Application of Site-Specific Calibration Data Using the CALUX by XDS Bioassay for Dioxin-like Chemicals in Soil and Sediment Samples

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The Chemically Activated Luciferase Gene Expression (CALUX) by Xenobiotic Detection Systems (XDS) bioassay was evaluated for the determination of the presence of dioxin and dioxin-like compounds in soil and sediment in two studies conducted under the U.S. Environmental Protection Agency's Superfund Innovative Technology Evaluation Monitoring and Measurement Technologies Program. In the first study, the results were compared with those generated by established laboratory methods (EPA Method 1613B) using high-resolution mass spectrometry (HRMS). The study results demonstrated that the technology could be used to screen for dioxin concentrations above and below threshold values (e.g., less than or greater than 1 or 50 picograms of toxicity equivalents per gram [pg TEQ/g]); however, the results were not linearly correlated to the HRMS results. A second study was initiated to evaluate performance on a site-specific basis. During the second study, the data from the XDS technology were evaluated in four ways: (1) uncalibrated to HRMS, (2) calibrated using an overall statistical model, (3) calibrated using statistical models generated on a site-specific basis, and (4) calibrated using site-specific calibration factors. The results showed that TEQ data produced by the XDS technology were more precise than the data reported during the first study. The second study also demonstrated that site-specific statistical models were better tools for understanding the relationship between the XDS and HRMS data than a single overall model generated from data from multiple sites. Ultimately, site-specific calibration was shown to be the best approach because it was a simple and accurate way of correcting the XDS data and improving comparability with HRMS.

Introduction

Conventional analytical methods for the determination of concentrations of dioxin and dioxin-like compounds in environmental samples are considered time-consuming and costly. U.S. Environmental Protection Agency (EPA) standard methods require solvent extraction of the sample, processing the extract through multiple cleanup columns, and analysis by gas chromatography (GC)/mass spectrometry (MS) using EPA methods such as 8280, 8290, or 1613 to quantify specific congeners of dioxin. Budgetary constraints and lengthy turnaround times for data reporting often limit the use of these methods to characterize or monitor for dioxin at contaminated sites. High-resolution mass spectrometry (HRMS) analyses using EPA methods 1613 or 8290 can cost from $800 to 1200 per sample, depending on the complexity of the sample, the level of quality assurance/quality control incorporated into the analyses, and the reporting requirements. A more simple and cost-effective analytical method would allow site personnel to assess the extent of contamination or direct and monitor remediation with less expensive and timelier data.

The EPA's Office of Research and Development (ORD), National Exposure Research Laboratory (NERL), contracted with Battelle (Columbus, Ohio) to investigate whether commercially available bioanalytical methods produced quantitative results comparable to HRMS data under the EPA's Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) Program. Technologies evaluated under the SITE MMT Program are expected to provide better, faster, or more cost-effective methods for the production of real-time data during site characterization and remediation activities. One of the technologies evaluated under the SITE MMT Program, Xenobiotic Detection Systems Chemically Activated Luciferase Gene Expression (CALUX by XDS), is a bioassay based on a generically engineered murine cell line that carries a firefly luciferase reporter gene driven by an aryl hydrocarbon receptor (AhR) dependent promoter that is activated when the cells are exposed to dioxins and other dioxin-like chemicals (*1*). The performance of the CALUX by XDS was evaluated in two studies. The first study was a 2004 field demonstration conducted in Saginaw, MI. The results of the first study, published as an EPA report (*2*), suggested that CALUX by XDS could be used to screen for dioxin concentrations above and below threshold values (e.g., less than or greater than 1 or 50 picograms of toxicity equivalents per gram [pg TEQ/g]). However, the XDS values did not demonstrate a high linear correlation with HRMS method 1613B data that were generated for comparison. After publication of these results and presentations of the information at seminars and conferences, the user and regulatory community showed significant interest in evaluation of the performance on a site-specific basis. Consequently, in May 2006, a second study was launched to evaluate the CALUX by XDS on a site-specific basis. This paper presents a comparison of the results from the two SITE MMT studies, demonstrating how relative performance was changed through the use of a site-specific calibration procedure. While there are many excellent articles in the literature that compare the CALUX bioassay data with HRMS results for TEQ measurement in environmental samples (*1, 3–6*), none of these studies use a site-specific calibration procedure.

