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Innovative Technology Verification Report

Technologies for Monitoring and Measurement of Dioxin and Dioxin-like Compounds in Soil and Sediment

Hybrizyme Corporation
AhRC PCR™ Kit

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Hybrizyme Corporation AhRC PCR™ Kit

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Notice

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's natural resources. Under the mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development (ORD) provides data and scientific support that can be used to solve environmental problems, build the scientific knowledge base needed to manage ecological resources wisely, understand how pollutants affect public health, and prevent or reduce environmental risks.

The National Exposure Research Laboratory is the Agency's center for investigation of technical and management approaches for identifying and quantifying risks to human health and the environment. Goals of the Laboratory's research program are to (1) develop and evaluate methods and technologies for characterizing and monitoring air, soil, and water; (2) support regulatory and policy decisions; and (3) provide the scientific support needed to ensure effective implementation of environmental regulations and strategies.

The EPA's Superfund Innovative Technology Evaluation (SITE) Program evaluates technologies designed for characterization and remediation of contaminated Superfund and Resource Conservation and Recovery Act sites. The SITE Program was created to provide reliable cost and performance data in order to speed the acceptance and use of innovative remediation, characterization, and monitoring technologies by the regulatory and user community.

Effective monitoring and measurement technologies are needed to assess the degree of contamination at a site, provide data that can be used to determine the risk to public health or the environment, and monitor the success or failure of a remediation process. One component of the EPA SITE Program, the Monitoring and Measurement Technology (MMT) Program, demonstrates and evaluates innovative technologies to meet these needs.

Candidate technologies can originate within the federal government or the private sector. Through the SITE Program, developers are given the opportunity to conduct a rigorous demonstration of their technologies under actual field conditions. By completing the demonstration and distributing the results, the Agency establishes a baseline for acceptance and use of these technologies. The MMT Program is managed by the ORD's Environmental Sciences Division in Las Vegas, Nevada.

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Abstract

A demonstration of 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, at Green Point Environmental Learning Center from April 26 to May 5, 2004. This innovative technology verification report describes the objectives and the results of that demonstration, and serves to verify the performance and cost of the Hybrizyme Corporation AhRC PCR™ Kit. Four other technologies were evaluated as part of this demonstration, and separate reports have been prepared for each technology. The objectives of the demonstration included evaluating the 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 the 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 Hybrizyme Corporation AhRC PCR™ Kit is a technology that reports the concentration of aryl hydrocarbon (Ah) receptor binding compounds in a sample, and the units are reported as Ah Receptor Binding Units (AhRBU). At the time of the demonstration, this particular test was intended for use as a screening tool to rank samples from those inducing the greatest Ah receptor (AhR) activity to those inducing the least AhR activity rather than to provide highly accurate toxicity equivalents (TEQ). The developer's goal is a highly portable screening technology that can help to determine areas of greatest concern for cleanup at a site and can help to minimize the number of more expensive analyses needed for specific analytes. It has been suggested that correlation between the Hybrizyme AhRBU results and HRMS TEQ results could be established by first characterizing a site and calibrating the Hybrizyme results to HRMS results. This approach was not evaluated during this demonstration. Since the technology measures an actual biological response, it is possible that the technology may give a better representation of the true toxicity from a risk assessment standpoint. Therefore, the technology's results were compared to the HRMS D/F and PCB data as well as polynuclear aromatic hydrocarbon (PAH) data in terms of ranking sample concentrations from low to high, rather than in a quantitative fashion of AhRBU vs TEQ. PAH concentrations were included in the comparison because Hybrizyme's kit responds to these compounds. The suite of PAHs that were quantified in the samples using gas chromatograph/mass spectrometry. The PAHs were a selected target list for this demonstration and likely do not include all of the PAHs that are responsive to this kit. The HRMS reference D/F and PCB data were generated by AXYS Analytical Services, using EPA Methods 1613B and 1668A.

