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Performance of the CAPE Technologies DF1 Dioxin/Furan Immunoassay Kit for Soil and Sediment Samples

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Prepared by

Battelle
505 King Avenue
Columbus, Ohio 43201

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Stephen Billets
Environmental Sciences Division
National Exposure Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Las Vegas, NV 89119

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Abstract

A demonstration of screening technologies for determining the presence of dioxin and dioxin-like compounds in soil and sediment was conducted under the U.S. Environmental Protection Agency's (EPA's) Superfund Innovative Technology Evaluation Program in Saginaw, Michigan in 2004. The objectives of the demonstration included evaluating each participating technology's accuracy, precision, sensitivity, sample throughput, tendency for matrix effects, and cost. The test also included an assessment of how well the technology's results compared to those generated by established laboratory methods using high-resolution mass spectrometry (HRMS). The demonstration objectives were accomplished by evaluating the results generated by each technology from 209 soil, sediment, and extract samples. The test samples included performance evaluation (PE) samples (i.e., contaminant concentrations were certified or the samples were spiked with known contaminants) and environmental samples collected from 10 different sampling locations. The PE and environmental samples were distributed to the technology developers in blind, random order. One of the participants in the original SITE demonstration was CAPE Technologies, which demonstrated the use of the DF1 Dioxin/Furan Immunoassay Kit.

The developers and potential users of the technologies provided feedback after the demonstration. There was significant interest in evaluating the performance of these technologies on a site-specific basis. This would more closely represent the expected application of the technologies than was the case during the original demonstration, which targeted technology performance when challenged with a broad range of sample types. Consequently, a second test (referred to as the "site-specific study") was conducted in which the developers were given a total of 112 samples that were segregated by site of origin. In contrast to the original demonstration, in which all sample information was unknown, environmental information for each site was provided to the developers to more closely represent the background information that would be available to contractors supporting a site-specific application. Each batch included some samples previously analyzed as part of the SITE Dioxin Demonstration and some unique samples in archive that were not used as part of the SITE Dioxin Demonstration, along with replicates and quality control (QC) samples. Only dioxin and furan concentrations were evaluated in this study. The developers were given the HRMS data from the SITE Dioxin Demonstration so that they would have the opportunity to utilize a site-specific calibration and knowledge regarding typical congener patterns at a particular site. Data analysis focused on analytical performance on a site-specific basis, and included an evaluation of comparability to the HRMS total dioxin/furan toxicity equivalents ($TEQ_{D/F}$) results over a range of TEQ concentrations from 10 to 12,000 picogram/gram, precision on replicate analyses, and QC sample results.

This report describes the experimental design of the site-specific study, the analytical methods used, and comparisons of the $TEQ_{D/F}$ results from the HRMS data to those reported by CAPE Technologies. The data generated and evaluated during the site-specific study showed that the TEQ data produced by the DF1 was more comparable to the HRMS $TEQ_{D/F}$ data than was the data reported during the original SITE demonstration. The quantitative correlation with HRMS $TEQ_{D/F}$ was 0.94 for all the samples in the site specific study. The average percent recovery value was 122% with a range between 48% and 354%. The average relative standard deviation for the site specific study was 26%, with a range between 6% and 63%. These results show that the DF1 kit could be used as an effective screening tool to determine areas of greatest concern for cleanup at a site and could help to minimize the number of more expensive analyses needed for specific analytes, particularly considering that the cost and the time to analyze samples is significantly less than that of HRMS analyses.

Contents

Page

List of Tables

List of Figures

Abbreviations, Acronyms, and Symbols

AhR	aryl hydrocarbon receptor
ASE	accelerated solvent extraction
ATSDR	Centers for Disease Control's Agency for Toxic Substances and Disease Registry
D/F	dioxin/furan
D/QAPP	demonstration and quality assurance project plan
DQO	data quality objective
EIA	enzyme immunoassay
EPA	Environmental Protection Agency
ERA	Environmental Resource Associates
g	gram
GC	gas chromatography
GPC	gel permeation chromatography
HRMS	high-resolution mass spectrometry
ITVR	innovative technology verification report
MDEQ	Michigan Department of Environmental Quality
MMT	Monitoring and Measurement Technology
MS	mass spectrometry
NERL	National Exposure Research Laboratory
NIST	National Institute for Standards and Technology
ORD	Office of Research and Development
PAH	polynuclear aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-*p*-dioxin/dibenzofuran
PE	performance evaluation
pg	picogram
ppt	parts per trillion; picogram/g; pg/g
QA/QC	quality assurance/quality control
RPD	relative percent difference
RSD	relative standard deviation
SITE	Superfund Innovative Technology Evaluation

TAT	turn around time
TCDD	tetrachlorodibenzo-*p*-dioxin
TCDF	tetrachlorodibenzofuran
TEF	toxicity equivalency factor
TEQ	toxicity equivalent
$TEQ_{D/F}$	total toxicity equivalents of dioxins/furans
WHO	World Health Organization

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Chapter 1
Introduction

1.1 SITE MMT Program Description

The U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), National Exposure Research Laboratory (NERL) contracted with Battelle (Columbus, Ohio) to conduct a demonstration of monitoring and measurement technologies for dioxin and dioxin-like compounds in soil and sediment. Testing of screening technologies for dioxin and dioxin-like compounds was conducted as part of the EPA Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) Program. The MMT Program evaluates technologies that sample, detect, monitor, or measure hazardous and toxic substances. These technologies are expected to provide better, faster, or more cost-effective methods for producing real-time data during site characterization and remediation efforts than conventional laboratory technologies. The purpose of the SITE MMT Program is to: (1) verify reliable performance by the technologies; (2) provide potential users with a better understanding of the technologies' performance under well-defined conditions; and (3) provide technology developers with documented results that will help promote the acceptance and use of their technologies.

1.2 Background of SITE Dioxin Demonstration

Conventional analytical methods for determining concentrations of dioxin and dioxin-like compounds are time-consuming and costly. For example, EPA standard methods require solvent extraction of the sample, processing the extract through multiple cleanup columns, and analyzing the cleaned fraction by gas chromatography (GC)/high-resolution mass spectrometry (HRMS). Turnaround times for HRMS results are typically three weeks. Use of these traditional methods for high volume sampling or screening a contaminated site often is limited by budgetary constraints. The cost of these analyses can range from $800 to $1,200 per sample, depending on the method selected, the level of quality assurance/quality control incorporated into the analyses, and reporting requirements. The use of a simple, rapid (i.e., real-time or near real-time), cost-effective screening tool would allow field personnel to quickly map the area of contamination at a site and could be used to direct or monitor remediation or risk assessment activities. This data could be used to provide immediate feedback on potential health risks associated with the site and permit the development of a more focused and cost-effective sampling strategy.

Five technology developers participated in the SITE MMT Dioxin Demonstration in 2004 (referred to as the "original SITE demonstration" throughout this report). The participating technologies included immunoassay test kits and aryl hydrocarbon receptor (AhR)-based bioassays. A field demonstration of the technologies was conducted in Saginaw, Michigan. A test suite of 209 soil, sediment, and extract samples with a variety of distinguishing characteristics, such as high levels of polychlorinated biphenyls (PCBs) and polynuclear aromatic hydrocarbons (PAHs), was analyzed by each developer as described in the project's demonstration plan (U.S. EPA, 2004). Samples were collected from 10 different sites around the country with a known variety of dioxin-contaminated soil and sediment. Samples were identified and supplied through EPA Regions 2, 3, 4, 5, and 7 and the Michigan Department of Environmental Quality (MDEQ). In addition to providing environmental samples, MDEQ also facilitated access to the field demonstration site and provided on-site technical and logistical support. The samples were homogenized and characterized by HRMS prior to use in the original SITE demonstration to ensure a variety of

homogeneous, environmentally derived samples with concentrations over a large dynamic range (< 50 to > 10,000 picogram/gram [pg/g]) were included. The environmental samples comprised 61% of the test samples (128 of the 209 samples) included in the original SITE demonstration. Performance evaluation (PE) samples were obtained from five commercial sources. PE samples consisted of known quantities of dioxin and dioxin-like compounds. Fifty-eight of the 209 demonstration samples (28%) were PE samples. Soil or sediment samples were extracted with toluene using Dean Stark Soxhlet extraction, and aliquots were provided to each of the five study participants to avoid possible variation due to sample heterogeneity. A total of 23 extracts (11% of the total number of samples) was included in the original SITE demonstration. For the 209 samples, sample type and sampling site were unknown to the developer during the analysis in order to challenge the technologies with a variety of matrices and potential interferences in an unbiased way. During the development of the demonstration plan, the possibility of identifying the environmental site to the developers was discussed, but the Demonstration Panel (which included all of the developers and approximately 20 EPA Regional experts) concluded that all sample analyses should be blind to the developers. Also, all developers refused additional sample information when it was offered to them prior to the demonstration. An EPA innovative technology verification report (ITVR) was published for each technology (U.S. EPA, 2005a, b, c, d, e). Each report is posted on the EPA SITE program Web Site (www.epa.gov/ORD/SITE/reports.html).

The results of the original SITE demonstration suggested that all of the technologies could be used in some capacity to screen for sample concentrations above and below threshold values (e.g., less than or greater than 1,000 pg/g toxicity equivalents (TEQ)). However, none of the tested technologies demonstrated a significantly high correlation with the HRMS data. After publication of the SITE reports and dissemination of the information through seminars and conference presentations, subsequent feedback from the developers and from potential users of the technologies indicated significant interest in evaluating the performance of these technologies on a site-specific basis. The consensus was that, if the technology developers had more information about the sample identities (for example, sample site) and had access to historical analytical information, the results from the screening technologies would be more highly correlated to the HRMS results. Since this type of information (sample location and dioxin congeners) would typically be made available during a site characterization, this approach was adopted and a second study was launched.