Analytical Methods

Table 1 summarizes the HRMS and XDS methods, which are similar in that they involve comparable sample extraction and cleanup procedures, but they differ in analytical finish. For the first SITE study, the HRMS data were generated following traditional EPA Method 1613B (*7*). The HRMS

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TABLE 1. Similarities and Differences between CALUX by XDS and HRMS Methods

method step	similarities	differences
sample preparation	solvent extraction	XDS extraction followed a modification of the EPA SW-846 Method 8290. Samples were extracted in an ultrasonic bath with a 20% solution of methanol in toluene and then twice with toluene. The extracts were filtered, pooled, and concentrated by vacuum centrifugation. HRMS extraction followed EPA Method 1613B sample preparation. Samples were extracted with methylene chloride using accelerated solvent extraction.
	chromatographic column cleanup techniques	XDS cleanup method employed a dual-column chromatographic procedure in which the sample was suspended in hexane and rapidly processed through a 33% sulfuric acid silica gel column in series with a patented XCARB activated carbon column to produce two extracts, one containing chlorinated dioxins/furans and one containing PCBs. HRMS cleanup method followed 1613B in which gel permeation chromatography, acid/base back extraction, carbon columns, multilayer silica, and alumina columns were used as needed.
sample analysis	laboratory-based methods technically trained operator	CALUX by XDS assay used a cellular culture that is incubated to produce optimal expression of the luciferase activity and the induction of luciferase activity (which is directly related to the amount of dioxin-like chemicals) is quantified using the luciferase assay kit from Promega. 1613B used HRMS.
quality control	reagent blanks laboratory control samples matrix spikes reference samples	^{13}C-labeled standards used for HRMS methods were not used for CALUX by XDS bioassay, but radioactively labeled standards were used with the XDS bioassay.
data presentation	results reported in TEQ	Results for the CALUX by XDS bioassay were based on relative potency values and called "bio-TEQ". Congener specific analysis for HRMS was based on World Health Organization's Toxic Equivalency Factors.

method estimates TEQs by calculation of the concentration of individual chemical congeners and then multiplication of this concentration by the relative toxicity of each congener. The relative toxicities are assigned using toxic equivalency factors (TEFs) determined by a World Health Organization (WHO) committee (*8*) on dioxin-like chemicals. The WHO TEFs were revised in 2006 (*9*), but the HRMS data in this manuscript were generated prior to the release of the 2006 values. For the second SITE study, the HRMS data were generated from a method used to characterize all of the samples prior to the first study that was based on Method 1613B, but with modifications (see Table S1 in the Supporting Information for a summary of the differences in the traditional and modified Method 1613B). The modifications, including the use of accelerated solvent extraction instead of Soxhlet extraction, lack of secondary column confirmation, analysis of less than 10 g of sample, and the use of estimated data when the calibration ranges were exceeded, were performed to reduce the time and cost of the analyses since the intent was to provide an initial characterization of the TEQ values, prior to analysis of the samples by traditional Method 1613B. It was demonstrated during the first study that data generated by modified Method 1613B (x) were comparable and highly correlated to the data generated by traditional Method 1613B (y) when plotted one-to-one ($y = 0.86x + 41$, $R^2 = 0.99$) (*2*). Therefore, for simplicity, all reference data are referred to as "HRMS" throughout this manuscript; no specific distinction is made between the traditional and modified 1613B methods.