Sample concentrations that were ranked by Hybrizyme from low to high were compared to the PE certified concentration and reference laboratory data, including contributions from PAHs where PAH data were available. The Hybrizyme ranking agreed with the certified values for higher concentration samples, but was inconsistent for lower concentration samples. The Hybrizyme technology's concentration ranking was consistent with the reference laboratory ranking for the environmental samples 70 to 90% of the time. The technology's calculated estimated method detection limit was 71 AhRBU. A significant effect was not observed for the reproducibility of Hybrizyme results by matrix type (soil, sediment, extract) or by PAH concentration, but a significant effect was observed for sample type (PE vs. environmental vs. extract) with the PE samples having a significantly higher mean RSD value (44%) compared to the environmental (19%) and the extract (14%) samples. The data generated and evaluated during this demonstration showed that the Hybrizyme technology could be used as an effective tool to rank sample concentrations from low to high AhR activity within a particular environmental site, particularly considering that the cost ($35,023 vs. $398,029) and the time (< two weeks vs. eight months) to analyze the 209 demonstration samples was significantly less than that of the reference laboratory.

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Abbreviations, Acronyms, and Symbols

Ah	aryl hydrocarbon
ANOVA	analysis of variance
AhR	aryl hydrocarbon receptor
AhRBU	Ah-receptor binding units
ATSDR	Agency for Toxic Substances and Disease Registry
CIL	Cambridge Isotope Laboratories
CoA	Certificate of Analysis
COC	chain of custody
CRM	certified reference material
DER	data evaluation report
D/F	dioxin/furan
DNA	deoxyribonucleic acid
DNR	Department of Natural Resources
D/QAPP	demonstration and quality assurance project plan
DRE	dioxin-responsive element
ELC	Environmental Learning Center
EMDL	estimated method detection limit
EMPC	estimated maximum possible concentration
EPA	Environmental Protection Agency
ERA	Environmental Resource Associates
g	gram
GC	gas chromatography
HPLC/GPC	high-performance liquid chromatography/gel permeation chromatography
HRGC	high-resolution capillary gas chromatography
HRMS	high-resolution mass spectrometry
i.d.	internal diameter
IDW	investigation-derived waste
ITVR	innovative technology verification report
kg	kilogram

Abbreviations, Acronyms, and Symbols (Continued)

L	liter
LRMS	low-resolution mass spectrometry
µL	microliter
µm	micrometer
m	meter
MDEQ	Michigan Department of Environmental Quality
MDL	method detection limit
mg	milligram
mL	milliliter
mm	millimeter
MMT	Monitoring and Measurement Technology
MS	mass spectrometry
NERL	National Exposure Research Laboratory
ng	nanogram
NIST	National Institute for Standards and Technology
NOAA	National Oceanic and Atmospheric Administration
ORD	Office of Research and Development
PAH	polynuclear aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-*p*-dioxin/dibenzofuran
PCP	pentachlorophenol
PCR	polymerase chain reaction
PE	performance evaluation
pg	picogram
ppb	parts per billion; nanogram/g; ng/g
ppm	parts per million; microgram/g; µg/g
ppt	parts per trillion; picogram/g; pg/g
QA/QC	quality assurance/quality control
RM	reference method
RPD	relative percent difference
RSD	relative standard deviation
SDL	sample-specific detection limit

Abbreviations, Acronyms, and Symbols (Continued)

SIM	selected ion monitoring
SITE	Superfund Innovative Technology Evaluation
SOP	standard operating procedure
SRM	Standard Reference Material®
TCDD	tetrachlorodibenzo-*p*-dioxin
TEF	toxicity equivalency factor
TEQ	toxicity equivalent
$TEQ_{D/F}$	total toxicity equivalents of dioxins/furans
TEQ_{PCB}	total toxicity equivalents of World Health Organization dioxin-like polychlorinated biphenyls
TOC	total organic carbon
total TEQ	total toxicity equivalents including the sum of the dioxin/furan and World Health Organization dioxin-like polychlorinated biphenyls
WHO	World Health Organization

Acknowledgments

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Chapter 1
Introduction

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. A field demonstration was conducted as part of the EPA Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) Program. The purpose of this demonstration was to obtain reliable performance and cost data on the technologies to provide (1) potential users with a better understanding of the technologies' performance and operating costs under well-defined field conditions and (2) the technology developers with documented results that will help promote the acceptance and use of their technologies.

This innovative technology verification report (ITVR) describes the SITE MMT Program and the scope of this demonstration (Chapter 1); a description of the Hybrizyme Corporation AhRC PCR™ Kit (Chapter 2); the demonstration site and the sampling locations (Chapter 3); the demonstration approach (Chapter 4); the confirmatory process (Chapter 5); the assessment of reference method data quality (Chapter 6); the performance of the technology (Chapter 7); the economic analysis for the technology and reference method (Chapter 8); the demonstration results in summary form (Chapter 9); and the references used to prepare this report (Chapter 10). Appendix A contains a verification statement; Appendix B contains supplemental information provided by the developer; Appendix C is a summary of method blank and batch duplicate data by the reference laboratory; and Appendix D contains a one-to-one matching of the developer and reference laboratory data.

