United States
Environmental Protection
Agency

Office of Research and
Development
Washington, DC 20460

EPA/540/R-07/001
January 2007

Interim Report on the Evolution and Performance of the Eichrom Technologies Procept® Rapid Dioxin Assay for Soil and Sediment Samples

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Contract No. 68-C-00-185

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Notice

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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 Hybrizyme Corporation, which demonstrated the use of the AhRC PCR™ Kit. The AhRC PCR™ Kit was a technology that reported the concentration of aryl hydrocarbon receptor (AhR) binding compounds in a sample, with units reported as Ah Receptor Binding Units (AhRBU). At the time of the original demonstration, this particular technology was intended for use as a screening tool to rank samples from those inducing the greatest AhR activity to those inducing the least AhR activity rather than to provide highly quantitative dioxin concentration in units of toxicity equivalents (TEQ). After the SITE Dioxin demonstration, this technology was exclusively licensed to Eichrom Technologies. Eichrom focused their efforts on developing optimal sample preparation procedures for the assay and reporting TEQ values instead of AhRBU. The technology is now marketed under the trade name Procept® Rapid Dioxin Assay.

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, 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 Eichrom Technologies Procept® Rapid Dioxin Assay. The data generated and evaluated during the site-specific study showed that the TEQ data produced by the Procept® Rapid Dioxin Assay was more comparable to the HRMS

$TEQ_{D/F}$ data than was the data reported using the Hybrizyme AhRC PCR™ Kit in the original SITE demonstration. The Procept® Rapid Dioxin Assay 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.

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Abbreviations, Acronyms, and Symbols

AhR	aryl hydrocarbon receptor
AhRBU	aryl hydrocarbon receptor binding units
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
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
PCR	polymerase chain reaction
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

Acknowledgements

This report was prepared for the U.S. Environmental Protection Agency (EPA) Superfund Innovative Technology Evaluation (SITE) Program under the direction and coordination of Stephen Billets of the EPA's National Exposure Research Laboratory (NERL)—Environmental Sciences Division in Las Vegas, Nevada. The EPA NERL thanks the technology developer, Eichrom Technologies, in particular Mike Fern and Dan McAlister, for their participation in this study and for their review of this report. The EPA NERL thanks the following peer reviewers for their review of this report: Andy Grange (US EPA NERL) and Al Taylor (Michigan Department of Environmental Quality). This report was prepared for the EPA by Battelle. Acknowledgment is given to Amy Dindal, who was the Battelle Project Manager, and to Elizabeth Thompson, Joe Tabor, and Mary Schrock, for their contributions to the preparation of this report.

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 demonstrate reliable performance by the technologies to provide (1) potential users with a better understanding of the technologies' performance under well-defined conditions and (2) 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 analytical method would allow field personnel to quickly assess the extent 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).

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.

The follow-on study (referred to as the "site-specific study") was conducted in May 2006. All past participants in the original SITE demonstration were invited to participate in 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. 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 Procept® Rapid Dioxin Assay by Eichrom Technologies, and the results for this technology are described in this report.

1.3 Description of Eichrom Technologies

Founded in 1990 to commercialize chemical separation technology developed at Argonne National Laboratory, Eichrom Technologies is a provider of products in the areas of radiochemistry, geochemistry, hazardous metals analysis, and environmental screening. Eichrom supplies a range of ion exchange resins, from industry standard cation and anion exchange resins, to proprietary chelating resins that have unusual selectivity for higher valence transition metals. In 2004 Eichrom Europe, a French subsidiary, established an analytical laboratory to monitor drinking water for gross alpha/beta, tritium and potassium-40. This lab also performs radionuclide-specific analysis for a variety of alpha- and beta-emitting isotopes, whether anthropogenic or naturally occurring.

In 2005 Eichrom signed a licensing agreement with Hybrizyme Corporation, which provided exclusive manufacturing and sales rights in the United States and Europe to Hybrizyme's AhRC PCR® Assay for polychlorinated dibenzodioxins and furans (PCDD/F). The Hybrizyme Corporation AhRC PCR® Assay was a technology that reported the concentration of AhR binding compounds in units reported as AhRBU. The technology was intended for use as a screening tool to rank samples from those inducing the greatest AhR activity to those inducing the least AhR activity, rather than to provide highly quantitative TEQ values. Hybrizyme's goal was a highly portable screening technology that could help determine areas of greatest concern for cleanup at a site and could help minimize the number of more expensive analyses needed for specific analytes. Hybrizyme's AhRC PCR® Assay was one of the five technologies evaluated in the original SITE demonstration. After the original SITE demonstration, Hybrizyme licensed this technology to Eichrom Technologies. Since that time, Eichrom has focused on developing optimal sample preparation procedures for the assay and reporting quantitative TEQ values. This technology is now marketed under the trade name Procept® Rapid Dioxin Assay and is designed for use in analytical chemistry labs.

1.4 Overview of the Report

This report describes the experimental design of the site-specific study. Detailed methods are provided for the Procept® Rapid Dioxin Assay and the HRMS methods are also discussed. Correlations between Eichrom $TEQ_{D/F}$ and HRMS $TEQ_{D/F}$ results are discussed along with the accuracy and precision of the test results. Operational factors such as cost comparisons, availability, turnaround times, and ease of use and training are also reported, although this information was provided by Eichrom and not independently verified.

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 Post. 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 $TEQ_{PCB,}$. 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 Post

The Winona Post site in Winona, Missouri, was a wood treatment facility that had been remediated. Contaminants at the site included PCP, dioxin, diesel fuel, and PAHs. 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 approximate 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. No PCBs or PAHs were identified in the soil.

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. Total PCBs from these previous measurements were in the low parts-per-trillion (ppt) range. 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. Average PCB concentrations ranged from 300 to 740 ppb. 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 are around 250 ppb. The fine-grained sediment and total organic carbon values were similar to percentages in Newark Bay.

2.3 Testing Protocol

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 so that they could perform site-specific calibrations, but no other HRMS data was provided to the developers.

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.

Eichrom received their samples on March 20, 2006 and reported results to Battelle for all 112 samples on May 1, 2006. After receiving the HRMS data, Eichrom elected to re-run all of their sample extracts, using an additional purification step (i.e., a larger silica column and increased elution solvent) and revised their reported data on June 2, 2006.

2.4 Data Analysis

As for the original SITE demonstration, HRMS analysis was used as the reference method against which all developer data was compared. 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. 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 any additional QC performed (for example: method blanks, positive controls, duplicates, 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 Procept® Rapid Dioxin Assay 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 concentration reported by Eichrom and TEQ_{HRMS} is the average HRMS concentration. Acceptable performance is generally in the range of 70 – 130% 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 Procept® Rapid Dioxin Assay'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%.

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

Chapter 3
Methods

This chapter describes the sample preparation, analytical, quality control, and data presentation methods used by Eichrom Technologies. Additionally the reference HRMS method is discussed. Each section will describe Eichrom's approach and the HRMS approach, followed by a description of the similarities and differences between the procedures. The Eichrom 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. Flowcharts of the Eichrom method are presented in Figures 1 and 2. A photo of the Eichrom assay is provided in Figure 3.