Estimations of TEQs based on biological response, such as from the CALUX by XDS system, are often referred to as "bio-TEQ" results because these estimates do not determine the TEQs of individual dioxin/furan congeners but, rather, are based on the biological response of the entire sample. XDS implements a methodology involving a cell-based method, coupled with processing of the samples, designed to remove most of the nondioxin-like chemicals that may activate the AhR before quantitative determinations of bio-TEQ. XDS recognizes that not all nondioxin-like chemicals will be removed, and additional cleanup for some compounds, such as polynuclear aromatic hydrocarbons (PAHs), may be necessary if the contaminants are present at high levels (*1*). More detailed information about the CALUX by XDS bioassay has been reported in the literature (*1*, *4*) and can be found on the company's web site (http://www.dioxins.com/pages/Publicationstechnical.shtml).

It should also be noted that both the HRMS and XDS methods are capable of analysis of separate fractions for polychlorinated biphenyls (PCBs). Since the focus of the second study was limited to analysis of dioxin/furan congeners, the results discussed in this manuscript are for dioxin/furan congeners only.

Experimental Design

In the first SITE MMT study, a total of 209 soil, sediment, and extract samples with a variety of distinguishing characteristics, such as high levels of PCBs and PAHs, were analyzed as described in the project's demonstration plan (*10*). Samples known to contain dioxin-contaminated soil and sediment were collected from 10 different sites around the country. Prior to use in the demonstration, the samples were homogenized and characterized by modified Method 1613B to ensure that the concentrations of dioxins were over a large dynamic range (<50 to >10 000 pg/g). Certified samples were included in the study as ongoing performance assessments for the reference method (see Table S2 in the Supporting Information for a brief description of the site locations and major contaminants for the environmental and certified samples). XDS was not given information

regarding the concentration levels, dioxin congener patterns, sample types, or sampling sites because technology developers did not feel that such qualitative or quantitative information about the samples collected from the sites was required. The XDS and HRMS data were compared on a sample-to-sample basis.

In the second SITE MMT study, a total of 112 samples were analyzed, segregated into five site batches. Soil and sediment samples were obtained from those archived after the first study, including some previously analyzed samples as part of the first study and some unique samples that were not used as part of the original study but were taken from the same sampling locations. Samples included in the site-specific calibration study experimental design were taken from five of the ten original study sites. The samples were stored in a walk-in freezer (approximately −20 °C) for approximately three years, since the time when the samples were collected for the first study. One sample from each site was reanalyzed by HRMS to confirm that the concentrations had not changed significantly (<20% relative percent difference [RPD]) since the initial analysis (see Table S3 in the Supporting Information for RPD values by site). Certified samples were not included in the second study because the focus was on site-specific calibrations. In contrast to the first study, where all sample information was unknown, relevant information regarding the environmental site for each batch was provided. XDS was provided with the TEQ concentration and congener data for one quality control (QC) sample per site batch; all other sample concentrations were unknown. XDS was given the HRMS data for the QC sample to simulate a confirmatory analysis that could be used to apply a site-specific calibration.

In both studies, four individual replicates of each environmental sample were included as blind samples so that the precision could be assessed. In addition, several replicates of an uncontaminated (blank) soil matrix were included. Other QC techniques integral to the method, including calibration and matrix spiking protocols, were also employed.

Results and Discussion

Comparison of Results from the First and Second Studies. The XDS results from the first study are fully described in an EPA report (*2*), which is posted on the EPA SITE program web site (www.epa.gov/ORD/SITE). The results from the second study are first reported in this document. The results of both studies are included to show the difference in the XDS results obtained by changing the analytical approach and modifying the data analysis.

