/F concentration may be attributed to differences in CR amongst the individual 2,3,7,8-PCDD/Fs. If the GC-HRMS data for the individual 2,3,7,8-substituted congener is used to predict the ELISA response, by multiplying the concentration of each congener with its CR and sum the contributions,

a better agreement is obtained, see Figure 2. A pronounced underestimation in three of the soil samples was due to high amounts of the congeners 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, and 1,2,3,4,7,8,9-HpCDF (for which the TEF-value is not correctly reflected by the cross-reactivity).

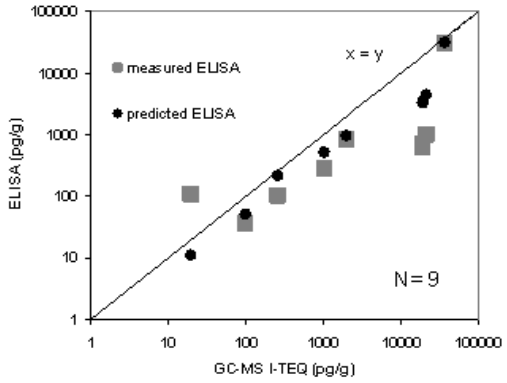

Figure 2. Correlation between ELISA derived TMDD equivalents (measured and predicted) and GC-HRMS results (I-TEQ).

The predicted ELISA values are all close to, or below, the actual TEQ-values for the samples. The difference between predicted and measured ELISA data can be explained by losses of analytes during clean-up. Hence, the ELISA response seems to derive solely from PCDD/Fs implying that immunoassay interferences were efficiently removed in the clean-up.

It has previously been shown that predicted ELISA values correlate very well, both with measured ELISA and GC-HRMS data, for sediment samples containing more than 100 pg TEQ/g.[6] Furthermore, the estimated method detection limit was about 20 pg TMDD-equivalents per gram sediment. The correlation between ELISA measured TMDD equivalents and GC-HRMS I-TEQ values in this study is defined by a correlation coefficient of 0.56 and a slope of 0.55 (after linear regression analysis of non-logarithmic data). This indicates that introduction of a response factor, linking the relationship between GC-HRMS and ELISA data given by the inverse value of the slope, would facilitate screening of PCDD/F contaminated soil samples.

However, as noted above, the PCDD/F pattern has a great impact on predicted ELISA performance. Site specific response factors are therefore recommended. This is especially important for matrices with expected differences in congener patterns, such as soils and sediments with input from a wide variety of pollution sources, and for which site specific influences on the PCDD/F transport and transformation are likely to take place. The patterns in matrices such as biota and flue gas are usually more constant due to metabolic action and a specific formation process, respectively. The correlation between predicted ELISA and I-TEQ values for 20 combustion samples (flue gas) is demonstrated by a correlation coefficient of 0.99 and a slope of 0.47 (Figure 3, left). The correlation between predicted ELISA and WHO-TEQ values for 30 biota samples (herring) is demonstrated by a correlation coefficient of 0.96 and a slope of 0.36 (Figure 3, right). Hence, the response factors between predicted ELISA and GC-HRMS values are 2.1 for flue gas, and 2.8 for herring. In theory, a sample size of 20 g herring would be sufficient to exceed the method detection limit. Confirmation of predicted response factors remain to be accomplished by ELISA analyses of flue gas and herring.

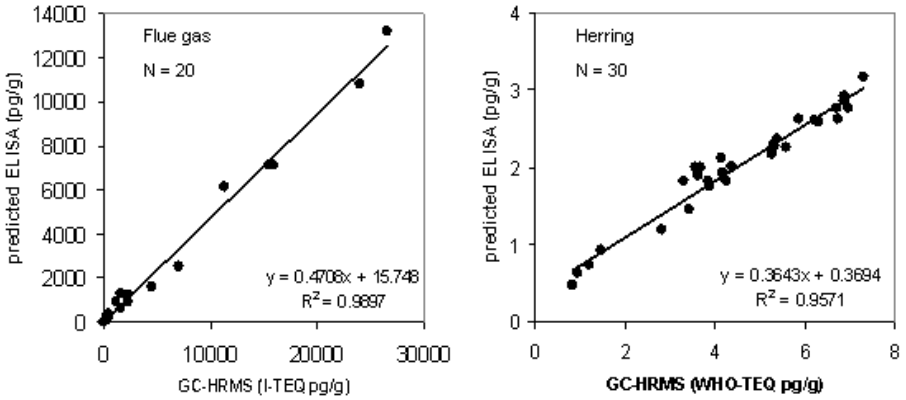

Figure 3. Correlation between predicted ELISA and GC-HRMS for flue gas (left) and herring (right).

Acknowledgements

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