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 the ITVRs (U.S. EPA, 2005a, b, c, d, e). As stated in Chapter 2, 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, 2005a, b, c, d, e). 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

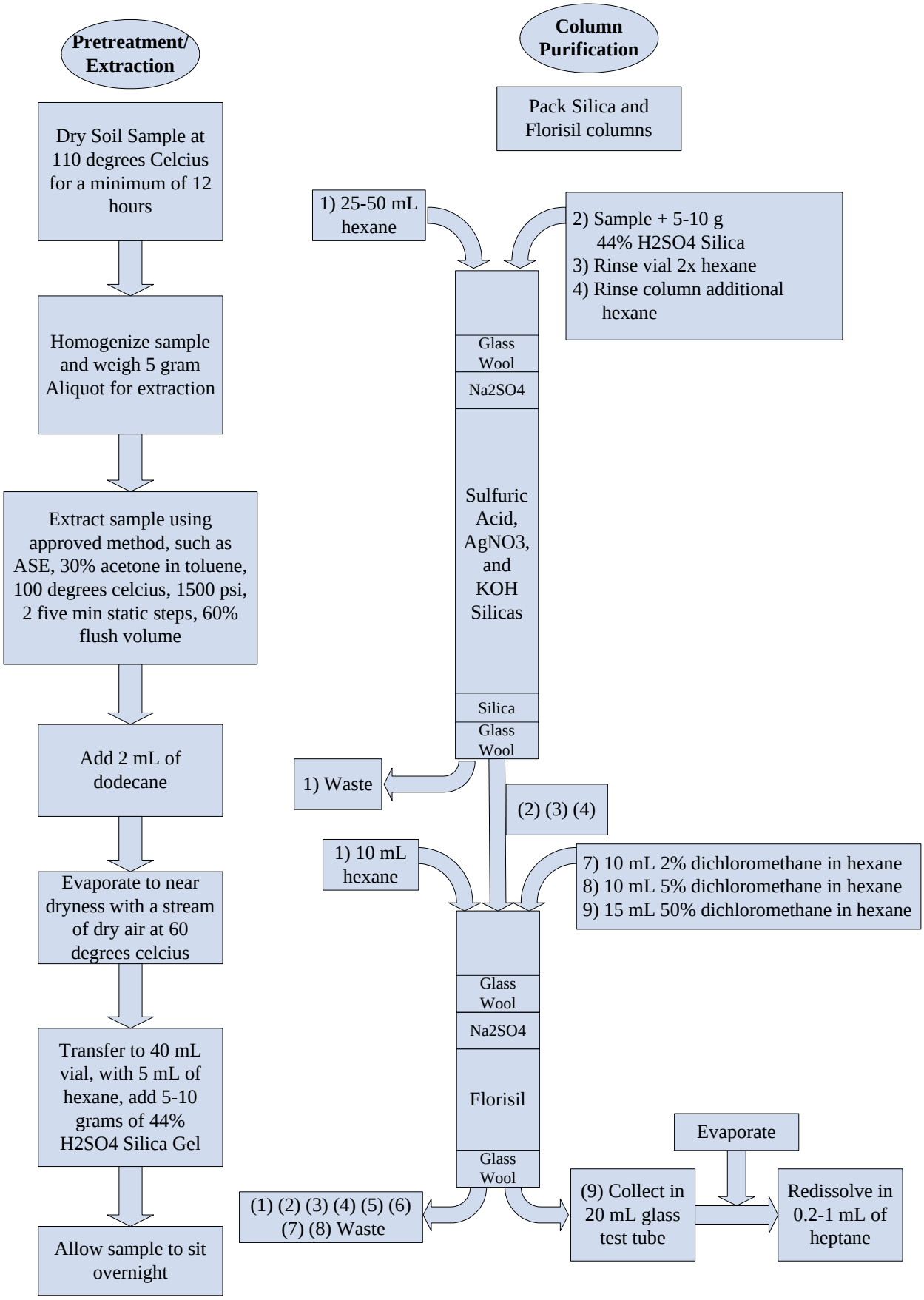

Figure 1. Procept® Sample Preparation and Cleanup

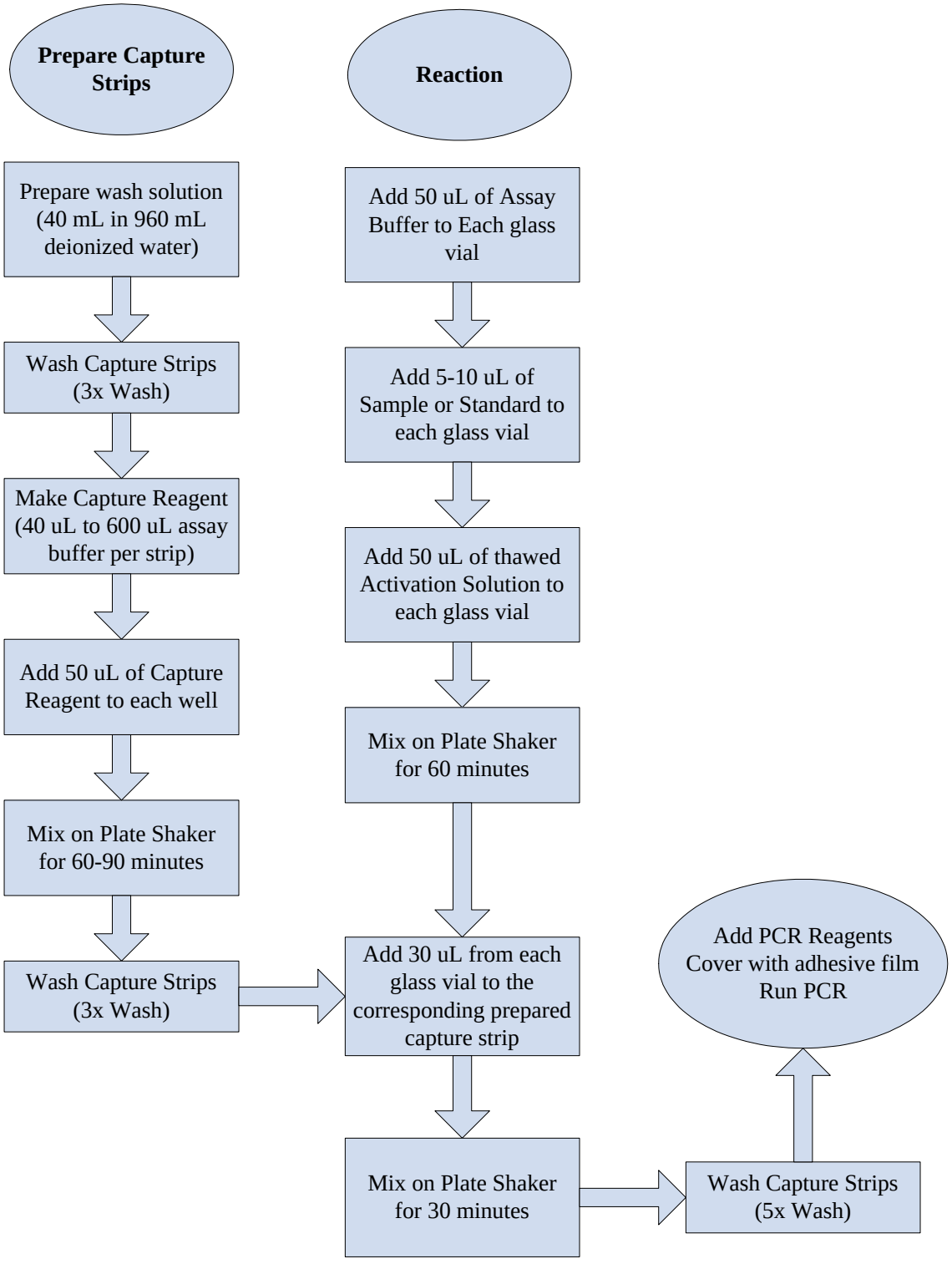

Figure 2. Procept®Assay Procedure

3.1 Sample Preparation

This section includes the sample extraction and cleanup methods employed.