In the first study, XDS provided bio-TEQ results using a single determination for each sample, operating the technology in the "screening" mode (i.e., the sample extract was analyzed once). XDS operated the technology in the screening mode because the initial study was conducted primarily as a field demonstration (although some samples were also analyzed in XDS's laboratory). Table 2 summarizes the performance of the CALUX by XDS technology in the first study, including precision (reported in terms of percent relative standard deviation [RSD]) and comparability (reported as the ratio of the XDS and HRMS values and called percent recovery [%R]). Table 2 represents a subset of the total data set from the first study because it includes only the TEQ data for samples that were also reported in the second study. As shown in Table 2, the range of RSD values for the XDS data was 13–84%. For comparison, the range of RSD values for the HRMS data was 2–28%. The range of %R values was 113–1611%, with an average %R value of 352%. Acceptable values for RSD are typically less than 25%, while %R values are typically between 75 and 125%. It is concluded that the XDS results were less precise and generally showed a significant high bias relative to the HRMS results.

TABLE 2. Comparison between HRMS and CALUX by XDS Results: First Study, Uncalibrated

	HRMS		XDS		
sample ID	av[a] (pg TEQ/g)	RSD (%)	uncalibrated av[b] (pg TEQ/g)	RSD (%)	%R[c]
Winona					
cell #12	7318	2	32 796	13	448
cell #2	9998	9	161 095	78	1611
Tittabawassee River					
DNR 1	475	10	1689	16	356
DNR 2	37	6	136	46	373
IMP 2	1062	26	2517	34	237
Solutia					
SS 2	65	13	218	24	338
SS 3	2923	5	4789	56	164
SS 4	2015	7	2282	84	113
Raritan Bay					
RB 1	11	5	24	18	227
RB 2	13	2	30	21	232
RB 6	11	5	28	23	265
Newark Bay					
NB 1	41	6	60	37	144
NB 5	16	28	30	32	185
NB 6	56	22	133	62	239

[a] Average based on analysis of four replicate samples. [b] Average based on analysis of screening results (single analysis) of four replicate samples. [c] %R = (av bio-TEQ result/av HRMS result) × 100%.

To reduce the variance of their results, XDS implemented its "comprehensive" analysis protocol (i.e., the sample extract was analyzed three times) in the second study to provide a more precise estimate of bio-TEQ. To minimize cost, all second-study analyses were performed in XDS's laboratories, which is preferred for the CALUX by XDS method unless a specially configured mobile laboratory is available. Table 3 lists the XDS bio-TEQ and HRMS TEQ results for each sampling site. In a manner similar to that in Table 2, comparability is expressed as percent recovery of the XDS results relative to the HRMS results. As shown in Table 3, across all sampling locations, the %R values for the XDS results relative to HRMS results ranged from 96 to 662%, with an average %R value of 279%. XDS results had the least comparability to the HRMS results at the Winona site, with percent recoveries ranging from 487 to 662%. The Winona site was also contaminated with pentachlorophenol and PAHs; it had the highest levels of PAHs of any of the sites evaluated in the second study. The Tittabawassee River and Raritan Bay sites also had poor comparability, with %R values ranging from 240 to 371% and 289 to 365%, respectively. Contributions from other contaminants for these sites (such as PCBs and PAHs) were low. XDS results for the Solutia and Newark Bay sites had the best comparability to the HRMS results, with percent recoveries ranging from 96 to 227% and 118 to 140%, respectively. These sites had low-level PCB contamination (1–100 pg TEQ/g) and low PAH contamination (<5 mg/kg). As a measure of precision, the RSDs for all sampling locations ranged from 1 to 28% for the HRMS data and 9 to 28% for the XDS data, with the exception of the Solutia site; in that case, the RSD ranged from 24 to 47% for the XDS data and 5 to 19% for the HRMS data.