1.1 Description of the SITE MMT Program

Performance verification of innovative environmental technologies is an integral part of the regulatory and research mission of the EPA. The SITE Program was established by the EPA Office of Solid Waste and Emergency Response and ORD under the Superfund Amendments and Reauthorization Act of 1986. The overall goal of the Program is to conduct performance verification studies and to promote the acceptance of innovative technologies that may be used to achieve long-term protection of human health and the environment. The program is designed to meet three primary objectives: (1) identify and remove obstacles to the development and commercial use of innovative technologies, (2) demonstrate promising technologies and gather reliable performance and cost information to support site characterization and remediation activities, and (3) develop procedures and policies that encourage use of innovative technologies at Superfund sites as well as at other waste sites or commercial facilities. The SITE Program includes the following elements:

- 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.

- Remediation Technology Program—Conducts demonstrations of innovative treatment technologies to provide reliable performance, cost, and applicability data for site cleanups.

- Technology Transfer Program—Provides and disseminates technical information in the form of updates, brochures, and other publications that promote the SITE Program and participating

technologies. It also supports the technologies by offering technical assistance, training, and workshops.

The MMT Program's technology verification process is designed to conduct demonstrations that will generate high-quality data so that potential users have reliable information regarding the technology performance and cost. Four steps are inherent in the process: (1) needs identification and technology selection, (2) demonstration planning and implementation, (3) report preparation, and (4) information distribution. The first step of the technology verification process begins with identifying technology needs of the EPA and regulated community. The EPA Regional offices, the U.S. Department of Energy, the U.S. Department of Defense, industry, and state environmental regulatory agencies are asked to identify technology needs for sampling, measurement, and monitoring of environmental media. Once a need is identified, a search is conducted to identify suitable technologies that will address the need. The technology search and identification process consists of examining industry and trade publications, attending related conferences, and exploring leads from technology developers and industry experts. Selection of technologies for field testing includes evaluation of the candidate technologies based on several criteria. A suitable technology for field testing

- is designed for use in the field or in a mobile laboratory,
- is applicable to a variety of environmentally contaminated sites,
- has potential for solving problems that current methods cannot satisfactorily address,
- has estimated costs that are lower than those of conventional methods,
- is likely to achieve equivalent or better results than current methods in areas such as data quality and turnaround time,
- uses techniques that are easier or safer than current methods, and
- is commercially available.

Once candidate technologies are identified, developers are asked to participate in a developer conference. This conference gives the developers an opportunity to describe their technologies' performance and to learn about the MMT Program.

The second step of the technology verification process is to plan and implement a demonstration that will generate representative, high-quality data to assist potential users in selecting a technology. Demonstration planning activities include a pre-demonstration sampling and analysis investigation that assesses existing conditions at the proposed demonstration site or sites. The objectives of the pre-demonstration investigation are to (1) confirm available information on applicable physical, chemical, and biological characteristics of contaminated media at the sites to justify selection of site areas for the demonstration; (2) provide the technology developers with an opportunity to evaluate the areas, analyze representative samples, and identify logistical requirements; (3) assess the overall logistical and quality assurance requirements for conducting the demonstration; and (4) select and provide the reference laboratory with an opportunity to identify any matrix-specific analytical problems associated with the contaminated media and to propose appropriate solutions. Information generated through the pre-demonstration investigation is used to develop the final demonstration design and to confirm the nature and source of samples that will be used in the demonstration.

Demonstration planning activities also include preparation of a demonstration plan that describes the procedures to verify the performance and cost of each technology. The demonstration plan incorporates information generated during the pre-demonstration investigation as well as input from technology developers, demonstration site representatives, and technical peer reviewers. The demonstration plan also incorporates the quality assurance (QA)/quality control (QC) elements needed to produce data of sufficient quality to document the performance and cost of each technology.

During the demonstration, each technology is evaluated independently and, when possible and appropriate, is compared to a reference technology. The performance and cost of one technology are not compared to those of another technology evaluated in the demonstration.