3.1.1 Procept® Rapid Dioxin Assay Method

3.1.1.1 Reagents

Hexane: CHROMOSOLV Plus for HPLC, 99.9% (Sigma no. 650579)

Heptane: Purification Grade, 99% (Sigma no. 644455)

Methylene chloride: Pesticide Residue Analysis Grade (Acros no. AC61016)

Toluene: CHROMOSOLV Plus for HPLC, 98.5% (Sigma no. 650544)

Acetone: Histological Grade, 99.5% (Sigma no. 534064)

Silica: For Column Chromatography 60 (Fluka no. 60741)

Florisil: 100-200 mesh (Sigma no. 20281)

Sulfuric acid: Reagent Grade, ACS (Acros no. AC42452)

Diatamaceous earth: Sample Dispersant (Dionex no. 062819)

DNase-free water: (Acros no. AC32739)

De-ionized water: Milli-Q2 System (or equivalent)

Potassium Hydroxide: Certified ACS Grade (Fisher no. P250-1)

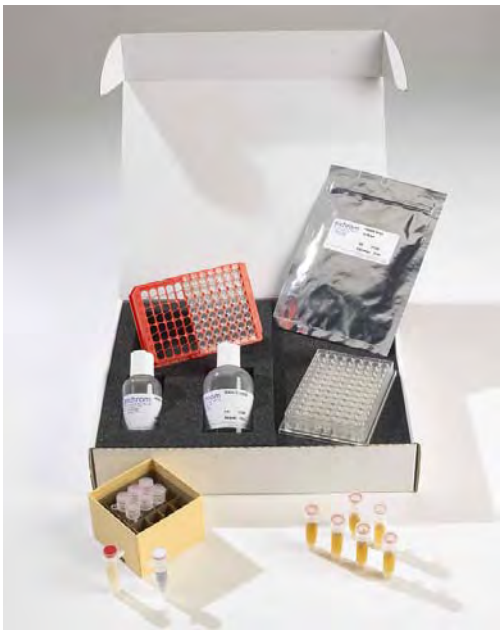

Figure 3. Procept® Rapid Dioxin Assay Kit

Sodium sulfate: Reagent Grade, ACS, anhydrous (Sigma no. 239313)

PCR Master Mix: Brilliant Plus QPCR Core Reagent Kit (Stratagene no. 929540)

Procept® Rapid Dioxin Assay: Eichrom Technologies, Inc.

3.1.1.2 Sample preparation

3.1.1.2.1 Determination of percent solids

3.1.1.2.2 Dry a glass vial at 110^{o}C for 12 hours; cool in a dessicator for each sample to be analyzed.

3.1.1.2.3 Weigh 5.0 grams of soil into the dried vial

3.1.1.2.4 Dry for a minimum of 12 hours at 110^{o}C and cool in a dessicator.

3.1.1.2.5 Calculate percent solids as follows:

% solids = (weight of sample after drying)/(weight of sample before drying) x 100% (eqn. 3-1)

3.1.1.3 Extraction

Any approved method for the extraction of polychlorinated dioxins and furans from soil can be used including Soxhlet and pressurized fluid extraction. The conditions for pressurized fluid extraction with a Dionex ASE100 used to generate the data in this report are given below.

3.1.1.3.1 Add 5 grams of diatomaceous earth sample dispersant to the dried soil sample and mix by capping the vial and shaking.

3.1.1.3.2 Place a glass fiber filter into a 34 mL stainless steel extraction cell and add 3 grams of diatomaceous earth sample dispersant.

3.1.1.3.3 Add the sample + dispersant to the extraction cell and fill the remaining volume with diatomaceous earth sample dispersant. Seal the cell by hand tightening the top and bottom caps.

3.1.1.3.4 Five minute static step. Flush 30% of the volume of the cell with 3:7 acetone: toluene. Second 5 minute static step. Flush 30% of the volume of the cell with 3:7 acetone:toluene. Flush for 60 seconds with nitrogen (no solvent).

3.1.1.4 Evaporation and solvent exchange

3.1.1.4.1 Add 2.0 mL of dodecane to the soil extract

3.1.1.4.2 Evaporate the acetone/toluene to approximately 10 mL with a gentle stream of air while heating at 50^{o}C (sand bath).

3.1.1.4.3 Transfer the extract to a 40 mL glass vial and rinse the 200 mL bottle twice with 5 mL of hexane.

3.1.1.4.4 Complete the solvent evaporation using a gentle stream of air while heating at 50^{o}C (sand bath) and add 5 mL of hexane.

3.1.1.5 Extract cleanup

3.1.1.5.1 Add 5 to 10 grams of 44% H_2SO_4 silica to the extract and allow the sample to sit overnight.

3.1.1.5.2 Pack and precondition a multilayer silica column and Florisil column (immediately prior to use):

- **Silica column** (50 mL glass serological pipette, bottom to top): glass wool plug, washed silica, 10% AgNO3 Silica, 2% KOH silica, washed silica, 44% H_2SO_4 silica, 22% H_2SO_4 silica, dry Na_2SO_4, glass wool plug. (See www.eichrom.com for the most up to date recommended column compositions and elution parameters)
- **Florisil column** (25 mL glass serological pipette, bottom to top): glass wool plug, 1.8 to 2.0 grams Florisil (washed by ASE, 50% methylene chloride in hexane, dried 24 hours at 140^{o}C, cooled and stored in a dessicator), 1.5 grams of dry Na_2SO_4, glass wool plug.
- Silica column prewashed with 25 to 50 mL of hexane
- Florisil column prewashed with 10 mL of hexane

3.1.1.5.3 Set up columns in series in a fume hood, with the silica column on top of the Florisil column and a 150 mL glass beaker beneath the Florisil column.

3.1.1.5.4 Slurry the extract/H_2SO_4 silica mixture and add it to the top of the silica column.

3.1.1.5.5 Use two portions of 5 mL hexane to complete the transfer of the extract to the silica column.

3.1.1.5.6 When the solvent level reaches 1 mm above the top of the silica column bed, add additional hexane to the silica column to complete the dioxin/furan elution.

3.1.1.5.7 When the solvent level reaches the top of the silica column bed, remove the silica column.

3.1.1.5.8 When the solvent level reaches the top of the Florisil column bed, rinse the column sequentially with 10 mL of 2% (v:v) methylene chloride in hexane and 10 mL of 5% (v:v) methylene chloride in hexane (waste).

3.1.1.5.9 Replace the 150 mL glass beaker with a 20 mL glass test tube and elute the dioxin and furans with 15 mL of 50% (w:w) methylene chloride in hexane.

3.1.1.5.10 Evaporate the sample to dryness using a gentle stream of air and transfer the residue to a 2 mL glass vial using methylene chloride.

3.1.1.5.11 Evaporate the sample to dryness using a gentle stream of air and dissolve the residue in 0.2 to 1.0 mL of heptane.

3.1.1.5.12 Add 0.3 mL of concentrated H_2SO_4 or 0.02 to 0.10 grams of 10% $AgNO_3$ silica, cap the vial with a PTFE lined cap and equilibrate overnight while mixing on an orbital plate shaker. (The equilibration with H_2SO_4 or $AgNO_3$ silica will remove any PAH compounds accumulated from the reagents used during the extract cleanup.

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 microliters (µL).

3.2 Sample Analysis

This section includes the determinative analytical methods employed.

3.2.1 Procept® Rapid Dioxin Assay Method

3.2.1.1 Preparation of Capture Strips

3.2.1.1.1 The wash solution is prepared by diluting 40 mL of the wash solution concentrate to 1 L with deionized water. The wash solution is then placed into a glass flask and used to prime the plate washer (BioTek ELx50, new buffer prime).

3.2.1.1.2 The desired number of capture strips are then placed into the orange rack and washed using the plate washer (3x wash) to remove the protective coating.