In the comparison of the RSD and %R values for XDS data in the two studies, the second study demonstrated a significant improvement in the precision of the XDS data. However, the HRMS TEQs and the XDS bio-TEQs for most samples still largely differed because the %R values were typically significantly higher than 100%. To account for some

TABLE 3. Comparison between HRMS and CALUX by XDS Results: Second Study, Uncalibrated

	HRMS		XDS		
sample ID	av[a] (pg TEQ/g)	RSD (%)	uncalibrated av[b] (pg TEQ/g)	RSD (%)	%R[c]
Winona					
cell #10	8648	28	57 238	14	662
cell #12	8831	1	51 597	22	584
cell #2	11 071	2	56 021	10	506
cell #4	11 410	4	55 599	18	487
cell #8	11 259	4	59 452	22	528
Tittabawassee River					
DNR 1	435	5	1613	9	371
DNR 2	42	23	127	23	304
FFP 1	3127	7	8828	23	282
FFP 2	1048	19	2511	17	240
IMP 2	808	10	2101	28	260
Solutia					
SS 1	846	18	840	38	99
SS 2	48	10	109	25	227
SS 3	3257	11	3946	24	121
SS 4	1833	19	2177	47	119
SS 5	1279	10	1234	28	96
SS 6	3951	5	3913	27	99
Raritan Bay					
RB 1	14	7	51	18	365
RB 2	12	8	39	10	325
RB 4	15	11	43	10	289
RB 5	14	3	43	14	310
RB 6	13	7	42	17	319
Newark Bay					
NB 1	45	26	61	13	135
NB 2	38	10	53	13	140
NB 3	32	6	39	19	123
NB 5	16	26	22	15	139
NB 6	62	14	73	9	118

[a] Average based on four replicate results. [b] Average based on analysis of comprehensive results (triplicate analysis) of four replicate samples. [c] %R = (av bio-TEQ result/av HRMS result) × 100%

of these differences, the bio-TEQ data set was statistically modeled to determine a relationship between the XDS bio-TEQ data and the HRMS TEQ data. In addition, the TEQ and bio-TEQ data were compared after a site-specific calibration factor was applied to the bio-TEQ data.

Statistical Modeling. Statistical modeling was performed to establish a relationship between the XDS bio-TEQ and HRMS TEQ values and to generate an equation to convert XDS data to HRMS and vice versa. A similar approach using a mathematical model to describe the relationship between XDS bioassay and HRMS data has been previously published by XDS (*1*).

Statistical tests during the model-fitting procedure indicated that the raw data were not normally distributed. After a linear model of the untransformed data was fitted, the residuals were tested for normality, and the null hypothesis that the residuals were normally distributed was rejected with a p-value of <0.01. Therefore, a linear scale was not used. A natural log (ln) transformation of both the HRMS and XDS values put the data on a more evenly spaced scale and produced residuals that appeared to be normally distributed. The transformed values of the replicates for each sample and method were averaged. Least squares linear regression analysis (*11*) was performed on the data. The average ln-transformed data were statistically modeled in two ways: as a whole data set across all sites (with XDS bio-TEQ as the independent variable and HRMS TEQ as the dependent variable) and with separate slopes for each site (where site and XDS bio-TEQ were both independent variables and HRMS TEQ was the dependent variable). Predictive equations and 95% prediction intervals were produced for each statistical model. The predictions can be transformed back from the log scale (exponential) so that 95% prediction intervals can be produced for HRMS values (see Figures S1–S6 in the Supporting Information for the plots and equations for the statistical modeling).

There was good correlation ($p < 0.0001$, $R^2 = 0.9708$) between the log-transformed bio-TEQs and HRMS TEQs with the data not segregated by site. However, when either the TEQs or the bio-TEQs were high, the 95% prediction intervals were rather wide. For example, when XDS reported a bio-TEQ of 10, the predicted HRMS value was 3 with a 95% prediction interval of 1–8, meaning a 95% certainty that HRMS TEQ will be between 1 and 8 pg/g. If XDS reported a bio-TEQ at a higher level, such as 60 000 pg/g, the predicted HRMS TEQ was 10 782 pg/g with a 95% prediction interval between 3772 and 30 820 pg/g.