Rather, demonstration data are used to evaluate theindividual performance, cost, advantages, limitations, and field applicability of each technology.

As part of the third step of the technology verification process, the EPA publishes a verification statement (Appendix A) and a detailed evaluation of each technology in an ITVR. To ensure its quality, the ITVR is published only after comments from the technology developer and external peer reviewers are satisfactorily addressed. All demonstration data used to evaluate each technology are summarized in a data evaluation report (DER) that constitutes a complete record of the demonstration. The DER includes audit reports, observer reports, completed data validation checklists, certificates of analysis, and the data packages (i.e., raw data) from the reference laboratory. The DER is not published as an EPA document, but a copy may be obtained from the EPA project manager.

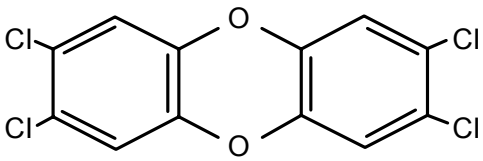
The fourth step of the verification process is to distribute demonstration information. To benefit technology developers and potential technology users, the EPA makes presentations, publishes and distributes fact sheets, newsletters, bulletins, and ITVRs through direct mailings and on the Internet. Information on the SITE Program is available on the EPA ORD Web site (http://www.epa.gov/ORD/SITE). Additionally, a Visitor's Day, which is held in conjunction with the demonstration, allows the developers to showcase their technologies and gives potential users the opportunity to have a firsthand look at the technologies in operation.

1.2 Scope of This Demonstration

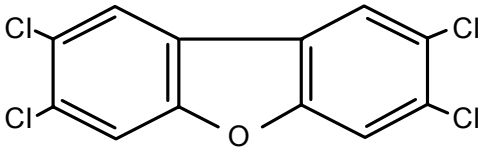
Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, commonly referred to collectively as "dioxins," are of significant concern in site remediation projects and human health assessments because they are highly toxic. Dioxins and furans are halogenated aromatic hydrocarbons and are similar in structure as shown in Figure 1-1. They have similar chemical and physical properties. Chlorinated dioxins and furans are technically referred to as polychlorinated dibenzo-*p*-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF). For the purposes of this document, they will be referred to simply as "dioxins," "PCDD/F," or "D/F." Dioxins and furans are not intentionally produced in most chemical processes. However, they can be synthesized directly and are commonly generated as by-products of various combustion and chemical processes.[1] They are colorless crystals or solids with high melting points, very low water solubility, high fat solubility, and low volatility. Dioxins and furans are extremely stable under most environmental conditions, making them persistent once released in the environment. Because they are fat soluble, they also tend to bioaccumulate.

There are 75 individual chlorinated dioxins and 135 individual chlorinated furans. Each individual dioxin and furan is referred to as a congener. The properties of each congener vary according to the number of chlorine atoms present and the position where the chlorines are attached. The congeners with chlorines attached at a minimum in the 2, 3, 7, and 8 positions are considered most toxic. A total of seven dioxin and ten furan congeners contain chlorines in the 2, 3, 7, 8 positions and, of these, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) is one of the most toxic and serves as the marker compound for this class.

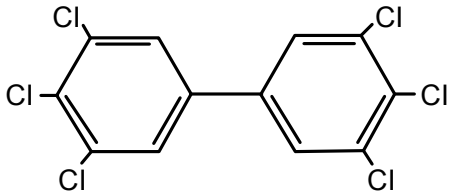
Certain polychlorinated biphenyls (PCBs) have structural and conformational similarities to dioxin compounds (Figure 1-1) and are therefore expected to exhibit toxicological similarities to dioxins as well.



2,3,7,8-Tetrachlorodibenzo-p-dioxin



2,3,7,8-Tetrachlorodibenzofuran



3,3',4,4',5,5'-Hexachlorobiphenyl

Figure 1-1. Representative dioxin, furan, and polychlorinated biphenyl structure.

Currently only twelve of the total 209 PCB congeners are thought to have "dioxin-like" toxicity. These twelve are PCBs with four or more chlorines with just one or no substitution in the ortho position, and which assume a flat configuration with rings in the same plane. These "dioxin-like" PCBs are often refered to as non-ortho and mono-ortho substituted coplanar PCBs.