3.2.1.1.3 The capture reagent is thawed and diluted in a glass test tube with the assay buffer (40 µL of capture reagent to 600 µL of assay buffer per capture strip).

3.2.1.1.4 Using an eight-channel automatic delivery pipette and 100 µL barrier pipette tips, 50 µL of the diluted capture reagent is added to each well of the capture strips.

3.2.1.1.5 The capture strips are then placed on the plate shaker (Heidolph Titramax 1000 or equivalent, speed set at 900) for 60 to 90 minutes.

3.2.1.2 Reaction of Samples and Standards with Ah-Receptor (Performed while capture strips are on the plate shaker)

3.2.1.2.1 For each capture strip used, a glass reaction vial is charged with 50 µL of assay buffer (8-channel automatic delivery pipette and 100 µL barrier pipette tips).

3.2.1.2.2 Five µL of the purified sample extract or standard is added to each glass vial (0.1 to 20 µL automatic delivery pipette and barrier pipette tips).

3.2.1.2.3 For each capture strip used, one vial of the activation solution (stored at -80^{o}C or in a liquid nitrogen dewar) is thawed and 50 µL is added to each glass reaction vial (8-channel automatic delivery pipette and 100 µL barrier pipette tips).

3.2.1.2.4 The rack of glass reaction vials is placed on the plate shaker for 60 minutes.

3.2.1.3 Addition of reaction mixture to capture strips

3.2.1.3.1 After 60 to 90 minutes on the plate shaker, the capture strips are washed using the plate washer (3x wash) to remove any excess capture reagent.

3.2.1.3.2 Using the 8-channel automatic delivery pipette and 100 µL barrier pipette tips, 30 µL of each solution from the glass reaction vials is added to each corresponding capture strip.

3.2.1.3.3 The capture strips are placed on the plate shaker for 30 minutes.

3.2.1.3.4 Following 30 minutes on the plate shaker, the capture strips are washed using the plate washer (5x wash). This takes approximately 15 minutes.

3.2.1.4 Polymerase Chain Reaction (PCR)

3.2.1.4.1 While the capture strips are on the plate washer, the PCR reagents are prepared per the manufacturers instructions. For each capture strip used, mix 175 µL of DNase free water, 140 µL of PCR master mix and 35 µL of primer probe solution in a glass test tube.

3.2.1.4.2 When the 5x wash program is complete, using the 8-channel automatic delivery pipette and 100 µL barrier pipette tips, 40 µL of the PCR reagent is added to each well of the capture strips.

3.2.1.4.3 The capture strips are sealed using optically clear adhesive film (Applied Biosystems part no. 4311971).

3.2.1.4.4 Two optical cover compression pads are placed on top of the sealed capture strips, and the capture strips are placed in the PCR instrument.

3.2.1.4.5 The quantitative PCR program is run using the following parameters:

Quantification dye:	FAM
Reference dye:	ROX
Thermal Profile:	2 minutes at 50^{o}C
	10 minutes at 95^{o}C
	Cycle between 15 seconds at 95^{o}C then 60 seconds at 60^{o}C (40 times)

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

3.3.1 Procept® Rapid Dioxin Assay Method

Since ^{13}C-labeled standards cannot be used to monitor recoveries through the sample preparation method used for the Procept® Rapid Dioxin Assay, it is important that samples are processed consistently. Also, it is recommended that a reagent blank and a known sample be processed with each batch of samples. The reagent blank can be generated by extracting diatomaceous earth or a soil sample known to be free of dioxin and furan contamination. The known sample can be a sample which has been analyzed for D/F contamination by HRMS or a blank soil spiked with a known quantity of dioxin and furan standards. Typical yields for the entire sample preparation method are 80 to 105% for diatomaceous earth spiked with a mixture of tetra-octa chlorinated dioxins and furans. In addition, for this site-specific study, Eichrom analyzed the QC sample unspiked as well as spiked in duplicate with a mixture of PCDD/F standards at a total TEQ value approximately ten times that of the unspiked QC sample. This mixture contained the following congeners: 2,3,7,8-TCDD (5% by mass); 1,2,3,7,8 PeCDD (5%); 1,2,3,7,8,9-HxCDD (5%); OCDD (60%); 2,3,7,8-TCDF (10%); 1,2,3,7,8-PeCDF (5%); 2,3,4,7,8-PeCDF (5%); and 1,2,3,4,7,8-HxCDF (5%).

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 ^{13}C-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 ^{13}C-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 Presentation Results

3.4.1 Eichrom Method

Whereas HRMS methods measure the concentration of seventeen individual PCDD/F congeners and then apply a toxicity equivalency factor (TEF) to calculate the TEQ value, the Procept® Rapid Dioxin Assay measures the total TEQ directly. As shown in Table 3-2, the response factors measured for the seventeen most toxic PCDD/F congeners on the Procept® Assay are presented along side the World Health Organization (WHO) 1998 TEF values (van den Berg, 1998) used to calculate TEQ from the HRMS congener data. Note that the updated WHO 2005 TEF values are presented for comparison, but these values were not available during the time of the original HRMS analysis, so the WHO 1998 TEF values were used (van den Berg, 2006). The agreement in magnitude is comparable for some compounds (e.g., 2,3,7,8-TCDD and 1,2,3,6,7,8-HxCDD), but quite different for others (e.g., 1,2,3,7,8-PCDD and OCDD).

Response factor values (shown in Table 3-2) for some of the non-dioxin/furan compounds, such as the PAHs, are quite high. For example, indeno-(1,2,3-cd)-pyrene, benzo(k)fluoranthene, benzo(b) fluoranthene, dibenzy(a,h)anthracene, and benzo(a)pyrene all have response factors to the Procept® Assay greater than 0.1. While these response factors are relatively high, the intent of the Procept® Rapid Dioxin Assay is to remove the PAHs during the sample preparation process. The efficiency of removal of non-dioxin/furan compounds was not evaluated in this study.

The quantity measured by the Procept® Rapid Dioxin Assay is the Threshold Cycle (Ct) of a polymerase chain reaction (PCR) growth curve. The Ct value can be converted to a TEQ value by generating a standard curve of Ct values for solutions of known TEQ. For samples with an unknown or variable ratio of PCDD/F congeners, the standard curve can be generated using serial dilutions of 2,3,7,8-TCDD. Due to differences between the WHO TEF values and the Procept® Assay Response factors for individual congeners, the most accurate results will be achieved by generating a standard curve from serial dilutions of a mix of PCDD/F standards mimicking the ratio typically observed in the samples. This was the approach taken for this study, since the samples results were calibrated based on the QC sample results.