When the TEQs were log-transformed and statistically modeled by site, there was a better fit ($p < 0.0001$, $R^2 = 0.9966$) than with the overall model ($p < 0.0001$, $R^2 = 0.9708$). Table 4 shows the 95% prediction intervals for each site, with the corresponding 95% prediction intervals for the overall model. Bio-TEQ values were selected to compare HRMS TEQ values generated by the site-specific and overall models. As shown in Table 4, using the same bio-TEQ values, there is less variability in the prediction intervals by statistically modeling the data based on site. In most cases, the variability is decreased by a factor of 2. In addition to decreased variability, a site-specific statistical model is more representative of site conditions than an overall model. Because the congener patterns and matrix interferences vary by site, no fixed relationship would exist between the HRMS-derived TEQ and the CALUX by XDS response; thus, a site-specific statistical model is a better representation of site conditions.

To test the site-specific statistical models using actual XDS bio-TEQ data, the unique data from the first study were inserted into the site-specific statistical models (generated using the second study's data). This was possible because the first study contained unique samples from the same sites used in the second study. HRMS TEQ values were predicted from the XDS bio-TEQ values using the site-specific models and compared with actual HRMS TEQ values generated in the first study. The site-specific models worked well for prediction of the HRMS TEQs from the first study's bio-TEQs for Raritan Bay samples, where 88% of the time the model was able to predict a TEQ within a difference of ≤30% from the HRMS average results in the first study. The site-specific statistical models were not as accurate at predicting the HRMS TEQs for the other sites. The site-specific statistical model predicted HRMS TEQ within a difference of ≤30% of the HRMS average value from the first study 38% of the time for Newark Bay, 25% of the time for Solutia, 50% of the time for Tittabawassee River, and 50% of the time for Winona. Each site-specific statistical model's ability to predict HRMS TEQ data compared with actual data from the first study was likely also impacted by sample analysis in the screening mode, which by design was less accurate and less precise than XDS's comprehensive mode designed to reduce variance. In summary, statistical modeling of the data was rather complex and did not prove to be highly accurate at predicting HRMS values, although site-specific modeling was preferred to overall modeling because of higher correlation and reduced variance.

Use of Site-Specific Calibration Factor. Site-specific calibration, used by EPA and other organizations (*12, 13*), involves calibration of an alternative technology using one or more sample results generated using a standard reference

TABLE 4. 95% Prediction Intervals for HRMS TEQs Based on XDS bio-TEQs Comparing Site-Specific Statistical Model and Overall Statistical Model

XDS (pg TEQ/g)	predicted HRMS (pg TEQ/g)		95% prediction interval	
simulated value	site-specific statistical model	overall statistical model	site-specific statistical model	overall statistical model
Winona				
50 000	9033	9944	5078–16 069	3502–45 849
Tittabawassee River				
50	19	24	11–32	9–110
1000	327	668	208–514	244–1830
8000	3149	3529	1894–5236	773–9082
Solutia				
50	30	24	18–51	9–110
1000	796	668	513–1235	244–1830
3500	3693	1938	2328–5856	382–4438
Raritan Bay				
40	13	18	8–21	8–90
50	15	24	9–25	9–110
Newark Bay				
25	19	10	11–31	5–60
50	38	24	25–59	9–110
70	55	37	34–89	13–148

method. It is most commonly used in conjunction with immunochemical techniques to normalize the assay cross-reactivity to site-specific contaminants (*14, 15*).