All past participants in the original SITE demonstration were invited to participate in the follow-on study (referred to as the "site-specific study"), and three developers did so. The study was conducted in the developer's laboratories, rather than a central demonstration site, since the experiences of the original SITE demonstration suggested that these were primarily laboratory-based technologies that could be mobilized in a field environment. The developers were given a total of 112 samples that were segregated by site and asked to report sample concentration in terms of total $TEQ_{D/F.}$ (Only dioxin and furan concentrations were evaluated due to the limited range of PCB concentrations in the samples that were available for this study). In contrast to the original SITE demonstration in which all sample information was unknown, environmental information for each site was provided to the developers. Samples were obtained from archived samples from the original SITE demonstration. Each batch included some samples previously analyzed as part of the original SITE demonstration and additional samples in archive along with replicates and one quality control (QC) sample per site batch. The developers were provided with the HRMS $TEQ_{D/F}$ concentration and dioxin congener data for the QC sample only. This provided the developers with an opportunity to calibrate their results on a site-specific basis using the HRMS data from the QC sample for each site. (Note, however, that CAPE Technologies elected to use site-specific samples from its archive from the original demonstration; see Section 3.3.1 for additional details.) The developers were asked to analyze the QC sample unspiked, then spike the QC sample with a known quantity of dioxin congeners (which congeners and at what concentration was left to the discretion of the developer) in duplicate to assess accuracy. Data analysis focused on analytical performance on a site-specific basis, and included an evaluation of comparability to the HRMS total dioxin/furan toxicity

equivalents ($TEQ_{D/F}$) results, precision on replicate analyses, and QC sample results. One of the participants in the site-specific study was the DF1 Dioxin/Furan Immunoassay kit by CAPE Technologies, and the results for this technology are described in this report.

1.3 Description of CAPE Technologies

CAPE Technologies was founded in 1996 to develop and market immunoassay test kits and supporting technology for analysis of dioxins and related compounds. The principal scientists responsible for the technology development leading to the current study have more than 50 person-years of experience in the design, development, validation, marketing, and technical support of immunoassays for environmental analysis. This collective experience encompasses five EPA 4000 series methods, including Method 4025 for dioxin/furan TEQ, which was accepted by EPA in 2001.

After Method 4025 was accepted, improved rapid sample preparation technology was developed by CAPE Technologies to extend the capabilities of the original Method 4025. This approach was utilized for both SITE demonstration studies and is generally referred to as modified Method 4025, or Method 4025m.

In addition to marketing of sample preparation kits and immunoassay test kits, CAPE Technologies performs analytical services using all of the kits it sells, including kits for dioxin/furan TEQ, dioxin-like PCB TEQ, and total PCBs.

In 2000, CAPE Technologies was selected by EPA Region 1 as Environmental Technology Innovator of the Year.

1.4 Overview of the Report

This report describes the experimental design of the site-specific study. Detailed methods are provided for the CAPE Technologies and the HRMS methods are also discussed. Correlations between DF1 $TEQ_{D/F}$ and HRMS $TEQ_{D/F}$ results are discussed along with the accuracy and precision of the test results. A comparison of the DF1 kit's performance in the original SITE demonstration (U.S. EPA, 2005d) and this site-specific study is also presented. Operational factors such as cost comparisons, availability, turnaround times, and ease of use and training are also reported, although the information was provided by CAPE Technologies and was not independently verified. Note that an independent assessment of ease of use and other operational factors was performed and reported with the original SITE demonstration (U.S. EPA, 2005d).

Chapter 2
Test Description

2.1 Experimental Design

Samples used in the site-specific study had been collected for the original SITE demonstration from a variety of dioxin-contaminated soil and sediment sampling locations around the country. Samples were identified and supplied by EPA Regions 2, 3, 4, 5, and 7 and the MDEQ. A mixture of soil and sediment samples that would bracket the Centers for Disease Control's Agency for Toxic Substances and Disease Registry (ATSDR) guidance levels (DeRosa, 1997) were used. The ATSDR decision framework specifies that sites with TEQ levels between 50 and 1,000 picogram per gram (pg/g) should be further evaluated and recommends action for levels above 1,000 pg/g (i.e., 1 part per billion (ppb)) TEQ. The samples were homogenized and characterized by HRMS prior to use in the original SITE demonstration to ensure inclusion of a variety of homogeneous, environmentally derived samples with concentrations over a large dynamic range (<50 to >10,000 picogram/gram [pg/g]). Procedures for homogenization and characterization are described in the demonstration/quality assurance project plan that can be found on the SITE Program's Web Site (www.epa.gov/ORD/SITE) (U.S. EPA, 2004). Samples included in the site-specific study experimental design were from five of the ten original SITE demonstration sites and represented different matrices, congener patterns, and potential interferences. The environmental sites included in the site-specific study were: Tittabawassee River, Newark Bay, Solutia, Raritan Bay, and Winona. The samples had been stored in a freezer (approximately -20 °C) at Battelle for approximately three years, since the time when the samples were collected for the original SITE demonstration. As shown in Table 2-1, one sample from each site was analyzed by HRMS to confirm that the concentrations had not changed significantly (>20% relative percent difference (RPD)) since the initial analysis.

Table 2-1. HRMS Holding Time Analysis for Archived Samples

Site	Original Total $TEQ_{D/F}$ (pg/g) from Characterization Analysis	2006 Total $TEQ_{D/F}$ (pg/g) from Holding Time Check	Relative Percent Difference (%)
Tittabawassee River	3127	2560	20
Newark Bay	38.3	36.7	4
Solutia	3951	4768	19
Raritan Bay	13.8	14.3	3
Winona	11259	10156	10

Only dioxin/furan (D/F) concentrations were evaluated in this study, as the PCB concentrations in the available environmental samples ranged from 0.5 to 40 pg/g TEQ_{PCB} and most concentrations were <10 pg/g $TEQ_{PCB,}$. (Site-specific PCB concentrations are listed in Section 2.2.) Consequently, the dynamic range of the PCB concentrations was inadequate for an effective evaluation of the technologies. A total of 112 samples were included in this study and evaluated by each technology. The distribution of samples amongst the five environmental sites and range of concentrations analyzed are described in Table 2-2. Five or six discrete sampling locations were included in each site batch. The samples in each site batch included those from sampling locations that were previously analyzed as part of the original SITE

demonstration. In addition, samples from locations within the site that were in archive that were not utilized as part of the original study were included. Four replicates of each environmental sample were included to determine analysis precision. Included in the number of samples per site are one to four replicates of an uncontaminated ("blank") soil matrix in each site batch. The sample concentrations and identities were unknown to the developers, but the samples were grouped by site batch, so that the developers knew which samples came from which site. The HRMS data for one sample from each site, the QC sample, was provided to the developers. For the QC samples only, the developers had access to all HRMS congener (dioxin, furan, PCB) data and supporting analytical information (e.g., PAH concentrations) that was available. The intention was that the QC samples would provide historical analytical information that could be used to calibrate the technology responses on a site-specific basis. The developers were also asked to spike the QC samples in duplicate to serve as a matrix spike/matrix spike duplicate. Congener and concentration selection for the spiking solution were the developer's choice.

It should be noted that it was not an objective of the demonstration to accurately characterize the concentration of dioxins, furans, and PCBs from a specific sampling site. It was, however, an objective to ensure comparability between technology samples and the HRMS analysis samples. This was accomplished by homogenizing each matrix, such that all sub-samples of a given matrix had consistent contaminant concentrations. As a result, homogenized samples were not necessarily representative of original concentrations at the site.

Table 2-2. Summary of Site-Specific Study Experimental Design

Site	Matrix	Approximate Range of Concentrations (pg/g $TEQ_{D/F}$)	# Samples [a]
Winona	Soil	8,000 – 12,000	21
Tittabawassee River	Soil	40 – 1,100	24
Newark Bay	Sediment	15 – 65	21
Raritan Bay	Sediment	10 – 15	21
Solutia	Soil	40 – 4,000	25
Total number of site-specific study samples			112

[a] # samples includes one blank sample per site, except for Tittabawassee River which had four blanks included

2.2 Site Descriptions

This section provides descriptions of each of the soil and sediment sites, including how the sites became contaminated and approximate dioxin concentrations, as well as the type and concentrations of other major constituents (such as PCBs, pentachlorophenol (PCP), and PAHs), where known. This information was provided by the site owners/sample providers (e.g., the EPA, the EPA contractors, and the MDEQ).

2.2.1 Soil Sampling Sites

2.2.1.1 Winona

The Winona site in Winona, Missouri, was a wood treatment facility that had been remediated. Contaminants at the site included PCP, dioxin, diesel fuel, and PAHs. PCB concentrations are between 0.9 - 2.2 pg/g TEQ. Over a period of at least 40 years, these contaminants were deposited into an on-site drainage ditch and sinkhole. Areas of contaminant deposition (approximately 8,500 cubic yards of soils/sludge) were excavated in late 2001/early 2002. This material was placed into an approximately 2½-acre treatment cell located on facility property. During 2002/2003, material at the treatment cell was treated through addition of amendments (high-ammonia fertilizer and manure) and tilling. Final

concentrations achieved in the treatment cell averaged 26 milligrams per kilogram (mg/kg) for PCP and from 8,000 to 10,000 for pg/g $TEQ_{D/F}$. Samples used for this study from this site were obtained from the treatment cell after these concentrations had been achieved.

2.2.1.2 Solutia

The chemical production facility at the Solutia site in Nitro, West Virginia, is located along the eastern bank of the Kanawha River, in Putnam County, West Virginia. The site has been used for chemical production since the early 1910s. The initial production facility was developed by the U.S. government for the production of military munitions during the World War I era between 1918 and 1921. The facility was then purchased by a small private chemical company, which began manufacturing chloride, phosphate, and phenol compounds at the site. A major chemical manufacturer purchased the facility in 1929 from Rubber Services Company. The company continued to expand operations and accelerated its growth in the 1940s. A variety of raw materials has been used at the facility over the years, including inorganic compounds, organic solvents, and other organic compounds, including Agent Orange. Agent Orange is a mixture of chemicals containing equal amounts of two herbicides: 2,4-D (2,4 dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5 trichlorophenoxyacetic acid). Manufacture of this chemical herbicide began at the site in 1948 and ceased in 1969. Dioxin contamination in the site soils was associated with the manufacture of 2,4,5-T, where dioxins are an unintentional by-product. The site has a dioxin profile from the ppt to low parts per billion (ppb) range. PCB concentrations ranged between 0.5 - 37 pg/g TEQ.