The software package for the PCR instrument will typically convert the Ct value of unknown samples to TEQ based on the standard curve generated for each Procept® Assay. However, this calculation can also be done independently using Microsoft Excel (or equivalent software) using the Ct value for the unknown sample extract and a standard curve generated by plotting Ct vs. log TEQ for a series of known standards:

$$TEQ_{extract} = 10^{((Ct - b)/m)} \qquad \text{(eqn 3-2)}$$

where Ct is the threshold cycle measured for the unknown sample extract, and m and b are the slope and y-intercept of the standard curve, respectively. The $TEQ_{extract}$ is then used to calculate the TEQ for the soil sample using the following equation:

$$TEQ_{soil} = (TEQ_{extract} \cdot V \cdot DF)/(M \cdot RF) \qquad \text{(eqn 3-3)}$$

Table 3-2. Comparison of Procept® Rapid Dioxin Assay Response Factors to Toxicity Equivalency Factors (TEF)

Congener [a]	WHO 1998 TEF [b]	WHO 2005 TEF [c]	Procept® Assay Response Factor
2,3,7,8 TCDD	1	1	1
1,2,3,7,8 PCDD	1	1	0.55
1,2,3,4,7,8 HxCDD	0.1	0.1	0.35
1,2,3,6,7,8 HxCDD	0.1	0.1	0.1
1,2,3,7,8,9 HxCDD	0.1	0.1	0.49
1,2,3,4,6,7,8 HpCDD	0.01	0.01	0.013
1,2,3,4,6,7,8,9 OCDD	0.0001	0.0003	0.0000028
2,3,7,8 TCDF	0.1	0.1	0.06
1,2,3,7,8 PCDF	0.05	0.03	0.14
2,3,4,7,8 PCDF	0.5	0.3	0.32
1,2,3,4,7,8 HxCDF	0.1	0.1	0.39
1,2,3,6,7,8 HxCDF	0.1	0.1	0.17
1,2,3,7,8,9 HxCDF	0.1	0.1	0.28
2,3,4,6,7,8 HxCDF	0.1	0.1	0.1
1,2,3,4,6,7,8 HpCDF	0.01	0.01	0.053
1,2,3,4,7,8,9 HpCDF	0.01	0.01	0.016
1,2,3,4,6,7,8,9 OCDF	0.0001	0.0003	0.00046
PCB-81 (3,4,4',5)	0.0001	0.0003	0.000045
PCB-77 (3,3',4,4')	0.0001	0.0001	0.000034
PCB-126 (3,3',4,4',5)	0.1	0.1	0.014
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	0.0000089
PCB-118 (2,3',4,4',5)	0.0001	0.00003	$<3 \times 10^{-7}$
PCB-114 (2,3,4,4',5)	0.0005	0.00003	0.00001
PCB-105 (2,3,3',4,4')	0.0001	0.00003	$<3 \times 10^{-7}$
PCB-167 (2,3',4,4',5,5')	0.00001	0.00003	0.000001
PCB-156 (2,3,3',4,4',5)	0.0005	0.00003	0.000029
PCB-157 (2,3,3',4,4',5')	0.0005	0.00003	0.000043
PCB-189 (2,3,3',4,4',5,5')	0.0002	0.00003	$<3 \times 10^{-7}$
Indeno(1,2,3-cd)pyrene	N/A	N/A	0.8
Benzo(k)fluoranthene	N/A	N/A	0.54
Benzo(b)fluoranthene	N/A	N/A	0.59
Dibenzo(a,h)anthracene	N/A	N/A	0.29
Benzo(a)pyrene	N/A	N/A	0.13
Benzo(a)anthracene	N/A	N/A	0.054
Chrysene	N/A	N/A	0.036
Benzo(g,h,i)perylene	N/A	N/A	0.0038

[a] Acenaphthylene, anthracene, fluorene, naphthalene, fluoranthene, phenanthrene, pyrene, acenaphthene, 2-methylnaphthalene, 2-chloronaphthalene, biphenyl, 2,4-dichlorophenol, 3,4-dichlorophenol and toluene showed no measurable response at 10 ppm.
[b] van den Berg, 1998
[c] van den Berg, 2006

where $TEQ_{extract}$ is the TEQ value measured for the sample extract, V is the volume, in milliliters, of solvent in which the extract was dissolved, DF is the dilution factor, M is the dry weight, in grams, of the sample which was processed to generate the extract and RF is the recovery factor calculated by processing a known sample through the sample preparation method, which in this case was the QC sample supplied with each sample batch.

The DF is only needed when multiple dilutions of the sample must be analyzed to achieve the range of quantification needed by the user. For a single dilution of a sample extract, the calibration curve for the Procept® Assay is normally linear over just greater than 2 orders of magnitude (for example 1 to 200 ppt). If the user wishes to measure a wider range of TEQ values, the sample must be diluted and re-assayed.

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.)

3.5 Comparison of Procept® and HRMS methods

The steps involved in the Procept® and HRMS methods are compared and contrasted in detail in Table 3-3. In this study, both the Procept® Rapid Dioxin Assay and characterization HRMS extraction methods employed accelerated solvent extraction (ASE), although the extraction solvents were different (Eichrom used 30% acetone in toluene; the characterization method used methylene chloride). The reference HRMS method used toluene extraction by Dean-Stark Soxhlet extraction. The Procept® method also states that Soxhlet extraction can be used. Sample cleanup for the Procept® Rapid Dioxin Assay and HRMS methods were similar, utilizing a series of silica and Florisil columns although the specific types, sizes, and volumes of extraction solvents varied between the methods. The difference between the Procept® assay and HRMS methods is most significant in the analytical step. The 1613B methods utilize HRMS, which is a laboratory-based, expensive analysis that allows for congener-specific analysis. The Procept® assay is analyzed using PCR which can be a field portable or laboratory-based instrument (although the extensive preparation and cleanup procedures described in Sections 3.1 and 3.2 suggest that this method fundamentally is a laboratory-based technique). Both HRMS and PCR techniques require a technically trained operator. The level of QC method criteria are much more stringent and involved for the HRMS methods, but some common QC techniques (blanks, laboratory control samples, matrix spikes) are applied in both techniques. The same data units, $TEQ_{D/F}$, are reported by both Procept® and HRMS, but the values are derived by different methods. The Procept® value is a total TEQ value that is calibrated based on the assay's response to the site-specific QC sample and therefore requires some percentage of HRMS confirmatory analyses (either concurrently or based on historical site information). The HRMS value is the sum of the 17 PCDD/F congener concentrations multiplied by the WHO TEF.

Table 3-3. Comparison between Procept® Rapid Dioxin Assay and Characterization HRMS Methods

Method Step	Similarities	Differences
Sample Preparation	Accelerated solvent extraction (ASE) is the extraction technique used by both methods.	Extraction solvent and ASE program **Eichrom**: Sample are mixed with diatomaceous earth and are extracted at 1500 psi and 100°C for two 5 minute static steps and 60% flush volume using 30% acetone in toluene and the extract collected in 200 mL glass bottles. Samples are concentrated by nitrogen blow down in a sand bath. **HRMS**: Samples are mixed with Hydromatrix and are extracted at 2000 psi and 125°C for a 7 minute heat time, a static time of 10 minutes and a flush volume of 60% using methylene chloride. Purge time is set to 120 seconds, and there are three static cycles. Samples are concentrated by TurboVap.
Sample Cleanup	Both Eichrom and the HRMS methods use a series of silica columns eluted with 50 mL hexane. Acid silica in the silica columns is 44% w/w. Both methods use serological pipettes for multilayer acid silica columns.	**Eichrom**: Additional cleanup with Florisil columns stacked under multilayer acid/base silica columns. Florisil columns are eluted with 15 mL of 50:50 (w/w) methylene chloride: hexane to remove dioxins and furans. **HRMS**: Additional cleanup methods are used including Gel Permeation Chromatography (GPC), acid/base back extraction, carbon column and silica and alumina columns. GPC- Extracts are brought to approximately 2 mL in methylene chloride. Each extract is transferred with four 1-mL aliquots of methylene chloride rinses to a GPC vial that has been pre-marked at 7 mL. Methylene chloride is added to the GPC vial to bring the total volume to the 7 mL mark on the vial. The extract is eluted according to the calibration data. Acid/Base back extraction- Extracts must not contain any Methylene chloride. The extracts are partitioned against 30mL of sulfuric acid solution and shaken for 2 minutes and the aqueous portion is discarded. The acid washing is repeated using 20-mL of sulfuric acid until no color is visible in the aqueous layer, to a maximum of four washings. The extract is partitioned against 20 mL of sodium chloride solution in the same way as with acid. The aqueous layer is discarded. The extract is partitioned against 15 mL of potassium hydroxide in the same way as with acid. The base washing is repeated until no color is visible in the aqueous layer, to a maximum of four washings. The partitioning is repeated against sodium chloride solution two times and the aqueous layer is discarded each time. Acid silica columns- HRMS uses only the 44% (w/w) in the silica columns. Eichrom used a layer of 22% w/w as well a layer of 44% (w/w). Both labs use 20-50 mL of hexane as the elution solvent Alumina columns- Alumina columns are stacked under the multilayer acid/base silica columns so that the eluant can drip directly onto the pre-rinsed alumina columns. 40 mL hexane: methylene cholride (50:50) is the final elution solvent. Final cleanup column used is 20% carbon: celite with 40 mL of toluene as the final elution solvent