In the second SITE MMT study, the site-specific calibration factor was derived from a QC sample in each environmental site by dividing the HRMS TEQ result by the XDS bio-TEQ result for the QC sample. Each XDS bio-TEQ result was then multiplied by that factor to generate a site-calibrated bio-TEQ result. Table 5 lists the averages of the bio-TEQ data after the site-specific calibration factors and multisample site-specific calibration factors were applied. The XDS bio-TEQ results are dramatically more comparable to the HRMS results after application of the site-specific calibration factors. Percent recoveries across all sampling locations ranged from 60 to 218% (compared with 96 to 662% as shown in Table 3), with an average %R value of 109% (compared with 279%). XDS results for Winona and Newark Bay ranged from 84 to 115% and 104 to 124%, respectively. XDS results for the Tittabawassee River ranged from 60 to 91%. The site-corrected XDS data for Solutia had the least agreement with the HRMS data, with percent recoveries ranging from 93 to 218%. Because one sample from a site may not be representative of all site conditions, after the data were received, three to five additional data points were used to refine the site-specific correction factor. Results were slightly more comparable when a multisample ($n = 5$) correction factor was applied to the Solutia data, with percent recoveries ranging from 80 to 189%. A similar improvement was achieved when a multisample ($n = 3$) correction factor was applied to the XDS data for Raritan Bay. Percent recoveries ranged from 121 to 152% for the site-corrected XDS data, and the recovery range improved to 97 to 123% when a multi-sample correction factor was applied to the data. When either the single- or multi-sample site-specific calibration correction for a given site shown in Table 5 is considered, the XDS bio-TEQ data generated %R values within 75 to 125% for 22 of 26 sample sets (85% of the time), compared with 27% of the time (7 out of 26 sample sets, shown in Table 3) when the XDS data were not calibrated using HRMS data.

To further evaluate this procedure, the site-specific calibration approach was applied to the first study's data. The results in Table 2 were calibrated using single-sample and multi-sample (where appropriate) site-specific calibration factors derived in the second study. Because XDS implemented a more precise analytical method (comprehensive versus screening) in the second study, application of the second study's calibration factor to the first study's data will not be ideally representative. Although the site-specific calibration factor is not representative of the specific analytical conditions of each method, a trend may be noted because application of the factor should demonstrate closer agreement to the HRMS data. Table 6 presents the results of this evaluation. All 14 of the %R values in Table 6 had lower %R values than when uncalibrated (Table 2), demonstrating closer agreement to the HRMS values. In addition, half-of the %R values in Table 6 fell between 75 and 125%, where only one value was in this range in Table 2. The average %R value was 143% compared with 352%. This assessment further substantiates that the site-specific calibration factor is viable for the transformation of the XDS bio-TEQ data into results more comparable to the HRMS data.

On the basis of the results of these studies, the uncorrected XDS bio-TEQ values and the HRMS TEQ values were not considered to be directly comparable. A site-specific statistical model proved to be a better tool for correlating the XDS and HRMS TEQ values than an overall model that used data from multiple sites. However, using a simplistic site-specific calibration factor, a technique recognized and applied by the EPA, yielded data that transformed the biologically-based CALUX by XDS data to better agree with chemically-derived HRMS estimates of contamination and, overall, was shown to be the best approach for improving the correlation between the XDS bio-TEQ and HRMS TEQ values. This data treatment was straightforward, and the site-specific calibration factor generated XDS bio-TEQ data within 25% of the HRMS TEQ data 85% of the time.

Because the direct linear correlation between CALUX by XDS technology and the HRMS method was not always within the normal acceptance range even after application of site-specific calibration factors, the XDS method warrants further investigation for other aspects beyond the scope of the studies described in this manuscript. For example, identification of other compounds contained in the XDS extracts that would respond to and be quantified by the bioassay method but are not being quantified by the HRMS method may reveal why the results of the two methods are not directly

TABLE 5. Comparison between HRMS and CALUX by XDS Results: Second Study, Site-Specific Calibration Factor