2.2.1.3 Tittabawassee River

The MDEQ sampled Tittabawassee River flood plain soils at three sites. The contamination source was possibly legacy contamination from chemical manufacturing. Individual samples were collected from two locations at Imerman Park in Saginaw Township. The first sample was taken near the boat launch, and the second sample was taken in a grassy area near the river bank. Previous analyses from these areas of this park indicated a range of PCDD/F concentrations from 600 to 2,500 pg/g. PCB concentrations were found to be between 1 and 8 pg/g TEQ. Individual samples were collected from two locations at Freeland Festival Park in Freeland, MI. The first sample was taken above the river bank, and the second sample was taken near a brushy forested area.

2.2.2 Sediment Sampling Sites

2.2.2.1 Newark Bay

Surrounded by manufacturing industries, Newark Bay is a highly contaminated area with numerous sources (sewage treatment plants, National Pollutant Discharge Elimination System discharges, and nonpoint sources). This bay is downstream from a dioxin Superfund site that contains some of the highest dioxin concentrations in the United States and also is downstream from a mercury Superfund site. The dioxin concentration in the area sampled for this demonstration was approximately 450 pg/g. PCB concentrations were found to be between 1 and 5 pg/g TEQ. Fine-grained sediments make up 50% to 90% of the dredged material. Average total organic carbon was about 4%.

2.2.2.2 Raritan Bay

Surrounded by industry and residential discharges, Raritan Bay has dioxin contamination, but to a lesser degree than Newark Bay. No major Superfund sites are located in the vicinity. Dioxin concentration should be significantly less than in Newark Bay. PCB concentrations were found to be around 2 pg/g TEQ. The fine-grained sediment and total organic carbon values were similar to percentages in Newark Bay.

2.3 Overview of Testing

Samples from each of the five sites were sent to each developer in a "site batch", the compositions of which are shown in Table 2-2. A total of 112 individual samples were provided to the developers. Samples within each site batch were distributed in blind, random order. Samples from a particular site were colored-coded (e.g., Site Batch #1 had green labels, Site Batch #2 had blue labels, etc.) to minimize sample mix-up. Site names for each batch were disclosed to the developers prior to shipment of samples. As stated earlier, the developers were provided with HRMS data for the QC samples, but no other HRMS data was provided to the developers. However, the developers had access to all samples and HRMS data from the original SITE demonstration, and CAPE Technologies elected to use archived samples and HRMS data as part of the site-specific calibration procedure.

The composition of each site batch is discussed in Section 2.1. Developers were given 25 g of each sample, except for the QC sample which was distributed in 50 g aliquots. Certified samples or Standard Reference Materials® were not included in the study since the developers performed site-specific calibrations. The developers were permitted and encouraged to calibrate their technologies for the D/F responses at each site based on the HRMS data from the original SITE demonstration. The developers had to specify the period of time required to analyze the samples.

CAPE Technologies received their samples on March 20, 2006 and reported results for the 112 samples on October 9, 2007. (Note that CAPE Technologies was not actively working on the sample analysis during this entire period of time between sample receipt and results reporting. See Section 4.4.4 for information on sample throughput time.) After receiving the HRMS data, CAPE Technologies further examined their results and found that some of the dilution runs selected for five Winona samples were different from dilutions selected for replicates within a sample set. (For example, three replicates were reported using data from the 10X dilution run but one replicate was reported using data from the sample run that was undiluted). For consistency, CAPE resubmitted data for these samples so that the same dilution level was used for all replicates from that site. This did not require additional analytical work but rather just a recalculation using results from the correct dilution. The revised data improved the precision and comparability to HRMS for the Winona samples. Only the revised data are reported here. CAPE Technologies reported their revised data for the Winona samples on October 26, 2007.

2.4 Data Analysis

Data analysis focused on analytical performance on a site-specific basis and included an evaluation of comparability to the HRMS total $TEQ_{D/F}$ result, precision on replicate analyses, and QC sample results. In addition, CAPE Technologies' results from the original demonstration were compared to results from the site-specific study where possible. Qualitative parameters such as ease of use, cost, and sample throughput were not assessed during this study, but information was provided by the developer for inclusion in the report. In addition to the $TEQ_{D/F,}$ sample results, the developer reported the results from additional QC performed (for example: method blanks, matrix spikes, etc.) that were analyzed as part of their method for the batches of analyses from each site.

2.4.1 Comparability

The percent recovery (R) of the DF1 Immunoassay Kit relative to the HRMS analysis was calculated from the following equation:

$$Recovery = \frac{TEQ_A}{TEQ_{HRMS}} \times 100 \qquad \text{(eqn. 2-1)}$$

where TEQ_A is the average measured TEQ concentration reported by CAPE Technologies and TEQ_{HRMS} is the average HRMS TEQ concentration. Acceptable performance is generally in the range of 75 – 125% relative recovery values.

2.4.2 Precision

The standard deviation (*S*) of the results for the replicate environmental samples was calculated and used as a measure of the DF1 Immunoassay Kit's precision. Standard deviation was calculated from the following equation:

$$S = \left[\frac{1}{n-1} \sum_{k=1}^{n} (C_k - \overline{C})^2 \right]^{1/2} \quad \text{(eqn 2-2)}$$

where n is the number of replicate samples, C_k is the concentration measured for the k^{th} sample, and $\overline{C}$ is the average concentration of the replicate samples. Precision was reported in terms of the relative standard deviation (RSD) as described in equation 2-3. A method is considered to have acceptable precision if the RSD values are less than 25%.

$$\text{RSD} = \left| \frac{\text{S}}{\overline{\text{C}}} \right| \times 100 \quad \text{(eqn. 2-3)}$$

Chapter 3
Methods

This chapter describes the sample preparation, analytical, quality control, and data presentation methods used by CAPE Technologies. Additionally the reference HRMS method is discussed. Each section will describe CAPE's approach and the HRMS approach, followed by a description of the similarities and differences between the procedures. The CAPE Technologies approach is described in greater detail than the HRMS method because it is assumed that the reader will have some basic knowledge of the HRMS method. While the CAPE method is presented in a procedural format (e.g., step-by-step instructions), it is not intended that a user would be able to use the kit from the steps presented in this report. Rather the intent is to provide the reader with an in-depth view of the steps, materials, and equipment involved so that the reader can get a sense of the skill level and resources required for use.

It should also be noted that the HRMS method for determining $TEQ_{D/F}$ described in this section is the same method that was used to generate the characterization concentrations prior to the original SITE demonstration. This method was a modification of EPA Method 1613B (U.S. EPA, 1994). Modifications to Method 1613B are allowed, provided that method performance specifications can be met. Differences in the method employed and traditional Method 1613B are summarized in Table 3-1. Both methods are described in detail in CAPE's report from the original SITE demonstration (U.S. EPA, 2005d). The modified 1613B method was used to characterize the $TEQ_{D/F}$ concentrations prior to the original SITE demonstration as a way to select samples for use in the demonstration and to ensure that the samples were homogenized to acceptable reproducibility criteria. This data set is referred to as "characterization" HRMS data. The samples selected for use in the original SITE demonstration were then analyzed using the traditional 1613B method. This data set is referred to as "reference" HRMS data. For samples that were analyzed by both methods, the results were highly correlated (coefficient of determination = 0.99), demonstrating that the characterization and reference 1613B methods produced comparable results (U.S. EPA, 2005d). Since the characterization HRMS data was generated on all collected samples, including samples that were and were not used in the original SITE demonstration, the characterization data were used for comparison with the developer results for the site-specific study.

Table 3-1. Summary of HRMS Method Modifications Relative to Traditional EPA Method 1613B

Characterization Analysis – Modified 1613B	Reference Analysis – Traditional 1613B
Accelerated solvent extraction with methylene chloride	Soxhlet-Dean Stark extraction with toluene
2,3,7,8-tetrachlorodibenzofuran (TCDF) concentrations not confirmed	2,3,7,8-TCDF concentrations confirmed
1 to 10 g was used, depending on what was known about the site	10 g always extracted. High concentration sites were extracted and then diluted before adding internal standard
Used extrapolation if calibration range was exceeded	All samples diluted so that peak areas were under calibration peak areas

3.1 Sample Preparation

This section includes the sample extraction and cleanup methods employed. Briefly, the procedure involves the following steps:

1. Weigh sample. Pre-dry if wet. Add sodium sulfate and mix. Add 1:1 hexane:acetone and extract sample by shaking 2-4 hours. Remove the supernatant hexane:acetone extract.

2. Evaporate an aliquot of the supernatant hexane:acetone extract using a hydrocarbon keeper such as tetradecane, redissolve in hexane, and load onto a coupled acid-silica:activated carbon mini-column.

3. Force hexane through the system until the sample has passed through carbon mini-column.

4. Transfer carbon mini-column to empty reservoir and wash with 1:1 toluene:hexane.

5. Reverse carbon mini-column on reservoir and elute with toluene.

6. Evaporate the toluene to exchange sample into water-miscible keeper solution.

7. Perform the immunoassay procedure.

8. Interpret the immunoassay results.

3.1.1 DF1 Immunoassay Kit

The following sample preparation steps were reprinted from CAPE Technologies Application Note (AN) 008, "Analysis of ppt (pg/g) range PCDD/Fs in soil and sediment using rapid extraction and rapid cleanup" which is found on their website (www.cape-tech.com) under Application Notes. Also included is a PowerPoint training presentation on AN-008 with photographs of the actual sample preparation procedures.

3.1.1.1 Weigh sample. Using wooden spatula from Sample Preparation Kit, mix sample thoroughly and weigh 5 g into 40 mL extraction vial from Sample Preparation Kit.

