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An enzyme-linked immunosorbent assay for the determination of dioxins in contaminated sediment and soil samples

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Abstract

A 96-microwell enzyme-linked immunosorbent assay (ELISA) method was evaluated to determine PCDDs/PCDFs in sediment and soil samples from an EPA Superfund site. Samples were prepared and analyzed by both the ELISA and a gas chromatography/high resolution mass spectrometry (GC/HRMS) method. Comparable method precision, accuracy, and detection level (8 ng kg^{-1}) were achieved by the ELISA method with respect to GC/HRMS. However, the extraction and cleanup method developed for the ELISA requires refinement for the soil type that yielded a waxy residue after sample processing. Four types of statistical analyses (Pearson correlation coefficient, paired *t*-test, nonparametric tests, and McNemar's test of association) were performed to determine whether the two methods produced statistically different results. The log-transformed ELISA-derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin values and log-transformed GC/HRMS-derived TEQ values were significantly correlated ($r = 0.79$) at the 0.05 level. The median difference in values between ELISA and GC/HRMS was not significant at the 0.05 level. Low false negative and false positive rates (<10%) were observed for the ELISA when compared to the GC/HRMS at 1000 ng TEQ kg^{-1}. The findings suggest that immunochemical technology could be a complementary monitoring tool for determining concentrations at the 1000 ng TEQ kg^{-1} action level for contaminated sediment and soil. The ELISA could also be used in an analytical triage approach to screen and rank samples prior to instrumental analysis.

Keywords: PCDD; PCDF; 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; TCDD; GC/HRMS; Immunoassay

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), are present in nearly all components of the global ecosystem (Safe, 1998). Seventy-five compounds represent the set of PCDD congeners, and 135 compounds comprise the PCDF congener set. Some of the PCDD and PCDF congeners are considered highly toxic. The most toxic of these compounds, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is classified as a human carcinogen (IARC, 1997; ATSDR, 1998) and by the EPA as a probable human carcinogen (USEPA, 1997). World-wide, PCDDs and PCDFs are formed mainly as byproducts of industrial processes involving chlorinated phenols, waste incineration (municipal waste, hospital waste, sewage sludge), open trash burning, metal smelting, production of chlorinated aromatic compounds, and bleaching of kraft pulp (Fielder, 2007). They can also be produced from natural processes (e.g. volcanic eruption or forest fire). Exposure to the PCDDs has been linked

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to adverse health effects such as severe skin disease (chloracne), birth defects, and increased risk of cancer (USEPA, 1992; ATSDR, 1998; Bernes, 1998; Warmer et al., 2002). Chloracne can be quite severe particularly when the exposure dose is extremely high as seen following the reported dioxin poisoning of Ukrainian President Viktor Yushenko whose symptoms also included slight paralysis. The environmental background toxic equivalent (TEQ) concentrations of PCDDs and PCDFs in non-contaminated soil are generally less than 10 ng TEQ kg^{-1} while much higher TEQ concentrations (>1000 ng TEQ kg^{-1}) have been reported for various contaminated sites in the US (USEPA, 2005a,b), Sweden (Persson et al., 2007), Korea (Im et al., 2002), and other locations. A threshold of 1000 ng TEQ kg^{-1} has been established in the US based on the decision framework of the US Department of Health and Human Services Agency for Toxic Substances and Disease Registry (ATSDR) for sites contaminated with PCDDs and PCDD-like compounds. The ATSDR action level is 1000 ng TEQ kg^{-1} for public health actions such as surveillance as determined per site. Non-occupational routes of exposure to the PCDDs include inhalation of contaminated air, ingestion of contaminated substances, and dermal contact. The major route (>90%) of exposure is from dietary ingestion of contaminated foods (IARC, 1997) and there is particular concern for breast-fed infants (Gies et al., 2007).

Conventional analytical methods for quantifying PCDD/PCDF levels in sediment and soil samples are time-consuming and costly. American and European standard methods require rigorous sample extraction, processing the sample extract through multiple cleanup columns, and analyzing the final fraction by gas chromatography/high resolution mass spectrometry (GC/HRMS) (USEPA, 1994, 1999a; European Committee for Standardization, 1997; Srogi, 2007). Rapid and cost-effective screening methods, as well as efficient high-sample capacity quantitative methods would facilitate large-scale environmental monitoring studies.

Immunoassays have been routinely used in medical and clinical settings for the quantitative determination of proteins, hormones and drugs with a molecular mass of several thousand Daltons. Immunoassay techniques including the enzyme-linked immunosorbent assay (ELISA) have proven useful for environmental monitoring studies (Van Emon and Lopez-Avila, 1992; Van Emon, 2001; Roda et al., 2006; Trang et al., 2007). The development of immunoassays for the PCDDs is complicated by their lipophilic properties, required method sensitivity, and the complex nature of environmental samples. Due to these difficulties, only a few studies have been reported in the development of antibodies and ELISA methods for PCDDs with mixed results (Stanker et al., 1987; Sanborn et al., 1998; Sugawara et al., 1998, 2002; Harrison and Carlson, 1999; Shan et al., 2001; SW846 Method 4025, 2002; Okuyama et al., 2004; Lo et al., 2005).