Method Step	Similarities	Differences
Sample Analysis	Both are laboratory-based methods which require technically trained operators.	Procept® uses PCR; 1613B uses HRMS
Quality Control	Both methods include reagent blanks, laboratory control samples, and matrix spike/duplicates performed on each batch (20-25) of samples.	Use of ^{13}C-labeled standards for HRMS methods cannot be used for Procept®
Data Presentation	Results reported in total $TEQ_{D/F}$	Congener specific analysis for HRMS; total $TEQ_{D/F}$ result for Procept® based on HRMS data for one or more confirmatory or quality control samples.

Chapter 4
Results and Discussion

4.1 Evolution of Procept® Rapid Dioxin Assay

The Hybrizyme Corporation AhRC PCR™ Kit was a technology that measured the concentration of AhR binding compounds in units reported as AhRBU. At the time of the original SITE demonstration, this particular test was intended for use as a screening tool to rank samples from those inducing the greatest AhR activity to those inducing the least AhR activity, rather than to provide highly accurate TEQ. The developer's goal was a highly portable screening technology to help determine areas of greatest concern for cleanup at a site and to help minimize the number of more expensive analyses needed for specific analytes. In March 2005, Hybrizyme licensed this technology to Eichrom Technologies. Eichrom focused on optimization of the sample preparation, which involved a more involved, laboratory-based approach. Eichrom analyzed the 112 samples for this study and reported results within one month. After reviewing the initial results, Eichrom determined that an additional purification step would improve the accuracy and precision of the results. Eichrom did not re-extract all 112 samples, but rather took the existing extracts through the additional purification step and re-assayed the samples by PCR. The results from Eichrom's second attempt at the site-specific study samples are evaluated in this report. While the Eichrom method has improved significantly from Hybrizyme's generic screening tool for AhR compounds to a quantitative technology for $TEQ_{D/F}$, the method is still being optimized. Eichrom expects to complete optimization of this method by the end of 2006.

4.2 Procept® Rapid Dioxin Assay Results

Eichrom's reported results for the site-specific study are summarized by site in Tables 4-1 through 4-5.

4.3 Discussion

An example of the results reported by Hybrizyme in the original SITE demonstration is shown in Table 4-6. The Hybrizyme technology did not report TEQ concentrations, but rather was intended to serve as an indicator of high levels of AhR binding compounds. Once Eichrom obtained exclusive licensing of the technology, Eichrom's focus was on the sample preparation aspect, since it was apparent that TEQ data could not be derived using Hybrizyme's method (i.e., a quick extraction using a cocktail of solvents followed by an acid-wash cleanup). Eichrom arrived at a sample preparation procedure that was similar to the HRMS method, but used essentially the same detection assay as was performed with the Hybrizyme method.

The results shown in Tables 4-1 through 4-5 demonstrate the comparability to the HRMS results and the precision of the method. For the Winona Post samples (Table 4-1), the percent recovery (%R) values were 30%, 74%, 78%, 86%, and 93%. This indicated that all but one sample set (Cell #12) were reported with results that were fairly consistent with the HRMS method. These results also indicated that the sample results reported by Eichrom were usually lower than the HRMS, with all %R values less than 100%. The relative standard deviation (RSD) values were between 30% and 46%. Note that all of the sample concentrations at this site were the highest among the five sites (approximately 10,000 pg/g $TEQ_{D/F}$).

Table 4-1. Winona Post Sample Results

			pg/g TEQ	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**Procept®**
Cell #10	1	W-13	8648	8909
	2	W-9		6134
	3	W-17		6723
	4	W-20		3980
Average				6436
Standard Deviation (SD)				2027
Relative standard deviation (RSD)				31%
% Recovery				74%
Cell #12	1	W-15	8831	2622
	2	W-18		1132
	3	W-5		2844
	4	W-7		4173
Average				2693
SD				1246
RSD				46%
% Recovery				30%
Cell #2	QC	W-1	11,071	12,325
	1	W-10		7204
	2	W-2		8071
	3	W-12		6725
Average				8581
SD				2557
RSD				30%
% Recovery				78%
Cell #4	1	W-14	11,410	9314
	2	W-11		16,339
	3	W-4		11,160
	4	W-21		5506
Average				10,580
SD				4504
RSD				43%
% Recovery				93%
Cell #8	1	W-16	11,259	11,110
	2	W-3		9855
	3	W-19		4668
	4	W-8		13,174
Average				9702
SD				3624
RSD				37%
% Recovery				86%
ERA Blank		W-6	ND	3

ND = not detected

Table 4-2. Tittabawassee River Sample Results

			pg/g TEQ	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**Procept®**
ERA Blank	1	TR-4	ND	3
	2	TR-16		1
	3	TR-7		11
	4	TR-18		1
DNR 1	1	TR-3	435	1243
	2	TR-11		394
	3	TR-23		546
	QC	QC TR-1		365
Average				637
Standard Deviation (SD)				412
Relative standard deviation (RSD)				65%
% Recovery				146%
DNR 2	1	TR-13	42	37
	2	TR-19		41
	3	TR-21		3
	4	TR-5		51
Average				33
SD				21
RSD				63%
% Recovery				79%
FFP 1	1	TR-10	3127	4705
	2	TR-20		9486
	3	TR-8		4829
	4	TR-15		4452
Average				5868
SD				2417
RSD				41%
% Recovery				188%
FFP 2	1	TR-6	1048	719
	2	TR-17		1249
	3	TR-14		1193
	4	TR-2		729
Average				972
SD				288
RSD				30%
% Recovery				93%
IMP 2	1	TR-22	808	8556
	2	TR-24		6865
	3	TR-12		1252
	4	TR-9		1871
Average				4636
SD				3626
RSD				78%
% Recovery				574%

ND = not detected

Table 4-3. Newark Bay Sample Results

			pg/g TEQ	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**Procept®**
ERA Blank		NB-12	ND	5
NB 1	1	NB-3	45	92
	2	NB-13		129
	3	NB-6		162
	4	NB-7		92
Average				119
Standard Deviation (SD)				34
Relative standard deviation (RSD)				29%
% Recovery				264%
NB 2	1	NB-14	38	100
	2	NB-16		103
	3	NB-9		58
	4	NB-19		66
Average				82
SD				23
RSD				28%
% Recovery				216%
NB 3	1	NB-8	32	25
	2	NB-5		22
	3	NB-21		84
	QC	NB-1 QC		17
Average				37
SD				31
RSD				84%
% Recovery				116%
NB 5	1	NB-17	16	30
	2	NB-2		13
	3	NB-11		20
	4	NB-20		28
Average				23
SD				8
RSD				35%
% Recovery				143%
NB 6	1	NB=10	62	137
	2	NB-15		150
	3	NB-4		NA
	4	NB-18		86
Average				124
SD				34
RSD				27%
% Recovery				200%

ND = not detected
NA = Eichrom did not report data for this sample due to a broken sample vial