3.1.1.2. Extract sample. Add 10-20 g anhydrous sodium sulfate to extraction vial. Add 3 steel mixing balls from the Sample Preparation Kit, then 20 mL of 1:1 hexane:acetone. Cap vials tightly and extract by shaking 2 to 4 hours at 350 rpm on orbital platform shaker. Extraction vials should lie flat on their sides for maximum agitation. If the sample sodium sulfate mixture is not completely homogeneous and free flowing within the solvent during extraction, then additional drying is required. Either a new aliquot can be extracted or more sodium sulfate can be added to the first attempt. In the latter case, the sample should be shaken for 2 to 4 hours after the sample-sodium sulfate mixture becomes homogeneous and free flowing within the solvent.

3.1.1.3. Spin extract and store. Centrifuge extraction vial for 10 to 15 minutes at 1000 x g or less. Caution: Exceeding this force during centrifugation can cause breakage of glass vials. Remove a portion of the supernatant hexane:acetone extract to a clean glass vial with Teflon lined cap for storage. The concentration of soil matrix in the extract will be 0.25 mg soil equivalent per µL.

3.1.1.4. Choose sample load and evaporate aliquot of extract. This extract cleanup protocol is designed for processing only a portion of the sample extract. Different sensitivities can be achieved by using different volumes of extract in this step to set up different sample loads. Using glass capillary micropipettor, add the chosen amount of hexane:acetone extract and 250 µL of tetradecane or similar hydrocarbon keeper to a glass tube or vial and evaporate. (Note: The analyst should consider significantly

different sample loads to be separate methods with respect to blanks, spikes, and other quality assurance samples.)

3.1.1.5a. Prepare coupled carbon-acid silica column system using vacuum. If a vacuum source is not available, go to step 3.1.1.5b below. If a vacuum source such as a pump is available, set it up outside fume hood so that flexible tubing can be run easily from intake into hood, with exhaust vented to hood. Remove endcaps from acid silica column. Remove a carbon mini-column (from the Sample Preparation Kit) from its pouch and twist square cut end firmly onto tip of acid silica column. Insert Luer plug into slant cut end of carbon column and place in rack. Add 10 mL of hexane to acid silica column and twist stopper/stopcock assembly firmly into top of column. Connect stopcock to vacuum source and depressurize headspace above hexane for 15-30 seconds. Bubbles should stream up through hexane, rapidly at first, then more slowly. Disconnect vacuum and let solvent flow downward into acid silica. When solvent flow has nearly stopped, reconnect vacuum, briefly repeat depressurization, then disconnect vacuum again. This time hexane should flow all the way through the acid silica column and into the carbon column. The acid silica column should appear translucent and should not have any air bubbles visible. The carbon column should appear uniformly black. Remove Luer plug from tip of carbon column. A few mL of hexane should remain in reservoir above acid silica. The reservoir and column assemblies can be left alone at this point until all are assembled and ready for step 3.1.1.6. DO NOT ALLOW TOP OF ACID SILICA COLUMN TO GO DRY.

3.1.1.5b. Prepare coupled carbon-acid silica column system without vacuum. This step is needed only if a vacuum source is not available for starting column flow. Remove endcaps from acid silica column and place in rack. Add 10 mL of hexane to acid silica column and allow to flow by gravity until hexane begins to drip from the column tip. Remove a carbon mini-column (from Sample Preparation Kit) from its pouch and use a Pasteur pipet to fill square cut end with hexane. As hexane is dripping from the tip of the acid silica column, place minicolumn firmly onto tip with a twisting action (gloves are essential for grip as well as skin protection). Be sure top of mini-column is full of hexane so that it can be attached without air bubbles. Flow will nearly stop, with solvent front advancing very slowly through carbon mini-column. Add more hexane if necessary to keep a few mL in reservoir. Twist stopper/stopcock assembly firmly into top of reservoir. The reservoir and column assemblies can be left alone at this point until all are assembled and ready for step 3.1.1.6. DO NOT ALLOW TOP OF ACID SILICA COLUMN TO GO DRY.

3.1.1.6. Complete prewash. A few mL of hexane should remain above the acid silica column. If not, then add hexane as needed. Insert stopper/stopcock assembly if not already in place. Using 20 mL syringe, pressurize the reservoir, close the stopcock, and remove the syringe. The hexane should flow immediately through the column at 0.5 to 2.0 mL/min. Catch solvent in waste basin. Stop prewash with 0.5-2 mL (2-5 mm height) of hexane remaining above bed, then remove stopcock from top of column. This procedure should be done one column at a time to avoid drying of columns. DO NOT ALLOW TOP OF COLUMN TO GO DRY; IT IS CRITICAL TO AVOID AIR BUBBLES IN ACID SILICA COLUMN.

3.1.1.7. Load sample. If hexane level drops to the top of the bed before loading sample, add more. Using a glass Pasteur pipet, add sample in tetradecane or other hydrocarbon keeper from step 3.1.1.4 to the top of the acid silica column. Gently rinse the sides of the sample tube with 2 mL of hexane, then add to acid silica column, rinsing the sides thoroughly. Repeat wash of sample tube with another 2 mL of hexane and add to acid silica column. Twist stopper/stopcock assembly firmly into top of column and pressurize as before to push sample and washes into acid silica bed. Catch solvent in waste basin. Stop flow by opening stopcock just before solvent level reaches top of bed. DO NOT ALLOW TOP OF ACID SILICA BED TO GO DRY.

3.1.1.8. Wash acid silica column. Remove stopper/stopcock assembly and add 10 mL of hexane to acid silica column. Replace stopper/stopcock assembly and pressurize as before. Catch solvent in waste basin. Maintain pressure to keep hexane flowing at 0.5 to 2.0 mL/min. Stop flow by opening stopcock just before solvent level reaches top of bed. Repeat twice for a total wash volume of 30 mL of hexane. On final portion only, maintain pressure to keep hexane flowing all the way through the acid silica column. When air penetrates the neutral silica layer (bottom 1-2 cm layer at bottom of column, just above tip of column), release pressure to stop flow. IT IS NECESSARY TO RUN THE SOLVENT ALL THE WAY THROUGH THE ACID SILICA BED, BUT DO NOT ALLOW TOP OF CARBON MINI-COLUMN TO GO DRY.

3.1.1.9. Remove carbon mini-column and wash. Remove carbon mini-column from the acid silica column, attach square cut end to a clean and empty reservoir, and place in rack over waste capture basin. Add 6 mL of 1:1 toluene:hexane and pressurize as before. Catch solvent in waste basin. When solvent level reaches tip of reservoir, release pressure to stop flow. DO NOT ALLOW TOP OF CARBON MINI-COLUMN TO GO DRY.

3.1.1.10. Elute sample. Remove carbon mini-column from tip of reservoir and replace on same reservoir in reverse orientation, slant cut end first. Add 12 mL of toluene and pressurize as before. Capture eluate in clean 16 x 125 mm borosilicate glass tube, allowing air to drive last of toluene through carbon mini-column.

3.1.1.11. Add keeper and evaporate solvent. Keeper solution (80:20 methanol:polyethylene glycol [PEG] + 100ppm Triton X-100) is made by adding methanol to a stock vial which is part of the DF1 kit. Add 62.5 µL of PEG-Triton-methanol keeper solution to each evaporation tube containing a toluene eluate. Evaporate the toluene at 70-90°C under a gentle stream of nitrogen as described in the immunoassay kit insert IN-DF1, section I3. When only keeper remains, centrifuge at 1-2000 x g for 2 minutes to concentrate all of the sample at the bottom of the tube.

3.1.1.12. Dilute sample with methanol. Add 50 µL of methanol (setting 2.5 of Repeater Plus pipettor with 1.0 mL tip) to each evaporation tube and mix vigorously for 15 seconds. Let stand for 15-30 seconds to allow liquid to flow back to bottom of tube, then remove 50 µL for enzyme immunoassay (EIA) analysis. Dilution and EIA loading should be done in batches of 4 samples or fewer to minimize concentration changes due to methanol evaporation before pipetting. Add the sample directly to the water in EIA tube, not above the water or onto side of EIA tube. Mix each tube individually as soon as sample is added.

3.1.2 Characterization HRMS Method

3.1.2.1 Sample Extraction Depending on the anticipated levels of dioxins from preliminary information received from each sampling location, 1 to 10 grams (g) of material were taken for analysis from each aliquot, spiked with $^{13}C_{12}$-labeled internal standards, and extracted with methylene chloride using accelerated solvent extraction techniques. (The accelerated solvent extraction technique is a deviation from Method 1613B, which calls for a Soxhlet/Dean-Stark extraction with toluene for a total of 16 to 24 hours.)

3.1.2.2 Sample Cleanup The sample extracts were processed through various cleanup techniques, which included gel permeation chromatography or acid/base washes, as well as acid/base silica and carbon cleanup columns. As warranted, based on sample compositions, some samples were put through additional acid silica cleanup prior to the carbon column cleanup. $^{13}C_{12}$-labeled recovery standards were added, then the extracts were concentrated to a final volume of 20 to 50 µL.

3.2 Sample Analysis

This section describes the determinative analytical methods employed.

3.2.1 DF1 Immunoassay Kit

A flowchart of the CAPE Technologies DF1 Immunoassay Kit assay procedure is presented in Figure 1.

3.2.1.1 Warm reagents. Bring all reagents to ambient temperature. Before use, mix all reagents briefly by gently inverting several times.

3.2.1.2. Prepare wash 1. Locate the vial labeled "0.5 mL neat Triton X-100". Make a wash solution of 100 ppm (0.01% v/v) Triton in reagent grade or bottled distilled water by adding 10 µL of Triton X-100 to 100 mL of water and mixing thoroughly (this will typically take several minutes on a magnetic stirrer). This amount is sufficient for 20 tubes (20 tubes x 4 washes per tube x 1 mL/wash/tube = 80 mL nominal). This wash can be prepared in larger volumes and stored at room temperature.