Streamlined sample preparation and cleanup procedures are typically easier to achieve for ELISA methods than for instrumental detection. However, simplified sample preparations must be adequate to remove matrix interferences from complex sample media. One of the important tasks in assay development is to determine an optimal solvent system for both analyte solubility and assay performance. This can be very challenging for analyzing compounds such as the PCDDs and PCDFs having a high lipophilicity, in an aqueous-based method such as ELISA. Previously, a sensitive polyclonal antibody-based ELISA method was reported (Sanborn et al., 1998; Sugawara et al., 1998; Shan et al., 2001). Different solvent systems including combinations of methanol and dimethyl sulfoxide (DMSO) to enhance analyte solubility, in combination with phosphate buffer for antibody performance, were evaluated and applied successfully to milk, soil, and biota samples (Shan et al., 2001; Sugawara et al., 2002; Nichkova et al., 2004; Nording et al., 2006). In these previous studies, cleanup procedures were required and comparable results were obtained between the ELISA and GC/HRMS methods for a small sample set ($n < 20$). A more in depth statistical analysis of the ELISA and GC/HRMS data from a large number of real-world samples was undertaken here to more completely assess the performance of the ELISA relative to the GC/HRMS method.

The main objective of the work reported here was to determine whether the ELISA method can be used as a monitoring tool for PCDDs/PCDFs in contaminated sediment and soil samples. The ELISA and GC/HRMS data were obtained for a large set of sediment and soil samples from an USEPA Superfund site. Results are discussed from the statistical analyses of the ELISA-derived TCDD equivalents (ELISA-EQ) data as well as from the GC/HRMS-generated TEQ values for these samples. These data were used to evaluate the performance of the ELISA method.

2. Materials and methods

2.1. Sample preparation for ELISA

Sediment and soil samples were collected from an USEPA Superfund site undergoing investigation for long-term remedial action. Throughout the investigation, removal actions were taken to reduce immediate threats. The site is along the Woonasquatucket River in North Providence, Rhode Island, USA. The Woonasquatucket River has played a key role in industrial activities since the American Industrial Revolution and now is heavily polluted with PCDDs and other contaminants (Stabler, 1908). Portions of the river are currently undergoing cleanup and restoration (USEPA, 1999b; Corcoran, 2007). Samples received from the site included: (1) surface sediment grab samples (up to 0.5 feet in depth), (2) soil samples collected from various boring depth intervals, (3) surface soil samples from a floodplain, and (4) sediment core samples from fresh water ponds at various depths. Aliquots of these samples were dried, homogenized, sieved and extracted with hexane by sonication. The hexane extracts were cleaned by a

multilayered acid/base silica gel column followed by an activated carbon column. The target fractions (in toluene) from the carbon column cleanup were solvent exchanged into a solution containing 50% DMSO and 50% phosphate buffered saline (PBS) with 0.01% Triton X-100 (PBST) referred to as DMSOT. Detailed sample preparation procedures were as described in Nichkova et al. (2004).

2.2. ELISA procedures

The ELISA coating antigen was a PCDD hapten that preserved key structural features for target recognition (7,8-dichloro(5,6)(1,4)dioxino(2,3-*b*)pyridine-3-carboxylic acid) that was covalently bound to bovine serum albumin (BSA, Sigma Chemical Co., St. Louis MO, USA). Each well of a high-binding 96-microwell plate (Maxisorp I, Nunc, Roskilde, Denmark) was coated with 100 μl of the coating antigen (50 ng ml^{-1}) and incubated overnight at 4 °C. A colorimetric inhibition assay format was followed as described in Nichkova et al. (2004). The absorbance of each well was determined in a dual-wavelength mode (450–650 nm) using a SpectroMax Plus microplate reader (Molecular Devices, Sunnyvale, CA, USA). The reader was interfaced to a personal computer and data processing was performed with SOFTmax Pro software (Molecular Devices, Sunnyvale, CA, USA).

2.3. Sample preparation for GC/HRMS

Sediment and soil samples were extracted according to procedures described in Misita et al. (2003) using a pressurized fluid extraction system (ASE 200, Dionex Corp., Sunnyvale, CA, USA) equipped with 33 ml extraction cells. An aliquot (10 g, wet weight) of each sample was mixed with Hydromatrix (Varian, Inc., Palo Alto, CA, USA) prior to placement into the extraction cell. The bottom of the extraction cell was first covered with a cellulose fiber filter. Half of the sample mixture was placed in the extraction cell prior to spiking with an internal standard solution containing 15 $^{13}C_{12}$-labeled PCDD/PCDF congeners including $^{13}C_{12}$-TCDD (Cambridge Isotopes, Andover, MA, USA). The remaining half of the sample mixture was added to the cell, and additional Hydromatrix was added to completely fill the cell. The extractions were performed with toluene (~50 ml) at 2000 psi and 150 °C for two cycles of 5 min with 60% flush. Each extract was then spiked with $^{37}Cl_4$-labeled TCDD (Cambridge Isotopes, Andover, MA, USA) for monitoring the recovery of the analytes through the cleanup procedures. Each extract was acid/base washed and then processed through acid/base silica, alumina, and carbon columns following the general procedures described in USEPA Method 1613B (USEPA, 1994). The extracts were spiked with the recovery standards 1,2,3,4-TCDD-$^{13}C_{12}$ and 1,2,3,7,8,9-HxCDD-$^{13}C_{12}$ (Cambridge Isotopes, Andover, MA, USA) and concentrated to a final sample volume of 20 μl. Some samples were further diluted to 30 μl to improve the chromatography.

2.4. GC/HRMS analysis

Extracts were analyzed either on a VG Autospec or Autospec Ultima high resolution mass spectrometer (Waters Corp., Milford, MA, USA) equipped with a Hewlett Packard 5890 GC (Agilent Technologies, Foster City, CA, USA). Analyses were performed in the selected ion-monitoring mode at a resolution of 10 000 or greater. A J&W Scientific DB5 (60 m × 0.32 mm × 0.25 μm) column (Agilent Technologies, Foster City, CA, USA) was used for initial analysis of the seventeen PCDD/PCDF congeners. As a DB5 column does not completely resolve 2,3,7,8- tetrachlorodibenzofuran (TCDF) from all other tetrachlorinated dibenzofurans, a second column confirmation was conducted as needed using a J&W Scientific DB-225 (30 m × 0.32 mm × 0.25 μm) column (Agilent Technologies, Foster City, CA, USA). Calculations were performed using OpusQuan, a software program specifically designed for PCDD/PCDF analysis by VG Co. Ltd. (Manchester, UK).