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). The use of a simple, rapid, 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. At this time, more affordable and quicker analytical techniques will not replace HRMS. However, before adopting an alternative to traditional laboratory-based methods, a thorough assessment of how commercially available technologies compare to conventional laboratory-based analytical methods using certified, spiked, and environmental samples is warranted. A summary of the demonstration activities to evaluate measurement technologies for dioxin and dioxin-like compounds in soil and sediment is provided below. The experimental design and demonstration approach are described in greater detail in Chapter 4 and was published in the Demonstration and Quality Assurance Project Plan (D/QAPP).[2]

1.2.1 Organization of Demonstration

The key organizations and personnel involved in the demonstration, including the roles and responsibilities of each, are fully described in the D/QAPP.[2] EPA NERL had overall responsibility for this project. The EPA reviewed and concurred with all project deliverables including the D/QAPP and the ITVRs, provided oversight during the demonstration, and participated in the Visitor's Day. Battelle served as the verification testing organization for EPA/NERL. Battelle's responsibilities included developing and implementing all elements of the D/QAPP; scheduling and coordinating the activities of all demonstration participants; coordinating the collection of environmental samples; serving as the characterization laboratory by performing the homogenization of the environmental samples and confirming the efficacy of the homogenization and approximate sample concentrations; conducting the demonstration by implementing the D/QAPP; summarizing, evaluating, interpreting, and documenting demonstration data for inclusion in this report; and preparing draft and final versions of each developer's ITVR. The developers were five companies who submitted technologies for evaluation during this demonstration. The responsibilities of the developers included providing input to, reviewing, and concurring with the D/QAPP; providing personnel and supplies as needed for the demonstration; operating their technologies during the demonstration; and reviewing and commenting on their technologies' ITVRs. AXYS Analytical Services, Ltd. was selected to serve as the reference analytical laboratory. AXYS analyzed each demonstration sample by EPA Method 1613B[3] and EPA Method 1668A[4] according to the statement of work provided in the D/QAPP. The Michigan Department of Environmental Quality (MDEQ) hosted the demonstration, coordinated the activities of and participated in Visitor's Day, and collected and provided some of the environmental samples that were used in the demonstration. The Dioxin SITE Demonstration Panel served as technical advisors and observers of the demonstration activities. Panel membership, which is outlined in the D/QAPP, included representation from EPA Regions 1, 2, 3, 4, 5, 7, and 9; EPA Program Offices; the MDEQ; and the U.S. Fish and Wildlife Services. Members, of the panel participated in five conference calls with the EPA, Battelle, AXYS, and the developers. The panel contributed to the experimental design and D/QAPP development; logistics for the demonstration, including site selection; sample collection, reference laboratory selection, and data analysis and technology evaluation procedures. As an example of the significant impact the panel had on the demonstration, it was the EPA members of the panel who suggested expanding the scope of the project from focusing exclusively on dioxins and furans, to also include PCBs and the generation of characterization data for polynuclear aromatic hydrocarbons (PAHs).

1.2.2 Sample Descriptions and Experimental Design

Soil and sediment samples with a variety of distinguishing characteristics such as high levels of PCBs and PAHs were analyzed by each participant. Samples were collected from a variety of dioxin-contaminated soil and sediment sampling locations around the country. Samples were identified and supplied through EPA Regions 2, 3, 4, 5, and 7 and the MDEQ. The samples were homogenized and characterized by the characterization laboratory prior to use in the 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 128 of the 209 samples included in the demonstration (61%). 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. A suite of solvent extracts was included in the demonstration to minimize the impact of sample homogenization and to provide a uniform matrix for evaluation. A total of 23 extracts (11% of the total number of samples) was included in the demonstration. The demonstration samples are described in greater detail in Section 4.3.

1.2.3 Overview of Field Demonstration

All technology developers participated in a pre-demonstration study where a representative subset of the demonstration samples was analyzed. The pre-demonstration results indicated that the Hybrizyme technology was suitable for participation in the demonstration. The demonstration of technologies for the measurement of dioxin and dioxin-like compounds was conducted at the Green Point Environmental Learning Center (ELC) in Saginaw, Michigan, from April 26 to May 5, 2004. Five technologies, including immunoassay test kits and aryl hydrocarbon (Ah) receptor-binding technologies, participated in the demonstration. The operating procedures for the participating technologies are described in the D/QAPP.