3.2.1.3. Prepare tubes. Place the anti-Dioxin antibody coated tubes in the rack and label them. Put the standard tubes first, from low to high concentration, then the sample tubes.

3.2.1.4. Prerinse tubes. Rinse tubes once by filling each tube with reagent grade or bottled distilled water. Dump water out and tap inverted tubes on absorbent material to remove excess water.

3.2.1.5. Add sample diluent. Insert the 10 or 12.5 mL pipet tip labeled "sample diluent" into the Repeater pipettor and set volume to 500 µL. Dispense one 500 µL aliquot from bottle of "Sample Diluent" into each tube.

3.2.1.6. Add standards. Using a glass capillary positive displacement pipettor, pipet 50 µL of standard solution into each EIA standard tube. The solutions must be dispensed directly into the liquid and not above the liquid surface or onto the side of the tube. Immediately after addition, mix each tube briefly until appearance is homogeneous. The mixing should be vigorous enough to visibly swirl the liquid around the bottom of the tubes.

3.2.1.7. Add samples. Using a glass capillary positive displacement pipettor, pipet 50 µL of prepared sample into each EIA sample tube. If dilutions are run, they must be prepared directly before addition to the EIA tube, for maximum accuracy in the measurement and delivery of small volumes. The solutions must be dispensed directly into the liquid and not above the liquid surface or onto the side of the tube. Immediately after addition, mix each tube briefly until appearance is homogeneous. Mix the rack of tubes by shaking for 10 seconds after adding the last sample. The mixing should be vigorous enough to visibly swirl the liquid around the bottom of the tubes. Incubate at room temperature for 2 to 24 hours. For longer incubation times, cover the rack of tubes or place in a closed plastic bag or other airtight container with limited headspace. The amount of time taken for addition of negative control, standard and sample has little effect on the results because of the long sample incubation. (It is preferred to incubate overnight at this point rather than 2 hours because of the slight improvement in sensitivity [up to two-fold] with the longer incubation). Also, results may be affected by proportionally higher variations in incubation time among samples, due to the sample addition process. The residual sample in each sample evaporation tube should be allowed to evaporate in case it is needed for subsequent dilution analysis.

3.2.1.8. Wash 1. Dump or aspirate the EIA tube contents into a suitable waste container. Tap inverted tubes on absorbent material to remove excess liquid. Insert a 50 mL pipet tip into the Repeater pipettor and set volume to 1.0 mL. Dispense one 1 mL aliquot of 100 ppm Triton X-100 in water (made in step J2

above) into each tube. Dump or aspirate the EIA tube contents into a suitable waste container. Repeat this wash step three more times for a total of 4 washes. Be certain to shake or tap out as much wash solution as possible on each wash, especially the last one.

3.2.1.9. Add conjugate. Insert the 10 or 12.5 mL pipet tip labeled "conjugate" into the Repeater pipettor and set volume to 500 µL. Dispense one 500 µL aliquot of "Competitor-HRP Conjugate" into each tube. Incubate tubes at room temperature for 15 minutes. Timing for this step is the most important of the EIA steps. Rapid and accurate addition of conjugate and consistent incubation times are necessary to maintain equal treatment within and among runs.

3.2.1.10. Wash 2. Repeat the wash procedure described in step 8 above except use reagent grade or bottled distilled water with no detergent added.

3.2.1.11. Add substrate. Insert the 10 or 12.5 mL pipet tip labeled "substrate" into the Repeater pipettor and set volume to 500 µL. Dispense one 500 µL aliquot of "HRP Substrate Solution" into each tube. Incubate at room temperature for 30 minutes.

3.2.1.12. Add stop solution. Insert the 10 or 12.5 mL pipet tip labeled "stop" into the Repeater pipettor and set volume to 500 µL. Dispense one 500 µL aliquot of "Stop Solution" into each tube. The Stop Solution converts the developed color to yellow. If Stop Solution is not added, all tubes will eventually turn dark blue. Read the tubes as soon as possible after stopping; the yellow color is stable for only 30 minutes.

3.2.1.13. Read OD values. To use the Artel Differential Photometer, add at least 1 mL of reagent grade or bottled distilled water to a blank test tube and insert the tube into the left well of the photometer. Wipe dry the outside of each EIA tube, insert tube into the right well of the photometer, and record the absorbance (optical density [OD]) of each tube. Alternatively, read the absorbance of each sample at 450 nm using a tube reader, conventional spectrophotometer, or microplate reader.

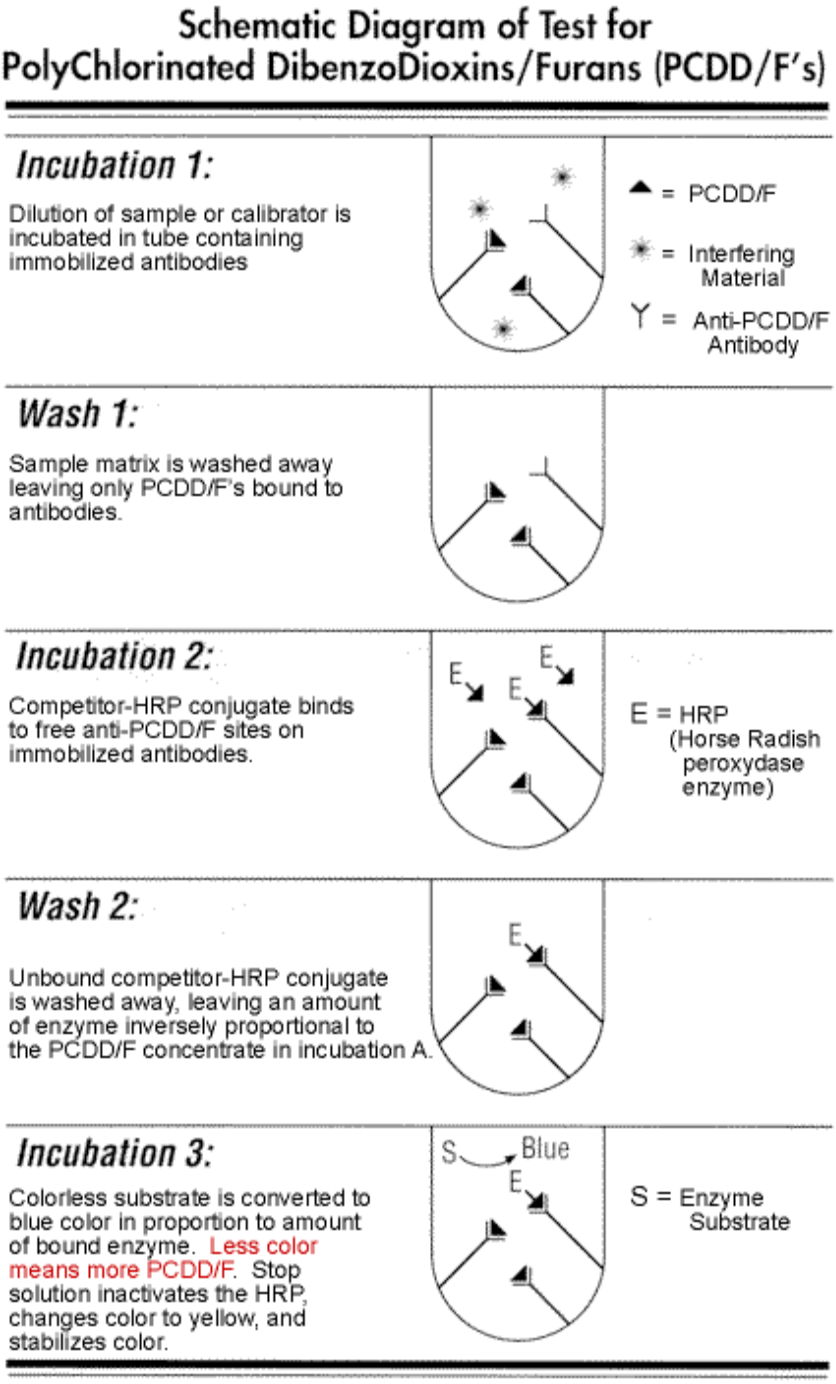

Figure 1. Schematic Diagram of DF1 Immunoassay Kit Assay

3.2.2 Characterization HRMS Method

Each extract was analyzed by GC/HRMS in the selected ion monitoring mode at a resolution of 10,000 or greater. A DB-5 column was used for separation of the seventeen PCDD/F congeners. The instrument was calibrated for PCDD/F at levels specified in Method 1613B with one additional calibration standard at concentrations equivalent to one-half the level of Method 1613B's lowest calibration point. Method

1613B relative response factor criteria was used for the calibration curve in which the relative response factors (RRF) were calculated for each analyte at each calibration level (RRF= (summed area of the native * concentration of the labeled analog)/(summed area of the labeled analog * concentration of the native)). An average RRF and a percent relative standard deviations (%RSD) were calculated for each analyte by averaging the calibration levels for that analyte. The % RSD criteria must be below 20% for the native analytes quantified by isotope dilution and 35% for the labeled analytes quantified by internal standards. Continuing calibration solutions were monitored at the beginning and end of each 12-hour analysis. A window-defining and column performance solution was also analyzed at the beginning of each sequence to verify that all of the 17 PCDD/F isomers were within the acquisition windows and that there was a 25% valley between 2,3,7,8 TCDF and its closest eluting isomer. PCDD/F data were reported as both concentration (pg/g dry) and TEQs (pg TEQ/g dry).

3.3 Quality Control and Calibration

3.3.1 DF1 Immunoassay Kit Method

Quality assurance samples to verify method performance include unspiked and spiked method blanks. Reference soils can also be included (such as NIST Standard Reference Materials) but were not included in the site-specific study since the congener patterns of a reference soil would not approximate the congener patterns of sites under evaluation. QC samples were provided with each sample batch. However, CAPE elected to use three samples from the original SITE demonstration from CAPE's sample archive for each site to calibrate the responses on a site-specific basis.