2.5. Data analysis

The immunochemical and instrumental analyses were performed at separate facilities using separate aliquots of each soil and sediment sample. Samples were mixed, prior to aliquoting, but no measure of heterogeneity was performed. The heterogeneity may have contributed to data variation. The ELISA-derived TCDD-equivalent levels (ELISA-EQ) were used in the statistical analysis. Following convention, the TEQ value for each congener was generated by multiplying the measured GC/HRMS concentration of each individual congener with its corresponding toxic equivalent factor (TEF) value (Van den Berg et al., 1998). A total TEQ value was obtained through summation of all 17 individual congener TEQ values. Since detection limits for all 17 PCDD/PCDF congeners were available in this study, non-detectable values were replaced with one-half the detection limit for statistical analysis. Preliminary reviews of simple descriptive statistics and scatter plots of the ELISA and GC/HRMS data showed that these data had positively skewed distributions, suggesting that a log-normal distribution would be a more appropriate model for statistical analysis than a normal distribution.

To address the final aspect of this evaluation (i.e. determining the presence of statistically significant differences between ELISA and GC/HRMS results), four types of statistical analyses were performed: (1) a test of significant linear correlation among the log-transformed data, (2) a paired *t*-test on the log-transformed data, (3) nonparametric tests of location, and (4) McNemar's test of association. The Pearson correlation coefficient measured the extent of a general linear association between the log-transformed ELISA and GC/HRMS data, and a parametric statistical test was performed to determine whether the calculated value of this correlation coefficient was significantly positive (Snedecor and Cochran, 1989). For each sample, the paired

t-test (Hollander and Wolfe, 1973) was used to determine whether the geometric mean of the ratio of the GC/HRMS TEQ levels and the ELISA-EQ levels differed significantly from one (at a 0.05 significance level). Two types of nonparametric tests (the Wilcoxon signed-rank test and the sign test) were performed on the sample-specific differences between ELISA and GC/HRMS results. These nonparametric tests were conducted to determine if the median difference was significantly different from zero (Rosner, 2000). Similar to the paired *t*-test, the Wilcoxon signed-rank test was applied to differences between log-transformed measurements, as this test assumes that the differences have a symmetric distribution. In contrast, the sign test does not make this assumption and is therefore performed on nontransformed data. Finally, McNemar's test of association was performed to determine whether there was any significant difference between the two methods in the proportion of samples having measurable TEQ levels and ELISA-EQ levels that were at or above a specified threshold. A series of threshold levels (50, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 ng kg^{-1}) were evaluated.

3. Results and discussion

3.1. ELISA method performance

Each sediment and soil sample was analyzed by the ELISA method in triplicate. Data acceptance criteria for the 96-microwell plate assay were established and used as guidance throughout the analysis. The four parameter curve-fit values of: (a) upper asymptote, (b) slope, (c) IC_{50}, and (d) lower asymptote were generated for each calibration curve. The percentage relative standard deviation (%RSD) values of the triplicate analyses (performed on the same ELISA plate) were less than ±20% for all samples and standard solutions. Day-to-day variation of the ELISA method based on 25 standard curves run on different days (expressed as the %RSD of the IC_{50}) was ±23.3%. The percent difference of the duplicate ELISA analyses of the same sample performed on different dates ranged from 7.2% to 29%. The correlation coefficient for each calibration curve was greater than 0.99. Recoveries of the back-calculated standard solutions were greater than 80%. If the ELISA result was outside the calibration range, the sample extract was diluted and re-assayed. All sample extracts were analyzed by at least three different dilutions. The dilution resulting in an absorbance closest to the absorbance at the IC_{50} was reported. The calculated value for the sample from the three dilutions analyses did not vary by more than 30%. Method blanks and negative control sample values were below the assay detection limit (4 pg ml^{-1}). The overall method accuracy and precision (including extraction, cleanup and detection) for the ELISA method were determined. Comparable results with the conventional GC/HRMS method were achieved, yielding an overall method accuracy of greater than 60%, and overall method precision within ±30% for the sediment and soil samples.

The ELISA method used the less-toxic TMDD (2,3,7-trichloro,8-methyl-dibenzodioxin) as a surrogate standard for TCDD in the calibration solutions for quantification. As the TCDD results were found to be approximately 130% of the TMDD response in the ELISA, TMDD measurements were multiplied by a conversion factor of 1.3 to obtain TCDD equivalent measurements (Sugawara et al., 2002; Nichkova et al., 2004). Most of the PCDDs and PCDFs with a high TEF value (>0.1) showed high to moderate cross-reactivity in the ELISA (Shan et al., 2001). The assay has a high cross-reactivity (110–115%) for two brominated dioxins, 2-bromo-3,7,8-triCDD and 2,3-dibromo-7,8-diCDD, and fairly low cross-reactivity for three coplanar polychlorinated biphenyls (PCBs), PCB77 0.1%, PCB126 <0.01% and PCB169 <0.01%. Neither the two brominated dioxins nor any PCBs were measured by GC/HRMS in this study.