The technologies were operated by the developers. Because the sample throughput of the technologies varied widely, it was at the discretion of the developers how many of the 209 demonstration samples were analyzed in the field. Results from the demonstration samples, in comparison with results generated by AXYS using standard analytical methods, were used to evaluate the analytical performance of the technologies, including the parameters of accuracy, precision, and comparability. Observations from the field demonstration were used to assess sample throughput, ease of use, health and safety aspects, and the field portability of each technology. The performance evaluation of the Hybrizyme Corporation AhRC PCR™ Kit is presented in this ITVR. Separate ITVRs have been published for the other four participating technologies.

Chapter 2
Description of Hybrizyme Corporation AhRC PCR™ Kit

This technology description is based on information provided by Hybrizyme and only editorial changes were made to ensure document consistency. Actual cost and performance data, as reported and observed during the demonstration, will be provided later in this document. The AhRC PCR™ assay couples the aryl hydrocarbon receptor (AhR) with polymerase chain reaction (PCR) technology to produce a method for analyzing dioxins and related compounds in environmental or food samples. The AhRC PCR™ kit can be shipped worldwide and yields results within hours. It is ideally suited for stationary or mobile laboratories.

2.1 Company History

In 1995, Hybrizyme Corporation licensed the worldwide rights to the gene encoding the AhR from Northwestern University to develop a rapid and cost-effective test for dioxins. The AhR was found to mediate most, if not all, of the harmful effects associated with dioxin-like compounds. Hybrizyme pioneered the development of a dioxin assay that utilized the specificity of the AhR with the detection capabilities of PCR.

In 1998, Hybrizyme entered into a licensing and OEM agreement with PerkinElmer Life Sciences to produce environmental and food immunoassay kits that would compliment the company's receptor-based technology. The company's product line includes complementary immunoassay products consisting of the DELFIA PCB Food Kit, DELFIA PCB Soil Kit, and DELFIA TCDD kit. The PCB food test has been validated for use in Europe, and the efficacy of the PCB soil test was demonstrated through the EPA's Environmental Technology Verification program.

2.2 Product History

Hybrizyme's goal was to develop a quick and inexpensive screen for dioxin-like compounds based on the molecular events responsible for their toxicity. Although Hybrizyme has a patented yeast-based system for screening dioxins, the company's aim was to develop an assay that did not require a living organism. This allows the test to be shipped throughout the world and used immediately upon arrival or as needed.

When the AhR binds to a dioxin-like molecule, it is irreversibly transformed into a protein that attaches to a specific sequence of deoxyribonucleic acid (DNA) called the dioxin-responsive element (DRE). Binding of the AhR to the DRE initiates a cascade of biochemical effects in humans and animals that lead to toxicological consequences.

The AhRC PCR assay is based on the ability of the transformed AhR to bind to a DNA-probe containing the DRE sequence. The advantages of the AhRC PCR assay result, in part, from the high degree of specificity that is required for the transformed receptor to bind the DRE-probe and the unmatched sensitivity of PCR to measure the bound DRE-probe. The amount of DRE-probe measured by PCR is directly proportional to the amount of dioxin in the sample.

The AhRC PCR™ kit has been validated in Japan for the quantitative determination of dioxins and furans in exhaust gas, fly ash, and sediment. This method utilizes accelerated solvent extraction and chromatographic clean up procedures.

A more rapid version of the AhRC PCR assay has been developed that requires limited sample cleanup. The test detects not only dioxin and furans but also coplanar

PCBs and carcinogenic PAHs. This method is designed as a cost-effective tool for quickly mapping large areas and establishing a relative concentration gradient for these toxicants. In addition, the method profiles the collected samples from nondetects to highly contaminated, offering a more effective use of expensive and time-consuming HRMS high-resolution capillary gas chromatography (HRGC).

2.3 Technology Description

This procedure uses Hybrizyme's AhRC PCR™ kit (Figure 2-1) to detect molecules in a test sample that bind to the AhR and is reported in Ah-receptor binding units (AhRBU). At the time of the 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 toxicity equivalents (TEQs). Since the technology measures an actual biological response, it is possible that the technology may give a better representation of the true toxicity from a risk assessment standpoint. The developer's goal is a highly portable screening technology that can help to determine areas of greatest concern for cleanup at a site and can help to minimize the number of more expensive analyses needed for specific analytes.