3.3.2 Characterization HRMS Method

The characterization HRMS method followed the Method 1613B QC requirements. Some of the critical QC criteria included:

- All initial calibrations met the criteria for response factor RSD and minimal signal-to-noise ratio requirements for the lowest calibration point.
- Continuing calibrations were performed at the beginning and end of every 12-hour analysis period and were required to meet performance criteria.
- Column performance was checked at the beginning of each 12-hour analytical period and met method criteria.
- Instrument resolution was documented at the beginning and end of each 12-hour period with one exception.
- Method 1613B 13C-labeled internal standard was added to each sample prior to extraction to evaluate sample extraction recovery.
- Method 1613B recovery standard was added to the GC vials and was used to calculate the percent recoveries for the internal standards and cleanup standards.
- Method 1613B requires that a 13C-labeled cleanup standard be added after sample extraction. However, the characterization laboratory has demonstrated a consistent quantifiable loss of analyte with GPC cleanup, therefore a GPC correction factor was applied to the sample weight extracted and the level of internal standard added to the samples prior to GPC cleanup. The cleanup standard was then added after the GPC step and was used to monitor loss during the remaining cleanup steps.
- Analysis of one method blank with every extraction batch was required to demonstrate freedom from contamination.
- One laboratory control spike, an on-going precision and recovery (OPR) sample, was also processed with every extraction batch. Native and labeled compounds were required to pass the Method 1613B limits for OPR.
- A decane blank was analyzed after the analysis of the OPR to monitor for carryover.

3.4 Data Analysis

3.4.1 DF1 Immunoassay Kit

The following steps are involved with determining quantitative results for the DF1 kit.

3.4.1.1 Open Calculation Module C (Microsoft Excel workbook downloadable from CAPE Technologies web site [www.cape-tech.com] or available by email from CAPE Technologies). Select "Introduction" worksheet and read the information on background and procedure, then select the "DF1 low-mid ppt quantitative" worksheet. Install Excel "Solver" Add-In if it is not already done.

3.4.1.2 Enter optical density (OD) data for standards and samples into designated spaces.

3.4.1.3 Perform non-linear curve fitting procedure using Excel "Solver" function.

3.4.1.4 Enter sample load (e.g. 500 mg sample equivalent per EIA tube) and dilution factor.

3.4.1.5 Read raw original sample ppt values for each sample in designated row.

3.4.1.6 Based on previously analyzed calibration samples, modify calibration adjustment factor (CAF) which is applied to raw results above. Final interpretation of data will be based also on analyst experience, knowledge of samples, and especially on results for quality assurance samples.

3.4.2 Characterization HRMS Method

The concentrations of the seventeen individual PCDD/F congeners were calculated in pg/g dry weight, based on the calibration curve. The World Health Organization's 1998 TEF (van den Berg, 1998) were then applied to the concentrations and summed to calculate the total $TEQ_{D/F}$ value for each sample. Note that at the time of the original HRMS analysis, the WHO 2005 TEF values were not available. The WHO TEF values are presented along side the DF1 Immunoassay kit crossreactivities in Table 3-2.

Table 3-2. Comparison of World Health Organization (WHO) Toxicity Equivalency Factors (TEF) and DF1 Immunoassay Crossreactivity Values

Congener	WHO 1998 TEF [a]	WHO 2005 TEF [b]	DF1 Immunoassay Kit Crossreactivity
2,3,7,8 TCDD	1	1	1
1,2,3,7,8 PCDD	1	1	1
1,2,3,4,7,8 HxCDD	0.1	0.1	0.013
1,2,3,6,7,8 HxCDD	0.1	0.1	0.079
1,2,3,7,8,9 HxCDD	0.1	0.1	0.39
1,2,3,4,6,7,8 HpCDD	0.01	0.01	0.007
1,2,3,4,6,7,8,9 OCDD	0.0001	0.0003	<0.00001
2,3,7,8 TCDF	0.1	0.1	0.2
1,2,3,7,8 PCDF	0.05	0.03	0.046
2,3,4,7,8 PCDF	0.5	0.3	0.17
1,2,3,4,7,8 HxCDF	0.1	0.1	0.004
1,2,3,6,7,8 HxCDF	0.1	0.1	0.01
1,2,3,7,8,9 HxCDF	0.1	0.1	0.033
2,3,4,6,7,8 HxCDF	0.1	0.1	0.049
1,2,3,4,6,7,8 HpCDF	0.01	0.01	0.0002
1,2,3,4,7,8,9 HpCDF	0.01	0.01	0.009
1,2,3,4,6,7,8,9 OCDF	0.0001	0.0003	<0.00001
PCB-81 (3,4,4',5)	0.0001	0.0003	NA
PCB-77 (3,3',4,4')	0.0001	0.0001	0.004
PCB-126 (3,3',4,4',5)	0.1	0.1	0.005
PCB-169 (3,3',4,4',5,5')	0.01	0.03	<0.001
PCB-123 (2',3,4,4',5)	0.0001	0.00003	NA
PCB-118 (2,3',4,4',5)	0.0001	0.00003	NA
PCB-114 (2,3,4,4',5)	0.0005	0.00003	NA
PCB-105 (2,3,3',4,4')	0.0001	0.00003	NA
PCB-167 (2,3',4,4',5,5')	0.00001	0.00003	NA
PCB-156 (2,3,3',4,4',5)	0.0005	0.00003	NA
PCB-157 (2,3,3',4,4',5')	0.0005	0.00003	NA
PCB-189 (2,3,3',4,4',5,5')	0.0002	0.00003	NA

[a] van den Berg, 1998
[b] van den Berg, 2006

Chapter 4
Results and Discussion

4.1 CAPE Technologies DF1 Immunoassay Kit Results for Site-Specific Study

The results reported by CAPE Technologies for the site-specific study are described by site in Tables 4-1 through 4-5. In Table 4-6, the percent recovery (%R) and precision (relative standard deviation, RSD) values are summarized. Note that data calculations were made with all of the significant digits provided by CAPE Technologies; however, all data were rounded to whole numbers when reported in the data tables.

For the Winona samples (Table 4-1), the %R values were 83%, 71%, 48%, 79%, and 84%. This indicated that all sample sets were reported with results that were consistently lower than the HRMS method, with all %R values less than 100%. The relative standard deviation (RSD) values were between 18% and 32%. Note that all of the sample concentrations at this site were the highest among the five sites (approximately 10,000 pg/g $TEQ_{D/F}$).

For the Tittabawassee River samples (Table 4-2), the %R values were 354%, 126%, 208%, 210%, and 274%. This indicated that all sample sets were reported with results that were consistently higher than the HRMS method, with all % R values greater than 100%. The RSD values were between 12% and 63%.

For the Newark Bay samples (Table 4-3), the %R values were 85%, 145%, 80%, 171%, and 67%. This indicated sample sets from this site were reported both higher and lower than the HRMS method. The RSD values were between 6% and 50%. Note that all of the sample concentrations at this site were relatively low with the highest concentration reported by HRMS at 62 pg/g $TEQ_{D/F}$.

For the Raritan Bay samples (Table 4-4), the %R values were 84%, 88%, 72%, 70%, and 76%. This indicated that all of the sample sets were reported with results that were consistently lower than the HRMS method. However, since all samples were in the 10 – 15 pg/g $TEQ_{D/F}$ range, the magnitude of the bias was small (no more than 5 pg TEQ/g). The RSD values were between 12% and 34%. Results from the Raritan Bay site were the most accurate and precise of all site batches.

For the Solutia samples (Table 4-5), the %R values were 142%, 200%, 95%, 116%, 82%, and 68%. This indicated sample sets from this site were reported both higher and lower than the HRMS method. The RSD values were between 10% and 52%.

Results from the DF1 kit for the eight uncontaminated ("blank") samples that were included in the experimental design were reported with $TEQ_{D/F}$ values between 3 and 30. One blank sample analyzed with the Winona samples was reported as a non-detect (e.g., << 30) by the DF1 kit.

Table 4-1. Winona Sample Results

			pg TEQ/g	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**DF1[a]**
Cell #10	1	W-14	8648	8,392
	2	W-19		6,771
	3	W-8		5,170
	4	W-16		8,392
Average				7,181
Standard Deviation (SD)				1,543
Relative standard deviation (RSD)				21%
% Recovery				83%
Cell #12	1	W-9	8831	4,158
	2	W-5		5,170
	3	W-21		7,792
	4	W-2		8,070
Average				6,298
SD				1,934
RSD				31%
% Recovery				71%
Cell #2	QC	W-1	11,071	5,370
	1	W-18		5,934
	2	W-3		3,599
	3	W-17		6,431
Average				5,333
SD				1,235
RSD				23%
% Recovery				48%
Cell #4	1	W-15	11,410	7,792
	2	W-6		7,666
	3	W-13		9,833
	4	W-4		10,951
Average				9,061
SD				1,605
RSD				18%
% Recovery				79%
Cell #8	1	W-20	11,259	12,915
	2	W-12		10,951
	3	W-7		6,464
	4	W-11		7,541
Average				9,468
SD				2,990
RSD				32%
% Recovery				84%
ERA Blank		W-10	ND	<<30

ND = not detected

[a] Revised data provided by CAPE Technologies after original submission due to error in data analysis. See description of error in Section 2.3.