Previously, this ELISA was demonstrated as being a potential screening method for dioxins in fish, eggs, and milk when cleanup methods are used (Shan et al., 2001; Sugawara et al., 2002). Due to the complicated sediment and soil sample matrices in this study, cleanup steps were also necessary. An effective cleanup method consisting of a multi-layered silica gel column followed by a carbon column provided quantitative recoveries for the sediment samples for ELISA analysis (Nichkova et al., 2004). The hexane extraction employed yielded recoveries greater than 80% for samples with total organic content ranging from 0.6% to 22.5%. The cleanup procedure was demonstrated to effectively remove the matrix interferences in the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 1944 sediment sample. The ELISA results for SRM 1944 improved from 1900% (4600 ng kg^{-1} as TCDD equivalents) of the expected TEQ value to 108% (260 ng kg^{-1}) when cleanup procedures were employed. Thus, cleanup procedures are necessary as components other than PCDDs/PCDFs appear to be present in the SRM sample interfering with the assay. Although the sample preparation method used here was adequate, it may not be effective for other soil types or those with higher organic contents. Thus, it is advisable to conduct a small pilot study to evaluate the overall ELISA method performance before undertaking a large-scale study.

Eighty-two sediment and soil samples were originally designated for the method comparison study. ELISA data for seven sediment samples were outside quality control criteria, as the calculated value from the three sample dilutions varied by more than 30%. Observation of these seven sediment extracts showed all to have a waxy residue following cleanup, unlike the other 75 samples tested. Possibly, the sample preparation and cleanup method reported here is not appropriate for these waxy extracts. More efficient streamlined sample preparation and cleanup methods for complicated samples are currently under development.

For comparison purposes, the results from the remaining 75 samples were used for the statistical comparisons of ELISA and GC/HRMS data as representative of a more homogeneous sample set.

3.2. GC/HRMS method performance

The GC/HRMS instrumentation was calibrated for detection of PCDD/PCDF levels (0.5–2000 ng ml^{-1}) specified in EPA Method 1613B (USEPA, 1994) with one additional calibration standard equivalent to one-half the level of the lowest calibration point. This allowed for an expanded calibration range beyond method requirements. The calibration was verified every 12 h of instrument operation. The recovery of $^{13}C_{12}$-labeled internal standards was monitored in each sample and met the criteria stated in the EPA Method 1613B (USEPA, 1994). With every batch of 20 samples, quality control checks including one laboratory method blank, one laboratory control spike, one matrix spike, one matrix spike duplicate, one field sample duplicate, and one NIST SRM 1944 sediment sample were processed to monitor the accuracy and precision of the method. The overall method precision was within ±30% based on results of the percent difference of duplicate samples and duplicate matrix spike samples. The overall method accuracy was greater than 70% based on the recoveries of the matrix spike and SRM samples.

3.3. PCDD/PCDF congener distribution by GC/HRMS

The soil and sediment samples were collected from different locations at one Superfund site. Contaminated sites are frequently very heterogeneous which often pose challenges for sampling and analysis. The distributions of PCDD/PCDF congener profiles among the samples analyzed were used to determine site heterogeneity. Distributions of the 17 individual target PCDD/PCDF congeners were examined, with each expressed as a percentage of total PCDD/PCDF content (i.e. summing the 17 PCDD/PCDF congeners), in the samples. Three of the seven dioxin congeners (TCDD; 1,2,3,4,6,7,8-heptaCDD; and OCDD) and two of the 10 furan congeners (1,2,3,4,6,7,8-heptaCDF; OCDF) dominated the contribution to the total PCDD/PCDF content. However, the relative concentrations for these five dioxin/furan congeners varied in each sample. Among these five dioxin/furan congeners, TCDD showed a high response (130% of TMDD) in the ELISA, with the remaining congeners showing very low cross-reactivity (<0.5%). The corresponding WHO TEF values are 1.0 for TCDD; 0.01 (1% of TCDD TEF value) for 1,2,3,4,6,7,8-heptaCDD and CDF; and 0.0001 (0.01% of TCDD TEF value) for OCDD and OCDF.

Seven congener profiles were established for this set of samples according to the concentration distribution of individual PCDD/PCDF compounds as described in Table 1. The congeners OCDD/OCDF were the major components of all the profiles with the exception of profile #6 where TCDD was the major component. As expected, 99.6–99.9% of the total TEQ values from the profile #6 samples were from TCDD. Except for profiles 2, 5 and 7, TCDD was present at >5%. Profile #5 is the only instance where the percentage of furans dominated. This profile is characterized by a less than 5% contribution each from TCDD and 1,2,3,4,6,7,8-heptaCDD; 10–30% contribution each from OCDD and 1,2,3,4,6,7,8-heptaCDF; and a 25–60% contribution from OCDF. One sample (profile #7) was similar to profile #2, except that it contained larger amounts of 1,2,3,4,6,7,8-heptaCDF and OCDF. Thus, the samples from this Superfund site were very heterogeneous.

3.4. Statistical comparison of ELISA and GC/HRMS data

The relative toxicological and biological potency of the PCDDs/PCDFs in soil and sediment samples was ranked by the TEF approach using the GC/HRMS data. The ELISA-derived concentrations were corrected for TCDD/TMDD cross-reactivity and expressed as TCDD-equivalent concentrations. Since the antibody used in this ELISA is designed to bind to the most toxic congener (TCDD) and has cross-reactivities to other less toxic PCDD/PCDF congeners, the ELISA-EQ has the potential to be used as an indicator for overall PCDD/PCDF toxicity in the sample. The ELISA-EQ may contain the responses from other PCDD/PCDF congeners and brominated dioxins/furans present in the sample that are not measured by the GC/HRMS method. Preliminary evaluation of soil and sediment samples from this Superfund site indicated that brominated dioxins and furans were either non-detected in the samples or were present at least one order of magnitude lower than the chlorinated compounds. Thus, the analysis of brominated dioxins/furans was not performed. Currently, there are no established TEF values for the brominated dioxins/

Table 1
PCDD/PCDF congener distribution profiles in soil and sediment samples

Profile	Number of samples	TCDD (%)	1,2,3,4,6,7,8-HeptaCDD (%)	OCDD (%)	1,2,3,4,6,7,8-HeptaCDF (%)	OCDF (%)
1	29	15–40	<10	40–70	<10	<10
2	18	<5	10–20	60–90	<5	<5
3	18	5–15	<10	50–80	<10	<10
4	4	40–50	<10	40	<10	<10
5	3	<5	<5	10–30	10–30	25–60
6	2	>90	<2	<5	<2	<2
7	1	<2	10	55	20	10

furans. However, in the 2005 WHO reevaluation of human and mammalian TEFs for PCDDs, PCDFs, and coplanar PCBs, the expert panel recommended that high priority be given for the development of TEFs for the brominated dioxins/furans (Van den Berg et al., 2006).