	HRMS		XDS		
sample	av[a] (pg TEQ/g)	RSD (%)	site-specific calibrated av[b] (pg TEQ/g)	RSD (%)	%R[c]
Winona					
cell #10	8648	28	9921	14	115
cell #12	8831	1	8944	22	101
cell #2	11 071	2	9257	4	84
cell #4	11 410	4	9637	18	84
cell #8	11 259	4	10 305	22	92
Tittabawassee River					
DNR 1	435	5	396	10	91
DNR 2	42	23	32	23	76
FFP 1	3127	7	2222	23	71
FFP 2	1048	19	632	17	60
IMP 2	808	10	529	28	65
Solutia					
SS 1	846	18	807	38	95
SS 2	48	10	105	25	218
SS 3	3257	11	3790	24	116
SS 4	1833	19	2178	54	119
SS 5	1279	10	1185	28	93
SS 6	3951	5	3759	27	95
Solutia[d]					
SS 1	846	18	701	38	83
SS 2	48	10	91	25	189
SS 3	3257	11	3292	24	101
SS 4	1833	19	1891	54	103
SS 5	1279	10	1029	28	80
SS 6	3951	5	3210	34	81
Raritan Bay					
RB 1	14	7	21	18	152
RB 2	12	8	16	10	136
RB 4	15	11	18	10	121
RB 5	14	3	18	14	130
RB 6	13	7	19	1	145
Raritan Bay[d]					
RB 1	14	7	17	18	123
RB 2	12	8	13	10	110
RB 4	15	11	15	11	97
RB 5	14	3	15	14	105
RB 6	13	7	15	1	117
Newark Bay					
NB 1	45	26	54	13	119
NB 2	38	10	47	13	124
NB 3	32	6	36	22	112
NB 5	16	26	20	15	123
NB 6	62	14	65	9	104

[a] Average based on three to four replicate results. [b] Average based on analysis of comprehensive results (triplicate analysis) of four replicate samples. [c] %R = (av bio-TEQ result/av HRMS result) × 100%. [d] Calculated using a multisample site-specific calibration factor.

TABLE 6. Comparison between HRMS and CALUX by XDS Results: First Study, Site-Specific Calibration Factor

	HRMS		XDS		
sample ID	av[a] (pg TEQ/g)	RSD (%)	site-specific calibrated av[b] (pg TEQ/g)	RSD (%)	%R[c]
Winona					
cell #12	7318	2	5685	13	78
cell #2	9998	9	27 923	78	279
Tittabawassee River					
DNR 1	475	10	425	16	94
DNR 2	37	6	34	46	90
IMP 2	1062	26	633	34	60
Solutia					
SS 2	65	13	210 (182)[d]	24	325 (282)[d]
SS 3	2923	5	4600 (3996)	56	157 (137)
SS 4	2015	7	2192 (1904)	84	109 (95)
Raritan Bay					
RB 1	11	5	10 (8)	18	95 (76)
RB 2	13	2	13 (10)	21	97 (78)
RB 6	11	5	12 (9)	23	111 (89)
Newark Bay					
NB 1	16	28	27	32	164
NB 2	56	22	117	62	211
NB 3	41	6	53	37	127

[a] Average based on four replicate results. [b] Average based on analysis of comprehensive results (triplicate analysis) of four replicate samples. [c] %R = (av bio-TEQ result/av HRMS result) × 100%. [d] %R values in parentheses were calculated using a multisample site-specific calibration factor.

comparable. It is also possible that other factors inherent to the XDS method (such as selection of a dilution factor, which often varied within replicate sample analysis, and the precision of the method, which was sometimes above generally accepted levels) may influence the comparability.

On the basis of the findings of the studies, the XDS procedure appears to work best to screen samples or to monitor cleanup activities after HRMS data have been obtained to characterize the site and provide the necessary calibration data. Overall, HRMS and the CALUX by XDS technology can be used in conjunction to provide a useful tool for risk assessment and risk management decisions on remediation of hazardous waste and contaminated sites. The use of these two techniques in combination for estimation of potential human health hazards in contaminated sites would both speed remediation and reduce costs.

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Supporting Information Available

Equations, plots of the six statistical models (Figures S1–S6), and summary tables describing the difference between the traditional and modified Method 1613B (Table S1), the environmental and performance evaluation samples that were included in the studies (Table S2), and analytical confirmation data for the archived samples (Table S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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