Table 4-2. Tittabawassee River Sample Results

			pg TEQ/g	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**DF1**
ERA Blank	1	TR-11	ND	11
	2	TR-13		13
	3	TR-16		17
	4	TR-20		15
DNR 1	1	TR-19	435	1,933
	2	TR-12		1,523
	3	TR-23		1,766
	QC	QC TR-1		929
Average				1,538
Standard Deviation (SD)				439
Relative standard deviation (RSD)				29%
% Recovery				354%
DNR 2	1	TR-2	42	41
	2	TR-8		41
	3	TR-5		102
	4	TR-18		28
Average				53
SD				33
RSD				63%
% Recovery				126%
FFP 1	1	TR-22	3127	5,233
	2	TR-3		10,286
	3	TR-15		5,233
	4	TR-17		5,233
Average				6,496
SD				2,527
RSD				39%
% Recovery				208%
FFP 2	1	TR-9	1048	2,354
	2	TR-10		1,959
	3	TR-24		1,992
	4	TR-4		2,502
Average				2,202
SD				268
RSD				12%
% Recovery				210%
IMP 2	1	TR-6	808	1,842
	2	TR-14		2,996
	3	TR-21		1,933
	4	TR-7		2,084
Average				2,214
SD				531
RSD				24%
% Recovery				274%

ND = not detected

Table 4-3. Newark Bay Sample Results

			pg TEQ/g	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**DF1**
ERA Blank		NB-14	ND	10
NB 1	1	NB-16	45	26
	2	NB-9		34
	3	NB-3		52
	4	NB-20		41
Average				38
Standard Deviation (SD)				11
Relative standard deviation (RSD)				29%
% Recovery				85%
NB 2	1	NB-4	38	92
	2	NB-6		41
	3	NB-8		58
	4	NB-10		28
Average				55
SD				28
RSD				50%
% Recovery				145%
NB 3	1	NB-7	32	21
	2	NB-13		27
	3	NB-18		26
	QC	NB-1 QC		29
Average				26
SD				3
RSD				13%
% Recovery				80%
NB 5	1	NB-5	16	26
	2	NB-11		32
	3	NB-15		24
	4	NB-21		27
Average				27
SD				4
RSD				13%
% Recovery				171%
NB 6	1	NB-12	62	41
	2	NB-19		39
	3	NB-2		43
	4	NB-17		45
Average				42
SD				2
RSD				6%
% Recovery				67%

ND = not detected

Table 4-4. Raritan Bay Sample Results

			pg TEQ /g	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**DF1**
ERA Blank		RB-15	ND	3
RB 1	1	RB-8	14	8
	2	RB-18		15
	3	RB-6		9
	4	RB-12		15
Average				12
Standard Deviation (SD)				4
Relative standard deviation (RSD)				34%
% Recovery				84%
RB 2	1	RB-16	12	13
	2	RB-5		7
	3	RB-14		11
	4	RB-3		11
Average				11
SD				2
RSD				24%
% Recovery				88%
RB 4	1	RB-2	15	12
	2	RB-21		12
	3	RB-11		9
	4	RB-20		10
Average				11
SD				1
RSD				12%
% Recovery				72%
RB 5	1	RB-4	14	9
	2	RB-19		13
	3	RB-10		9
	4	RB-7		9
Average				10
SD				2
RSD				20%
% Recovery				70%
RB 6	1	RB-9	13	10
	2	RB-17		10
	3	RB-13		11
	QC	RB-1		8
Average				10
SD				1
RSD				12%
% Recovery				76%

ND = not detected

Table 4-5. Solutia Sample Results

			pg TEQ/g	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**DF1**
ERA Blank		S-18	ND	33
SS 1	1	S-3	846	1,688
	2	S-12		907
	3	S-20		1,155
	4	S-24		1,055
Average				1,200
Standard Deviation (SD)				341
Relative standard deviation (RSD)				28%
% Recovery				142%
SS 2	1	S-8	48	73
	2	S-17		81
	3	S-5		85
	4	S-23		145
Average				96
SD				33
RSD				35%
% Recovery				200%
SS 3	1	S-15	3257	3,544
	2	S-10		2,611
	3	S-6		2,470
	4	S-16		3,808
Average				3,108
SD				667
RSD				21%
% Recovery				95%
SS 4	1	S-22	1833	2,429
	2	S-13		2,470
	3	S-7		1,433
	QC	S-1		2,155
Average				2,122
SD				480
RSD				23%
% Recovery				116%
SS 5	1	S-2	1279	906
	2	S-4		1,082
	3	S-21		1,155
	4	S-25		1,055
Average				1,050
SD				104
RSD				10%
% Recovery				82%
SS 6	1	S-14	3951	2,214
	2	S-11		1,146
	3	S-9		2,920
	4	S-19		4,447
Average				2,682
SD				1,385
RSD				52%
% Recovery				68%

ND = not detected

Table 4-6. Summary of Site-Specific Study Results

Sample ID	HRMS		CAPE Technologies [a]		Recovery (%R)
	Average (pg TEQ/g)	Relative Standard Deviation (RSD, %)	Average (pg TEQ/g)	Relative Standard Deviation (RSD, %)	
Winona					
Cell #10	8648	28	7181	21	83
Cell #12	8831	1	6298	31	71
Cell #2	11071	2	5333	23	48
Cell #4	11410	4	9061	18	79
Cell #8	11259	4	9468	32	84
Tittabawassee River					
DNR 1	435	5	1538	29	354
DNR 2	42	23	53	63	126
FFP 1	3127	7	6496	39	208
FFP 2	1048	19	2202	12	210
IMP 2	808	10	2214	24	274
Newark Bay					
NB 1	45	26	38	29	85
NB 2	38	10	55	50	145
NB 3	32	6	26	13	80
NB 5	16	26	27	13	171
NB 6	62	14	42	6	67
Raritan Bay					
RB 1	14	7	12	34	84
RB 2	12	8	11	24	88
RB 4	15	11	11	12	72
RB 5	14	3	10	20	70
RB 6	13	7	10	12	76
Solutia					
SS 1	846	18	1200	28	142
SS 2	48	10	96	35	200
SS 3	3257	11	3108	21	95
SS 4	1833	19	2122	23	116
SS 5	1279	10	1050	10	82
SS 6	3951	5	2682	52	68

[a] As noted in Tables 4-1 through 4-5, QC samples were provided with each sample batch. As described in Section 3.3.1, CAPE Technologies elected to use three samples from the original SITE demonstration from CAPE Technologies' sample archive for each site to calibrate the responses on a site-specific basis.

In summary, sample $TEQ_{D/F}$ values measured using the immunoassay were reported consistently higher than those measured by HRMS for the Tittabawassee River site and consistently lower for the Raritan Bay and Winona sites. Overall there was no significant pattern of positive or negative bias relative to the HRMS method results, since the percent recovery values were both above and below 100%. This evaluation also demonstrated the need for a site-specific factor to convert the raw data generated by the DF1 into $TEQ_{D/F}$ data. This suggests that the need for independent HRMS confirmatory analysis would be appropriate at a level of at least 10%, since CAPE Technologies used the results from three archived samples to calibrate the batch of 20-25 samples (see description in Section 3.3.1). Possibly, more comparability to HRMS would be obtained with a greater percentage of HRMS confirmation analyses, but this was not evaluated in this study.

Figure 2 is a log-log plot of the DF1 and GC-MS TEQ results. The log scale was used since the data covered a large dynamic range. Data shown include the means of replicates for every sample in the site-specific study. Samples from each site are indicated by a unique symbol. The overall correlation coefficient (r) value was 0.94. Note that the line shown in the plot represents y=x (it is not a regression line).

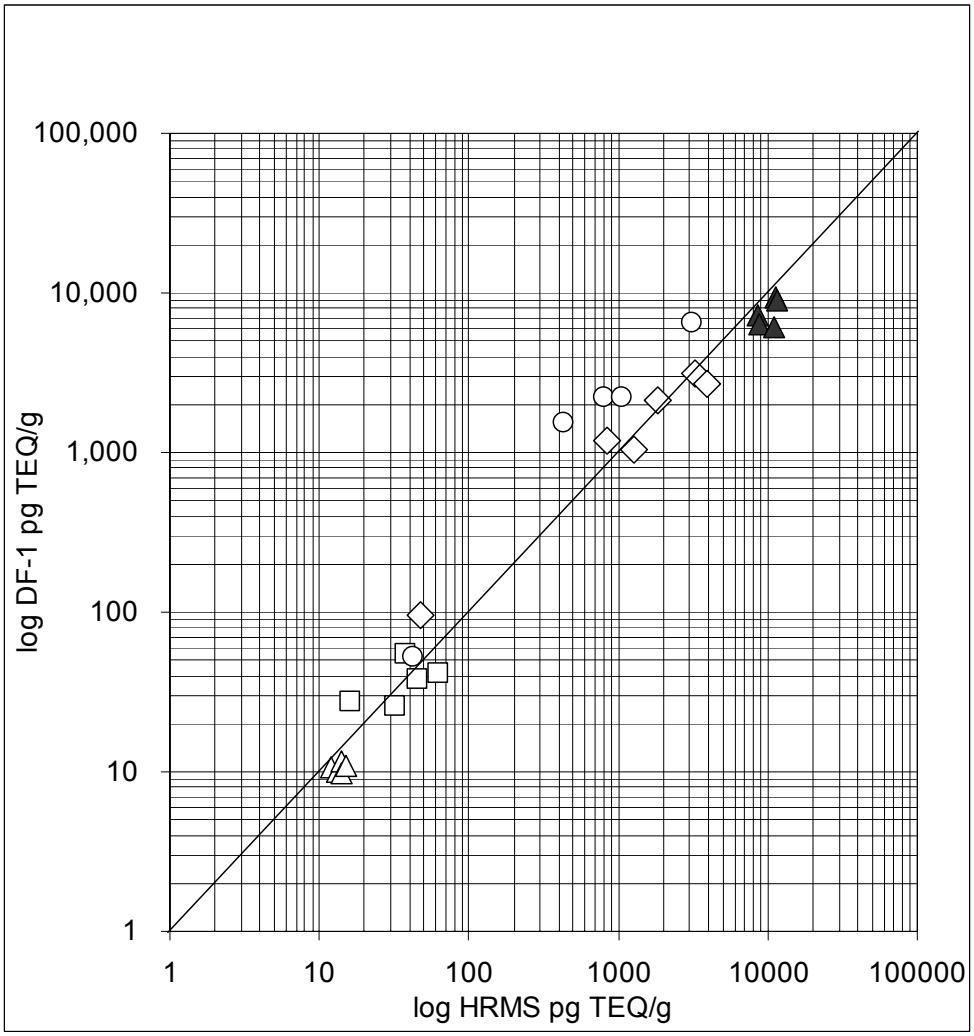

Figure 2. Plot of DF1 results versus HRMS results for the site-specific study.