Because there is a difference between TEF values and the ELISA cross-reactivities of the individual PCDD/PCDF congeners, statistical analyses were conducted on the GC/HRMS-derived TEQs and ELISA-EQ to determine the correlation between the measurements. Statistical analyses were also performed to determine if the ELISA-EQ can be used to address the relative toxicity of the samples similar to the TEQ approach used for GC/HRMS analysis. Because split samples were analyzed by both methods, statistical methodologies were used that would properly account for any underlying correlation expected to be present between the two measurements associated with the same physical sample.

Summary statistics (sample size, arithmetic and geometric means, standard deviation, minimum, 25th percentile, 50th percentile, 75th percentile, and maximum) for the ELISA and GC/HRMS results, are shown in Table 2 and are plotted in Fig. 1. Note that the points in Fig. 1 are clustered around the 45° line of equality, suggesting that there is good agreement in results between the ELISA and GC/HRMS methods among samples that had valid, quantitative results for both methods. While this clustering tends to be predominant across the entire range of data for both the ELISA and GC/HRMS methods, the plot shows some evidence that for samples having the lowest reported concentrations ($<100\ \text{ng kg}^{-1}$), the ELISA method yields slightly higher measurements than those reported by the GC/HRMS.

The estimate of the Pearson correlation coefficient between the log-transformed measurements of the two methods was $r = 0.787$, which was significantly greater than zero at the 0.05 level ($p < 0.0001$). Thus, there is very strong statistical evidence of a general linear association present in the reported log-transformed data between the ELISA and GC/HRMS methods for these samples.

Table 2
Summary statistics of ELISA and GC/HRMS results

Summary statistics[a]	ELISA, TCDD EQ (pg/g)	GC/HRMS, TEQ (pg/g)
Sample size	75	75
Arithmetic mean	1700	2400
Standard deviation	2400	3700
Geometric mean	460	410
Minimum	<8[b]	5.1
25th Percentile	97	61
50th Percentile	490	660
75th Percentile	2400	3700
Maximum	14000	17000

[a] An aliquot (10 g) of each soil and/or sediment sample was used for GC/HRMS analysis and another aliquot (1–5 g) of each sample was used for ELISA analysis.

[b] The ELISA results of two samples were detected below the working range and reported as <8 pg/g.

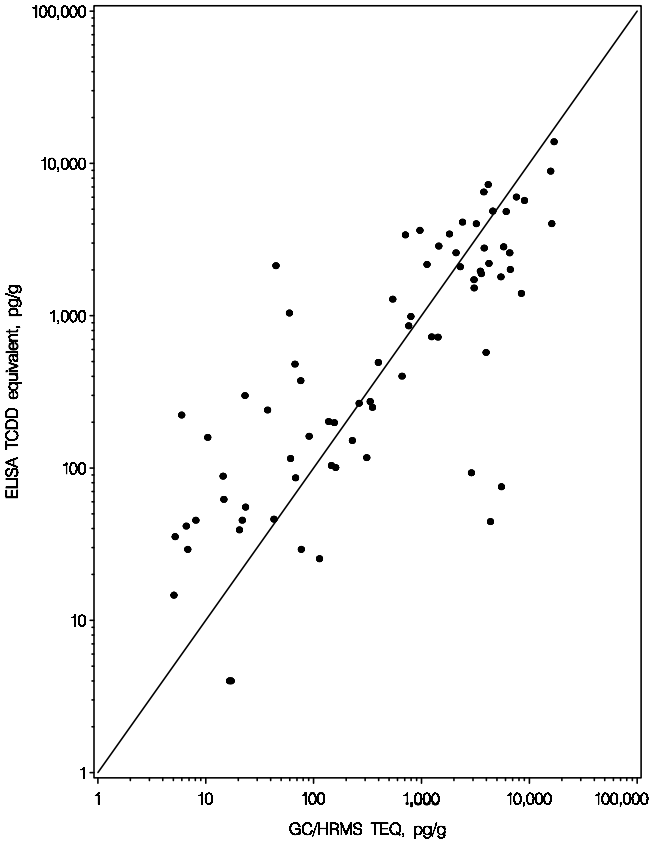

Fig. 1. Comparison plot of ELISA-EQ and GC/HRMS TEQ levels for soil and sediment samples with a 45° line of perfect agreement.

As calculated across the samples, the ratio of the geometric mean of a sample's ELISA measurement to its GC/HRMS measurement was 1.12, implying that on average, the ELISA measurements tended to be approximately 12% higher than the GC/HRMS measurements for a given sample. This could be due to the ELISA cross-reactivity to other compounds structurally related to TCDD such as brominated dioxins that are not measured by the GC/HRMS method. This ratio was not significantly different from one at the 0.05 level, according to a paired t-test performed on the log-transformed measurements ($p = 0.508$). Thus, the ELISA measurements were not statistically different from GC/HRMS measurements.