Table 4-4. Raritan Bay Sample Results

			pg/g TEQ	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**Procept®**
ERA Blank		RB-4		0.2
RB 1	1	RB-5	14	16.6
	2	RB-14		11.3
	3	RB-16		7.1
	4	RB-20		7.5
Average				11
Standard Deviation (SD)				4
Relative standard deviation (RSD)				42%
% Recovery				76%
RB 2	1	RB-19	12	6.7
	2	RB-3		24.9
	3	RB-18		7.4
	4	RB-9		4.5
Average				11
SD				9
RSD				87%
% Recovery				91%
RB 4	1	RB-6	15	9.2
	2	RB-11		14.4
	3	RB-21		2.7
	4	RB-2		12.4
Average				10
SD				5
RSD				53%
% Recovery				64%
RB 5	1	RB-17	14	4.6
	2	RB-13		3.7
	3	RB-7		12.6
	4	RB-10		8.2
Average				7
SD				4
RSD				56%
% Recovery				52%
RB 6	1	RB-15	13	4.7
	2	RB-12		10.2
	3	RB-8		13.0
	QC	RB-1		10.4
Average				10
SD				3
RSD				36%
% Recovery				74%

ND = not detected

Table 4-5. Solutia Sample Results

			pg/g TEQ	
Sample ID	**Replicate**	**Analysis Order**	**Average HRMS**	**Procept®**
ERA Blank		S-11		7
SS 1	1	S05	846	2302
	2	S-9		1096
	3	S-23		1795
	4	S-20		1207
Average				1600
Standard Deviation (SD)				560
Relative standard deviation (RSD)				35%
% Recovery				189%
SS 2	1	S-18	48	1289
	2	S-4		1208
	3	S-14		1418
	4	S-22		1255
Average				1293
SD				90
RSD				7%
% Recovery				2693%
SS 3	1	S-15	3257	3366
	2	S-2		3736
	3	S-10		4581
	4	S-25		6633
Average				4579
SD				1461
RSD				32%
% Recovery				141%
SS 4	1	S-7	1833	2653
	2	S-16		2032
	3	S-13		2461
	QC	S-1		2199
Average				2336
SD				275
RSD				12%
% Recovery				127%
SS 5	1	S-8	1279	2479
	2	S-17		1405
	3	S-12		2097
	4	S-21		1753
Average				1934
SD				460
RSD				24%
% Recovery				151%
SS 6	1	S-24	3951	5226
	2	S-6		4657
	3	S-3		4776
	4	S-19		8552
Average				5803
SD				1849
RSD				32%
% Recovery				147%

ND = not detected

Table 4-6. Hybrizyme AhRBU Results Compared to HRMS by Ranking--Original SITE Demonstration Data[a]

Environmental Site	Hybrizyme Ranking by Average AhRBU [b]	HRMS Ranking by Total Concentration (ng/g) [b]	Did Ranking Agree?
Newark Bay	1	1	No
	4	2	
	2	4	
	3	3	
Raritan Bay	1	1	No
	2	3	
	3	2	
Solutia	1	1	Yes
	3	3	
	2	2	
Tittabawassee River	3	3	No
	2	1	
	1	2	
Winona Post	1	1	Yes
	2	2	
	3	3	

[a] U.S. EPA 2005e

[b] Ranking of sample numbers within a site from low to high. Total concentration includes D/F and PCBs.

For the Tittabawassee River samples (Table 4-2), the percent recovery (%R) values were 79%, 93%, 146%, 188%, and 574%. This indicated that all but two sample sets were reported with results that were consistently higher than the HRMS method. The RSD values were between 30% and 78%.

For the Newark Bay samples (Table 4-3), the percent recovery (%R) values were 116%, 143%, 200%, 216%, and 264%. This indicated all of the sample sets were reported with results that were consistently higher than the HRMS method. The RSD values were between 27% and 35%, with the exception of one set, which had an RSD value of 84%. Note that all of the sample concentrations at this site were relatively low (< 100 pg/g $TEQ_{D/F}$).

For the Raritan Bay samples (Table 4-4), the percent recovery (%R) values were 52%, 64%, 74%, 76%, and 91%. This indicated that all of the sample sets were reported with results that were consistently lower than the HRMS method. The RSD values were between 36% and 87%.

For the Solutia samples (Table 4-5), the percent recovery (%R) values were 127%, 141%, 147%, 151%, 189%, and 2,693%. This indicated that all of the sample sets were reported with results that were consistently higher than the HRMS method. The RSD values were between 7% and 35%.

Procept® results for the eight uncontaminated ("blank") samples that were included in the experimental design were reported with $TEQ_{D/F}$ values between 0.2 and 11. None of these were reported as non-detects by the Procept® method.

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%, but three of the five sites had consistent results within the site (either all >100% or all < 100%). This evaluation also demonstrated the need for a site-specific factor (based on the QC sample results) to convert the raw data generated by the Procept® method into $TEQ_{D/F}$ data. This suggests that the need for independent HRMS confirmatory analysis would be appropriate at a level of 5% at the least; presumably, more comparability to HRMS

would be obtained with a greater percentage of HRMS confirmation analyses, but this was not evaluated in this study.

4.4 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 Eichrom Technologies and not evaluated independently by Battelle or EPA.

4.4.1 Cost of Procept® Rapid Dioxin Assay

The costs of running the Procept® Assay can be divided into three categories: capital equipment necessary to run the sample preparation and the assay itself, chemicals and supplies for the sample preparation and the assay, and labor necessary to perform the analysis. Labor costs are not described in this report, but costs for the other categories are described in this section.

Capital Equipment: Table 4-7 summarizes all of the pieces of equipment necessary to prepare samples and run the Procept® Assay. A range of estimated purchase prices is also shown. The total estimated acquisition costs to purchase all pieces of equipment new (new setup cost) would be in the range of $65,000 to $100,000. In practice, however, laboratories currently involved in dioxin analysis would already possess equipment related to sample preparation and storage and, as a result, they would not need to purchase all equipment in order to perform the Procept® Assay. Among the pieces of equipment likely to be owned already by a dioxin laboratory are an ASE system or a Soxhlet extraction system, a refrigerator/freezer (-20 °C), and a top loading balance. The acquisition cost of pieces of equipment specific to the Procept® Assay (addition to existing setup) range from $37,000 to $43,000.

Table 4-7. Capital Equipment Costs for the Procept® Assay

Capital Equipment	Cost	Incremental
Accelerated Solvent Extraction Instrument	$25,000 - $50,000	
PCR Instrument	$30,000 - $35,000	$30,000 - $35,000
Plate Washer	$3,000 - $4,000	$3,000 - $4,000
Plate Shaker	$1,000 - $1,500	$1,000 - $1,500
Automatic Delivery Pipets	$2,000	$2,000
Refrigerator/Freezer (-2°C)	$2,000 - $4,000	
Liquid Nitrogen Dewar	$600	$600
Top Loading Balance	$1,000	
	$65,000 - $100,000	$37,000 - $43,000

Chemicals and Supplies: The largest cost in this category is the cost of the Procept® Assay Kit. The list price of $2,400 is for a kit based on a 96-well plate. The number of samples that can be analyzed depends on several factors related to the data quality objectives (DQO) of the laboratory. The DQOs will drive decisions on the number of replicates of each sample and standard to be analyzed, as well as the necessity for other "QC" samples like blanks, spikes, etc. Assuming that each sample is analyzed in duplicate and that 16 wells of each plate are reserved to standards and other quality control samples, one kit will yield 40 analytical determinations, at a kit cost of $60. Table 4-8 includes the price of other disposable chemicals and supplies necessary for sample preparation and for running the kit itself. Total per sample cost for consumables is approximately $25, plus the cost of the kit.