Line represents y = x (not a regression line).

4.2 Comparison of CAPE Technologies DF1 Immunoassay Kit Results to Results from the Original Demonstration

The CAPE results from the original SITE demonstration are fully described in an EPA report (U.S. EPA, 2005d), which is posted on the EPA SITE program web site (www.epa.gov/ORD/SITE). The results of both studies are included to show the comparability of the CAPE results obtained by changing the analytical approach to include the use of a site-specific calibration factor.

In the first study, CAPE analyzed samples from all 10 environmental sites in random order, so no site-specific calibration factor could be used. Table 4-7 summarizes the performance of the CAPE Technologies kit in the first study, including precision and percent recovery. Table 4-7 represents a subset of the total dataset 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 4-7, the range of RSD values for the CAPE data was 17% to 116% (overall mean of 26%). For comparison, the range of RSD values for the HRMS data was 2% to 28%. The range of %R values was 18% to 582%.

The results from the first and second studies are presented site-by-side in Table 4-8. In the second study, the samples were segregated into site batches so that a site-specific calibration factor could be applied for each site. To determine the site-specific calibration factor, CAPE analyzed three samples from the same site that were analyzed in the original study. CAPE had access to HRMS congener and TEQ data for these samples and used the responses of these samples to calibrate the results of other samples analyzed from each site. When comparing the values from the two studies in Table 4-8, it is evident that the application of the site-specific calibration factor significantly improved some of the results. The Raritan Bay samples, for example, showed improved precision and accuracy for all samples. The Winona results were closer to the HRMS values, although they were still low by 30% and 50%. Precision was improved for the Newark Bay samples, with RSD values ranging from 6% to 29% compared to 21% to 62% for the original study. However, the accuracy of some results from the Solutia, Newark Bay, and Tittabawassee River sites were not improved or poorer in some cases. In addition, improvements in the sample cleanup protocol and sample evaporation protocol since the original demonstration are believed to be responsible for some of the observed changes in performance compared to the results from the original SITE demonstration.

Table 4-9 is a summary of the percent recovery and RSD values for both studies; HRMS RSD values are presented for comparison. The average percent recovery value for the original study was 206% compared with 122% of the site-specific study. The RSD value for the original study was 56% compared with 26% for the site-specific study. In comparing the RSD and %R values for the CAPE data in the two studies, the second study demonstrated a significant improvement. However, these data should be considered along with the individual data points presented in Table 4-8, since averages can normalize high and low bias.

Table 4-7. Summary of a Subset of Results from Original SITE Demonstration (Without Site-Specific Calibration)

Sample ID	HRMS		CAPE		Recovery (%R)
	Average (pg TEQ/g)	Relative Standard Deviation (RSD, %)	Average (pg TEQ/g)	Relative Standard Deviation (RSD, %)	
Winona					
Cell #10	-- [a]	--	--	--	--
Cell #12	7318	2	1319	91	18
Cell #2	9998	9	2011	116	20
Cell #4	--	--	--	--	--
Cell #8	--	--	--	--	--
Tittabawassee River					
DNR 1	475	10	713	87	150
DNR 2	37	6	202	56	547
FFP 1	--	--	--	--	--
FFP 2	--	--	--	--	--
IMP 2	1062	26	1020	NA [b]	96
Newark Bay					
NB 1	41	6	45	62	111
NB 2	--	--	--	--	--
NB 3	--	--	--	--	--
NB 5	16	28	25	29	154
NB 6	56	22	64	21	115
Raritan Bay					
RB 1	11	5	64	40	582
RB 2	13	2	43	17	333
RB 4	--	--	--	--	--
RB 5	--	--	--	--	--
RB 6	11	5	52	59	470
Solutia					
SS 1	--	--	--	--	--
SS 2	65	13	73	65	113
SS 3	2923	5	3035	27	104
SS 4	2015	7	1371	58	68
SS 5	--	--	--	--	--
SS 6	--	--	--	--	--

[a] Sample not included in original SITE demonstration.
[b] Two of four replicate results were reported semi-quantitatively (i.e., > 330 and < 280 pg TEQ/g) so RSD could not be calculated.

Table 4-8. Comparison of Original SITE Demonstration and Site-Specific Study Data

Sample ID	Original SITE Demonstration		Site-Specific Study		HRMS [a] (pg TEQ/g)
	Average (pg TEQ/g)	Relative Standard Deviation (RSD, %)	Average (pg TEQ/g)	Relative Standard Deviation (RSD, %)	
Winona					
Cell #12	1319	91	6298	31	8831
Cell #2	2011	116	5333	69	11071
Tittabawassee River					
DNR 1	713	87	1538	29	435
DNR 2	202	56	53	63	42
IMP 2	1020	NA [b]	2214	24	808
Newark Bay					
NB 1	45	62	38	29	45
NB 5	25	29	27	13	16
NB 6	64	21	42	6	62
Raritan Bay					
RB 1	64	40	12	34	14
RB 2	43	17	11	24	12
RB 6	52	59	10	12	13
Solutia					
SS 2	73	65	96	35	48
SS 3	3035	27	3108	21	3257
SS 4	1371	58	2122	23	1833

[a] HRMS data for characterization analysis

b Two of four replicate results were reported semi-quantitatively (i.e., > 330 and < 280 pg TEQ/g) so RSD could not be calculated.

Table 4-9. Summary Data for CAPE and HRMS Results for Original Demonstration and Site-Specific Study

Source	Study	% Recovery			RSD (%)		
		Average	Minimum	Maximum	Average	Minimum	Maximum
CAPE	Original Demonstration [a]	206	18	582	56	17	116
	Site-Specific Study	122	48	354	26	6	63
HRMS	Original Demonstration	-- [b]	--	--	11	2	28
	Site-specific Study	--	--	--	11	1	28

[a] Only includes samples that were also included in the site-specific study, as presented in Tables 4-7 and 4-8.
[b] Not appropriate since % Recovery values are based on HRMS results.

4.3 Operational Factors

Operational factors such as cost, availability of the technology, turnaround time, and training are described in this section. This information was provided by CAPE Technologies and not evaluated independently by Battelle or EPA. More detailed information on most of these factors, which were independently verified, is available in the final report from the original study (U.S. EPA, 2005d).

4.3.1 Cost of Analysis for DF1 Immunoassay Kit

The economic analysis developed for the original study largely applies here (U.S. EPA, 2005d). Cost of disposable materials for both sample preparation and immunoassay analysis is typically well below $100/sample. Exact cost will depend on project size and other variables. Cost of analytical services performed at CAPE Technologies depends on turnaround time, batch size, sensitivity target, and other factors, but is generally in the range of $150-300/sample. Note that the cost of the kit had not changed significantly since the original demonstration.

4.3.2 Cost Comparison to HRMS Method

The economic analysis developed for the original study found that the immunoassay costs were 15% of the HRMS method (U.S. EPA, 2005d). This is based on the total of all costs for both methods, including ^{13}C-labeled standards, equipment, setup, disposables, labor, and waste disposal. The ratio for the current study was not calculated, but should be in the same range. Many details of the comparison are given in the original study final report U.S. EPA, 2005d).

4.3.3 Availability of Technology

All technologies involved in both the original study and the site specific study are either owned by CAPE Technologies or are licensed by CAPE Technologies on a global exclusive basis, directly from the technology owner. Inventory of either completed kits or their components is always maintained at CAPE Technologies. Kits for sample preparation and immunoassay analysis are typically shipped within five business days after receipt of an order. Analysis performed by CAPE Technologies in their laboratory can generally be initiated within one day of sample receipt.

4.3.4 Turnaround

Turnaround time for analysis performed in an on-site field lab can be as little as 10 hours. A small batch of samples (roughly 10 or fewer) received in the early morning can be completed by the end of the day. More typical on-site workflow would be next day completion of the first batch and staggered overlap with subsequent batches. Based on the original demonstration, an experienced analyst sustained a processing rate of 19 test samples (plus QA samples) per 11-hour day working in the field operating in a mobile laboratory (U.S. EPA, 2005d).

Analysis performed off-site by CAPE Technologies can generally be completed within 5-10 business days after receipt of samples. Individual projects may differ from this based on sample numbers, requirements for drying or other pre-processing, and willingness to pay premiums for accelerated handling.

4.3.5 Training/Ease of Use for DF1 Immunoassay Kit

Training for field use is optional, but highly recommended. The sample preparation protocol represents most of the total work and difficulty, and is therefore the focus of training. Sessions range from 1.5 to 3 days and include one or two complete runs of sample preparation and immunoassay analysis. Best results are typically obtained by analysts with training and experience in other field analytical chemistry methods. Dioxin analysis experience is helpful, but not essential. Prior immunoassay experience is also helpful, but not required. Some laboratory experience is essential. General familiarity with non-dioxin 8000 series EPA SW-846 Methods (such as 8082 and 8270) is also helpful.

No training is required for samples sent to CAPE Technologies for analysis.

Chapter 5
Conclusions

For the majority of the samples, CAPE Technologies DF1 Immunoassay Kit was demonstrated to be more precise and comparable to HRMS results with the use of a site-specific calibration procedure. However, the accuracy of some results from the Solutia, Newark Bay, and Tittabawassee River sites were not improved or poorer in some cases. Slight modifications in the sample preparation procedure were also made in this study which contributed to changes in performance. These procedure changes were not independently evaluated so performance changes can not be specifically attributed to procedural changes or the site-specific calibration approach. Given the wide range of possible responses of unknown matrices to the immunoassay, calibrating the kit's response against a few HRMS data points from each site is the preferred approach. The cost of operating this technology is about 15% of the cost of HRMS at $150-$300/sample. Same day results can be obtained if the user performs the immunoassay in an equipped, on-site mobile laboratory.

Chapter 6
References

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