The nonparametric sign test found that the median difference between the (log-transformed) ELISA and GC/HRMS measurements among the samples was not significantly different from zero at the 0.05 level ($p = 0.731$). Likewise, the Wilcoxon signed-rank test concluded that the median difference in log-transformed ELISA and GC/HRMS measurements was not significantly different from zero at the 0.05 level ($p = 0.457$). These findings support the hypothesis that ELISA and GC/HRMS measurements for a given sample tend to be statistically equivalent.

Under the decision framework prepared by the ATSDR, sites contaminated with PCDDs, PCDFs, and related compounds with TEQ levels between 50 and $1000\ \text{ng kg}^{-1}$ should be further evaluated, and public health action

may be necessary for levels above 1000 ng kg^{-1} (DeRosa et al., 1997). Table 3 summarizes the number and percentage of the soil and sediment samples that fall within each of the four categories denoted by whether or not the reported sample concentrations were at or above a specified threshold under either method. For each comparative group included in Table 3, the exact *p*-value associated with McNemar's test was well above 0.10, implying that the proportion of samples with concentrations at or above the designated threshold level did not differ significantly between the two methods. This is seen by the values in the second and third columns of Table 3 being very similar for each comparative concentration group. If the GC/HRMS procedure represents a standard method, then the false negative and false positive rates for the ELISA at each comparative concentration level ranged from 4.0% to 8.0% and from 2.7% to 13.3%, respectively. For any screening method, a 0% false negative rate and a low false positive rate (<10%) are ideal. In the current sample sets, the false positive and false negative rates for the ELISA method were 6.7% and 8.0%, respectively at the concentration levels of 1000 ng kg^{-1}. The low false positive rate (6.7%) suggests that the ELISA method was an effective screening tool for monitoring PCDDs/PCDFs. Additional studies could be undertaken to investigate the causes for these false negative samples (6 out of 75) and to improve the ELISA method to perhaps achieve a 0% false negative rate at the 1000 ng kg^{-1} level. However, low-cost screening methods can provide a denser sampling grid which may compensate for the lack of a zero false negative rate.

The statistical analyses suggest that for the contaminated sediment and soil samples, the log-transformed measurements were highly linearly correlated between the ELISA and GC/HRMS methods. Sample-to-sample differences in these levels between methods were not significant at the 0.05 level. This is evidence that the two methods yield statistically similar outcomes within the range of concentrations (<8–14 000 ng kg^{-1} by ELISA and 6.8–17 000 ng kg^{-1} by GC/HRMS) represented by these samples. In general, the ELISA-EQ values were similar to the GC/HRMS-derived TEQ values, but the presence of certain PCDD/PCDF congeners and brominated dioxins and furans in the samples could contribute to the differences observed in the measurements between the two methods. We would expect the relationship in measurements between the two methods for the same sample would depend upon the true composition (mixture of the PCDD/PCDF congeners and other compounds with ELISA cross-reactivities) of the sample.

The GC/HRMS method for measuring PCDD/PCDF congeners is costly and time-consuming, involving extraction and multi-column cleanup steps. In this study, the estimated sample throughput by the ELISA method is similar to that for the GC/HRMS method, because similar cleanup procedures must also be performed for the ELISA. The required cleanup procedures somewhat negate the advantages (i.e. simple analysis, high-sample throughput) typically exhibited by an ELISA method. However, the high costs associated with using GC/HRMS instrumentation leads to overall lower costs for the ELISA method. The ELISA method can be easily integrated into most analytical laboratories, even in a mobile facility at remote locations. However, GC/HRMS instrumentation is not commonly available in a typical analytical laboratory due to its high initial operating costs and the need for highly specialized trained operators.

4. Conclusion

In this study an ELISA method for determining PCDDs and PCDFs in real-world soil and sediment samples was evaluated. Results suggest that the ELISA method could be a complementary and relative low-cost detection tool for determining PCDD/PCDF concentrations in site-specific contaminated sediment and soil samples. Note that the relationship between the ELISA and GC/HRMS data from one contaminated site may not apply directly to another contaminated site. Contaminated sites may contain different types of soil and sediment, different composition profiles of PCDDs, PCDFs and other contaminants.

Table 3
ELISA and GC/HRMS classification of sediment and soil samples at or above comparative concentrations

Comparative concentration (pg/g)	Number (%) of 75 sediment and soil samples with:			
	ELISA ⩾ conc.; GC/HRMS < conc. (false positive)	ELISA < conc.; GC/HRMS ⩾ conc. (false negative)	Both ELISA and GC/HRMS ⩾ conc. (true positive)	Both ELISA and GC/HRMS < conc. (true negative)
50	8 (10.7%)	3 (4.0%)	54 (72.0%)	10 (13.3%)
100	10 (13.3%)	4 (5.3%)	46 (61.3%)	15 (20.0%)
200	8 (10.7%)	5 (6.7%)	40 (53.3%)	22 (29.3%)
300	4 (5.3%)	6 (8.0%)	37 (49.3%)	28 (37.3%)
400	4 (5.3%)	3 (4.0%)	36 (48.0%)	32 (42.7%)
500	2 (2.7%)	4 (5.3%)	35 (46.7%)	34 (45.3%)
600	3 (4.0%)	5 (6.7%)	33 (44.0%)	34 (45.3%)
700	3 (4.0%)	4 (5.3%)	33 (44.0%)	35 (46.7%)
800	6 (8.0%)	6 (8.0%)	28 (37.3%)	35 (46.7%)
900	5 (6.7%)	6 (8.0%)	28 (37.3%)	36 (48.0%)
1000	5 (6.7%)	6 (8.0%)	27 (36.0%)	37 (50.7%)