The AhR mediates most, if not all, of the harmful effects associated with exposure to 2,3,7,8-substituted D/F. How tightly or loosely these compounds bind to the AhR is one of the determining factors of their toxicity. The AhR also binds to certain coplanar PCBs and carcinogenic PAHs, such as benzo-[a]-pyrene. Sample cleanup procedures can be employed so that all or a subset of these AhR-reactive compounds are detected by the assay.

For this demonstration, minimal cleanup procedures were performed on sample extracts and so the results obtained were expected to reflect all AhR-reactive compounds in the samples.

Samples were prepared using an extraction method designed for speed and simplicity while maintaining sample concentration. Two grams (g) of each sample were placed in a 40-milliliter (mL) vial and 20 mL of an extraction cocktail was added. The vials were placed in an ultrasonic bath for 10 minutes, followed by brief centrifugation to remove solids. The extract was decanted into a new vial and 20 mL of water added. Immediately following the addition of water, approximately 2 mL of hexane (present in the extraction cocktail) is partitioned from the solution. Because of their hydrophobic nature, most of the dioxin-like molecules originally in the 2 g of soil were now present in the 2 mL of hexane. A portion of the hexane was removed to a disposable glass tube and dried for analysis. Depending on the condition of the sample being analyzed, an acid wash was added as an additional step. The acid wash consisted of resuspending the dried extract in 1 mL of hexane, adding 2 mL of concentrated sulfuric acid, and vortexing for two minutes. The phases were allowed to separate for 10 minutes, and a portion of the hexane was removed to a disposable glass tube and dried for analysis. The dried sample extract was suspended in methanol for analysis by the AhRC PCR™ kit.

The AhRC PCR test was based on an assay format commonly used in research and in clinical applications ensuring that pipettes, tips, and other disposables would be commonly available and inexpensive. Sample extracts were added to 0.5-mL glass vials containing the assay

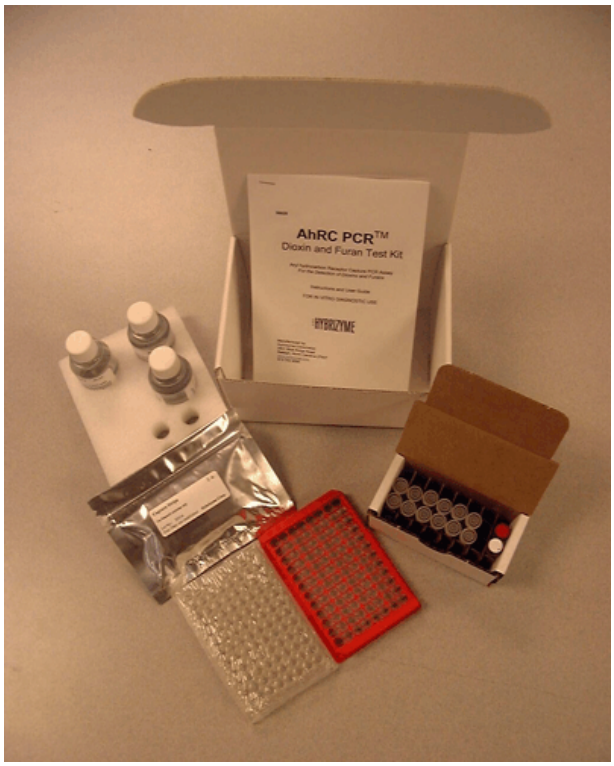


Figure 2-1. Hybrizyme's AhRC PCR™ Test Kit.

buffer. The glass vials were provided with each kit and come racked in a convenient frame for ease of use. After all of the sample extracts were added, activation solution containing the AhR and DRE-probe was also added to the glass vials and shaken at room temperature for one hour. The reaction mix was transferred from the glass vials to capture strips using a multichannel pipettor, and the capture strips were shaken at room temperature for an additional 30 minutes. During this time, the AhR/DRE-probe complexes were trapped onto the wells of the capture strip. The capture strips were washed to remove free DRE-probe, and PCR master mix was added. The strips were placed in a real-time thermocycler, and the amount of the DRE-probe was measured. The signal was directly proportional to the amount of dioxin in the samples. A user guide for the AhRC PCR™ kit can be found at www.hybrizyme.com.

The AhRC PCR™ assay was developed for real-time PCR systems such as the ABI PRISM 7000 (Figure 2-2), which can generate up to 96 results per run, or the Cepheid Smart Cycler (Figure 2-3), designed to be totally transportable.