Table 4-8. Chemicals and Supplies Cost for Procept® Assay

Chemicals	Amount per Sample
Toluene	100 mL
Acetone	30 mL
Heptane	100 mL
Hexane	50 mL
Methylene Chloride	10 mL
Florisil	2 grams
Silica	25 grams
Sulfuric Acid	25 grams
Potassium Hydroxide	1 gram
Sodium Chloride	10 grams
Sodium Sulfate Anhydrous	4 grams
Nitrogen Gas	1 tank/200 samples
Diatomaceous Earth	10 grams
PCR Reagents	
Deionized Water	
DNA-ase Free Water	
Disposable Supplies	**Amount per Sample**
0.5 – 20 uL Barrier Pipet Tips	3
0.1 – 100 uL Barrier Pipet Tips	3
0.1 – 200 uL Barrier Pipet Tips	1
100 – 1,000 uL Barrier Pipet Tips	1
Glass Transfer Pipets	2
Glass Test Tube	1
2 mL Glass Vial w/PTFE-Lined Cap	3
Glass Column (25 mL seralogical pipet)	1
Glass Column (50 mL seralogical pipet)	1
Glass Wool	0.1 grams
Chemical and Supplies Cost: $25/Sample	

4.4.2 Cost Comparison to HRMS Methods

This section presents the costs associated with the HRMS Method 1613B used to analyze the soil and sediment samples for dioxins and furans. Typical costs 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. Note that the HRMS cost per sample estimate includes everything to generate the sample result, where the costs listed for Eichrom in Section 4.4.1 include the consumables and capital equipment, but not the labor involved with the sample analysis.

4.4.3 Availability of Technology

Eichrom provides the Procept® Dioxin Assay as a kit that is available for purchase. Typical customers for this technology would include analytical laboratories. The manufacturing and quality control systems of this product are established and routine. Kits are available in three sizes: (1) a full 96-well format, (2) a half size kit with sufficient reagents for 48 wells, and (3) a one-fourth size kit with reagents for 24 wells.

The list price for a kit based on a 96-well plate is $2,400. The 48-well kit is $1,400, and the 24-well kit is $800.

4.4.4 Turnaround

The various steps of the sample preparation and Procept® assay are summarized in Table 4-9 with the amount of time that should be required to perform the step, both in terms of labor hours and in terms of elapsed time. The labor involved to perform the sample preparation and run the Procept® Assay itself have been estimated assuming a batch of 20 samples is processed simultaneously. The kit itself can accommodate larger batch sizes (up to 40 samples as indicated above.) The actual batch size chosen by a laboratory would depend on its staffing level and available equipment. The assumption of a batch size of 20 is based on Eichrom's experience with the ASE system and with the number of silica/Florisil column set ups that can fit inside a laboratory fume hood. Larger batch sizes would not require proportionally more labor or elapsed time. Approximately one-half of a labor hour per sample is necessary to perform the extraction, sample prep, and analysis. The elapsed time (or turnaround time, TAT) is a little more difficult to gauge. In Eichrom's experience with a single analyst working one shift, it takes slightly longer than 48 hours to complete the analysis. In laboratories where staffing is available for longer than an 8-hour work day, samples can be processed in less than 48 hours. The major constraint on TAT is the overnight sulfuric acid treatments, which are required to reduce background response for low level samples. Further optimization of this part of the sample preparation could result in significantly faster turnaround times.

Table 4-9. Estimation of Sample Turnaround Time Using Procept® Assay

Activity	Time Estimate	
	Hands On	**Elapsed**
ASE Extraction	1 hour	6 hours
Evaporation and Sulfuric Acid Treatment	1 hour	Overnight
Silica and Florisil Columns	3 hours	5 hours
Evaporation and Sulfuric Acid Treatment	1 hour	Overnight
Procept® Assay	2 hours	4 hours
Data Analysis	1 hour	1 hour
Total Man Hours	9 hours	
Per Sample (batch of 20)	~0.5 hours	
Total Elapsed Time: 1 shift		> 48 hours
Total Elapsed Time: 2 shift		< 48 hours

In comparison, a batch of 20 samples by the HRMS methods for a laboratory operating one shift generally takes 1 day for the ASE extraction, 1 day for GPC cleanup, two days for layered silica and carbon column cleanup, one day for final concentration and solvent exchange (a conservative total of 5 days for preparation) and a total of three days for sample analysis by HRMS, resulting in a total estimated eight-day TAT for a batch of 20 samples. Turnaround time could increase with a more rigorous QA review. Quicker than typical TATs for Method 1613B usually involves additional cost on a per sample basis.

4.4.5 Training/Ease of Use for Procept® Assay

The Procept® Assay is designed for use in analytical chemistry laboratories that currently perform dioxin testing. The sample preparation used is a simplified version of the typical silica/alumina/carbon column procedure that is widely used. Samples are extracted using toluene/acetone in a Soxhlet or ASE system.

Slightly smaller silica and Florisil columns are used and the carbon column is omitted. An additional step not typically employed in the EPA procedures (e.g., 1613B) is the use of sulfuric acid in batch contact with the dioxin (hexane) phase at two points in the procedure. However, all the steps in the sample preparation are easily carried out by any trained laboratory technician.

The Procept® Assay requires the use of multi-channel pipettes, a plate washer, plate shaker, a liquid nitrogen dewar and a real-time PCR instrument. These are items perhaps not typically used in a dioxin laboratory. All but the PCR require minimal training that can be accomplished in a matter of minutes or hours.

The real-time PCR instrument is a combination of a thermocycler to amplify and a detector to measure the fluorescence of each sample well in the 96-well plate. Software in the system determines the cycle in which the fluorescence crosses a "threshold" (C_t.) The C_t values are plotted versus TEQ for a set of TCDD standards. The TEQ for each sample is calculated by fitting its C_t on the standard curve. The training required for this instrument is typical of what is necessary to learn to operate any automated piece of laboratory equipment, such as an atomic absorption spectrometer or an inductively-coupled plasma instrument.

Chapter 5
Conclusions and Future Directions

As stated earlier, the Procept® Rapid Dioxin Assay has been licensed to Eichrom Technologies for approximately one year. Since obtaining the technology from Hybrizyme, Eichrom has made significant modifications to the front-end sample preparation procedures to improve the robustness of the method and to make the method more quantitative than the method that was being used by Hybrizyme. Eichrom participated in the site-specific study with a method that was an interim stage of development, nearing optimization, but not at the point of finalization of the method. Eichrom plans to continue working on the sample preparation and cleanup, such that the technology can produce results that are more highly correlated with HRMS methods with site-specific calibration. Eichrom plans to complete the method optimization by the end of 2006. It is unknown what impact the complete re-extraction of solid samples using the additional purification step would have had on the comparability and precision of the results; Eichrom also intends to investigate this further.

Eichrom anticipates that this technology will mostly be used by analytical laboratories prior to the more expensive HRMS analysis, given its lower cost and quicker analysis time. Prior to HRMS analyses, the Procept® Assay may be useful as a screening technique to provide gross estimates (none, a little, or a lot of toxicity) of the $TEQ_{D/F}$ present at a site. With site-specific calibration of the Procept® results using a one-point (e.g., quality control sample) HRMS result, it is a potential tool for providing an estimate of total $TEQ_{D/F}$ concentrations.

Eichrom has stated that future work will also involve evaluation of the Procept® assay's response to brominated and chloro-bromo D/F congeners, since it is speculated that these congeners will have some response to the assay due to the structural similarity to the chlorinated D/F congeners. However the degree of response relative to the chlorinated D/F congeners is unknown and needs to be investigated. Future work might also include evaluation of response factors for other compounds (e.g., 2,4-D) and evaluation of the effectiveness of removal of non-dioxin/furan compounds during the sample cleanup process.

Chapter 6
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