These differences could affect the relationship between the ELISA and GC/HRMS data. Thus, it may be necessary to characterize the site-specific relationship between the ELISA and GC/HRMS data from pilot-scale samples prior to full-scale application of the ELISA method. More studies are needed to develop and validate streamlined effective sample preparation procedures for PCDDs and PCDFs. This will improve the effectiveness of both the ELISA and GC/HRMS methods for screening PCDDs and PCDFs at sites with multiple contaminants and various physical characteristics. Recent investigations of a selective pressurized fluid extraction approach incorporating cleanup absorbents with the sample in an extraction cell for streamlining sample preparation procedures show promising results (Lundstedt et al., 2006; Haglund et al., 2007). A tiered approach employing the ELISA screen prior to the GC/HRMS method could provide improvements in overall PCDD/PCDF analysis. Screening would prioritize and eliminate samples not requiring any further analysis. The prioritization and reduction in sample load would increase the effectiveness of GC/HRMS instrumentation. The possibility of establishing an effective and relatively low-cost monitoring approach (a combination of ELISA and GC/HRMS methods) for PCDDs/PCDFs in sediment and soil from multiple contamination sites for future large-scale studies is warranted.

Notice

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References

ATSDR (Agency for Toxic Substances and Disease Control Registry), 1998. Toxicological Profile for Chlorinated Dibenzo-*p*-dioxins. US Department of Health and Human Service, Atlanta, GA.

Bernes, C., 1998. Persistent Organic Pollutants: A Swedish View of an International Problem. Swedish Environmental Protection Agency, Stockholm.

Corcoran, M.K., 2007. Geomorphic identification and verification of recent sedimentation patterns in the Woonasquatucket River, North Providence, Rhode Island, Final report, ERDC/GSL TR-07-2, March 2007. http://libweb.wes.army.mil/uhtbin/hyperion/GSL-TR-07-2.pdf.

DeRosa, C.T., Brown, D., Dhara, R., Garrett, W., Hansen, J.H., Jones, D., Jordan-Izaguirre, D., O'Conner, R., Pohl, H., Xintaras, C., 1997. Dioxin and dioxin-like compounds in soil, part 1: ATSDR interim policy guidance. Toxicol. Ind. Health 13, 759–768.

European Committee for Standardization, 1997. Stationary source emissions – determination of the mass concentration of PCDDs/PCDFs. EN 1948:2-3. European Committee for Standardization, Brussels.

Fielder, H., 2007. National PCDD/PCDF release inventories under the Stockholm convention on persistent organic pollutants. Chemosphere 67, S96–S108.

Gies, A., Neumeier, G., Rappolder, M., Konietzka, R., 2007. Risk assessment of dioxins and dioxin-like PCBs in food – comments by the German Federal Environmental Agency. Chemosphere 67, S344–S349.

Haglund, P., Sporring, S., Wiberg, K., Bjorklund, E., 2007. Shape-selective extraction of PCBs and dioxins from fish and fish oil using in-cell carbon fractionation pressurized liquid extraction. Anal. Chem. 79, 2945–2951.

Harrison, R.O., Carlson, R.E., 1999. Immunoassay analysis of dioxin in soil: validation of TEQ screening at 500 ppt using rapid extraction and cleanup. Organohal. Comp. 40, 31–34.

Hollander, M., Wolfe, D.A., 1973. Nonparametric Statistical Methods;. John Wiley and Sons, New York.

IARC (International Agency for Research on Cancer), 1997. IARC Monographs programme on the evaluation of carcinogenic risks to humans: polychlorinated-*p*-dibenzodioxins and polychlorinated furans, vol. 69, pp. 1–631.

Im, S.H., Kannan, K., Giesy, J.P., Matsuda, M., Wakimoto, T., 2002. Concentrations and profiles of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in soils from Korea. Environ. Sci. Technol. 36, 3700–3705.

Lo, C., Dermicheva, S., Reiner, E.J., 2005. In: Proceedings for 25th International Symposium on Halogenated Environmental Organic Pollutants and Persistent Organic Pollutants (POPs), ANA-Bioanalytical Approaches for POPs Detection, vol. 67, pp. 23–26.

Lundstedt, S., Haglund, P., Oberg, L., 2006. Simultaneous extraction and fractionation of polycyclic aromatic hydrocarbons and their oxygenated derivatives in soil using selective pressurized liquid extraction. Anal. Chem. 78, 2993.

Misita, M., Schrock, M., Tracy, K., Tabor, J., 2003. Simultaneous extraction of PCDD/PCDF and PCBs using accelerated solvent extraction for sediment, tissue, and sludge matrices. Organohal. Comp. 60, 37–40.

Nichkova, M., Park, E.K., Koivunen, M.E., Kamita, S.G., Gee, S.J., Chuang, J.C., Van Emon, J.M., Hammock, B.D., 2004. Immunochemical determination of dioxins in sediment and serum samples. Talanta 63, 1213–1223.

Nording, M., Nichkova, M., Spinnel, E., Persson, Y., Gee, S.J., Hammock, B.D., Haglund, P., 2006. Rapid screening of dioxin-contaminated soil by accelerated solvent extraction/purification followed by immunochemical detection. Anal. Bioanal. Chem. 385, 357–366.

Okuyama, M., Kobayashi, N., Takeda, W., Anjo, T., Matsuki, Y., Goto, J., Kambegawa, A., Hori, S., 2004. Enzyme-linked immunosorbent assay for monitoring toxic dioxin congeners in milk based on a newly generated monoclonal anti-dioxin antibody. Anal. Chem. 76, 1948–1956.

Persson, Y., Lundstedt, S., Oberg, L., Tysklind, M., 2007. Levels of chlorinated compounds (CPs, PCPPs, PCDEs, PCDFs and PCDDs) in soils at contaminated sawmill sites in Sweden. Chemosphere 66, 234–242.

Roda, A., Mirasoli, M., Michelini, E., Magliulo, M., Simoni, P., Guardigli, M., Curini, R., Sergi, M., Marino, A., 2006. Analytical approach for monitoring endocrine-disrupting compounds in urban waste water treatment plants. Anal. Bioanal. Chem. 385, 742–752.

Rosner, B., 2000. Fundamentals of Biostatistics, fifth ed. Duxbury Press, Massachusetts.

Safe, S.H., 1998. Development validation and problems with the toxic equivalency factor approach for risk assessment of dioxins and related compounds. J. Animal Sci. 76, 134–141.

Sanborn, J.R., Gee, S.J., Gilman, S.D., Sugawara, Y., Jones, A.D., Rogers, J., Szurdoki, F., Stanker, L.H., Stoutamire, D.W., Hammock, B.D., 1998. Hapten synthesis and antibody development for polychlorinated dibenzo-*p*-dioxin immunoassays. J. Agric. Food Chem. 46, 2407–2416.

Shan, G., Leeman, W.R., Gee, S.J., Sanborn, J.R., Jones, A.D., Chang, D.P.Y., Hammock, B.D., 2001. Highly sensitive dioxin immunoassay and its application to soil and biota samples. Anal. Chim. Acta. 444, 169–178.

Snedecor, G.W., Cochran, W.G., 1989. Statistical Methods, eighth ed. Iowa State University Press, Iowa.

Srogi, K., 2007. Overview of analytical methodologies for dioxin analysis. Anal. Lett. 40, 1647–1671.

Stabler, H., 1908. Report on the results of an examination of the conditions causing the pollution of the Moshassuck, Woonasquatucket, and Providence Rivers in the State of Rhode Island. E.L. Freeman Company, State Printers, 37. The Rhode Island State Board of Health, Providence, Rhode Island.

Stanker, L.H., Watkins, B., Rogers, N., Vanderlaan, M., 1987. Monoclonal antibodies for dioxin: antibody characterization and assay development. Toxicology 45, 229.

Sugawara, Y., Gee, S.J., Sanborn, J.R., Gilman, S.D., Hammock, B.D., 1998. Development of a highly sensitive enzyme-linked immunosorbent assay based on polyclonal antibodies for the detection of polychlorinated dibenzo-*p*-dioxins. Anal. Chem. 70, 1092–1099.

Sugawara, K., Saito, K., Ogawa, M., Kobayashi, S., Shan, G., Sanborn, J.R., Hammock, B.D., 2002. Development of dioxin toxicity evaluation method in human milk by enzyme-linked immunosorbent assay–assay validation for human milk. Chemosphere 46, 1471–1476.

SW846 Method 4025, 2002. Screening for polychlorinated dibenzodioxins and polychlorinated dibenzofurans (PCDD/Fs) by immunoassay, Revision 0. http://www.epa.gov/sw-846/main-htm.

Trang, N.T., Hau, V.T., Thin, B.V., To, L.V., Trang, N.T.T., Tuyen, B.C., Ha, P.N., Viet, P.H., Anh, P.T., Son, C.P.N., Allan, R., Mewett, K., Tran, H., Khurana, J., Wang, S., Kennedy, I.R., Lee, N.A., 2007. Application of a cyclodiene-specific ELISA to residue monitoring of agricultural produce and the environment in Vietnam. In: Kennedy, I.R., Solomon, K.R., Gee, S.J., Crossan, A.N., Wang, S., Sanchez-Bayo, F. (Eds.), Rational Environmental Management of Agrochemicals, Risk Assessment, Monitoring, and Remedial Action. American Chemical Society, Washington, DC, pp. 245–258.

USEPA, 1992. Health Assessment Document for 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) and Related Compounds –Volume I, II, and III. US Environmental Protection Agency, Washington, D.C., EPA/600/BP-92/001a,b,c.

USEPA, 1994. Method 1613, Revision B, tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS, EPA 821-B-94-005Q.

USEPA 1997. Addition of dioxin and dioxin-like compounds; modification of polychlorinated biphenyls (PCBs) listing; toxic chemical release reporting; community right-to-know. US Environmental Protection Agency. Federal Register. 62 FR 24887.

USEPA, 1999a. Method 1668, Revision A: chlorinated biphenyl congeners in water, soil, sediment, and tissue by HRGC/HRMS, EPA-821-R-00-002.

USEPA, 1999b. E.P.A. launches new round of sampling of Woonasquatucket River, March 2003. http://www.epa.gov.

USEPA, 2005a. Pilot survey of levels of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, polychlorinated biphenyls and mercury in rural soils of the US. US Environmental Protection Agency, Washington, DC, EPA/600/R-05/043F, http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=150944.

USEPA, 2005b. Innovative technology verification report: technologies for monitoring and measurement of dioxin and dioxin-like compounds in soil and sediment. Hybrizyme Corporation AhRC PCR™ Kit, EPA540/R051005. http://www.epa.gov/ORD/SITE/reports/540r05005/540r05005.pdf.

Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasagawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., Van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Persp. 106, 775–792.

Van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haw, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. Review: the 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.

Van Emon, J.M., 2001. Immunochemical applications in environmental science. J. AOAC Int. 84, 125–133.

Van Emon, J.M., Lopez-Avila, V., 1992. Immunochemical methods for environmental analysis. Anal. Chem. 64, 79A–88A.

Warmer, M., Eskenazi, B., Mocarelli, P., Gerthoux, P.M., Samuels, S., Needham, L., Patterson, D., Brambilla, P., 2002. Serum dioxin concentrations and breast cancer risk in the Seveso women's health study. Environ. Health Persp. 110, 625–628.