This is the developer method that was implemented during the field demonstration. This procedure is different from the one presented in the demonstration plan [2] where the developer originally intended to demonstrate a technology that reported TEQ results. A photo of the technology in operation during the demonstration is presented in Figure 2-4. Hybrizyme provided supplemental information about the performance of its technology during the demonstration and it is presented in Appendix B.

Figure 2-2. ABI Prism 7000.

Figure 2-3. Cepheid Smart Cycler.

Figure 2-4. Hybrizyme's AhRC PCR™ Test Kit in operation during the field demonstration.

2.4 Developer Contact Information

Additional information about this technology can be obtained by contacting:

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Chapter 3
Demonstration and Environmental Site Descriptions

This chapter describes the demonstration site, the sampling locations, and why each was selected.

3.1 Demonstration Site Description and Selection Process

This section describes the site selected for hosting the demonstration, along with the selection rationale and criteria. Several candidate host sites were considered. The candidate sites were required to meet certain selection criteria, including necessary approvals, support, and access to the demonstration site; enough space and power to host the technology developers, the technical support team, and other participants; and various levels of dioxin-contaminated soil and/or sediment that could be analyzed as part of the demonstration. Historically, these demonstrations are conducted at sites known to be contaminated with the analytes of interest. The visibility afforded the sites is a valuable way of keeping the local community informed of new technologies and to help promote the EPA's commitment to promote and advance science and communication.

After review of the information available, the site selected for the demonstration was the Green Point ELC site, located within the city of Saginaw, Michigan. The Saginaw city-owned, 76-acre Green Point ELC, formerly known as the Green Point Nature Center, is managed by the Shiawassee National Wildlife Refuge. The Green Point ELC is situated within the Tittabawassee River flood plain. The MDEQ found higher than normal levels of dioxins in soil and sediment samples taken from the flood plain of the Tittabawassee River. The flood plain is not heavily laden with PCBs; however, low levels of PCBs have been detected in some areas. Soil samples taken from areas outside the flood plain were at typical background levels. The source of the contamination was speculated to be attributed to legacy contamination from chemical manufacturing.

To summarize, Green Point ELC was selected as the demonstration site based on the following criteria:

- Access and Cooperation of the State and Local Community—Representatives from the MDEQ, EPA Region 5, and the local U.S. Fish and Wildlife Services supported the demonstration, providing site access for the demonstration, logistical support for the demonstration, and supported a Visitor's Day during the demonstration.

- Space Requirements and Feasibility—The demonstration took place in the parking lot adjacent to the Green Point ELC, not directly on an area of contamination. The site had electrical power and adequate space to house the trailers and mobile labs that were used for the demonstration. Furthermore, the site was close to an international airport. The weather in Michigan at the time of the demonstration was unpredictable; however, all participants were provided heated containment (a mobile laboratory or construction trailer).

- Site Diversity—The area encompassing the Green Point site had different levels and types of dioxin contamination in both the soil and sediment that were used to evaluate the performance of the technologies.

The demonstration was conducted at the Green Point ELC over a 10-day period from April 26 to May 5, 2004. All technologies were operated inside trailers equipped with fume hoods or inside mobile laboratories. As such, the ambient weather conditions during the demonstration had little impact on the operation of the technologies, since all of the work spaces were climate-controlled with

heat and air conditioning. The outdoor weather conditions were generally cool and rainy, but the developers kept their working environment at comfortable temperatures (16 to 18°C). The low temperature over the 10-day demonstration period was 2°C, the high temperature was 26°C, and the average temperature was 9°C. Precipitation fell on eight of the 10 days, usually in the form of rain, but occasionally as sleet or snow flurries, depending on the temperature. The largest amount of precipitation on a given demonstration day was 0.50 inches.

3.2 Description of Sampling Locations

This section provides an overview of the ten sampling sites and methods of selection. Table 3-1 summarizes each of the locations, what type of sample (soil or sediment) was provided, the number of samples submitted from each location, and the number of samples included in the demonstration from each location. Samples were collected from multiple sampling sites so that a wide variety of matrix conditions could be used to evaluate the performance of the technologies in addressing monitoring needs at a diverse range of Superfund sites.

Samples consisted of either soil or sediment and are described below based on this distinction. 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 reference laboratory 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.

3.2.1 Soil Sampling Locations

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

3.2.1.1 Warren County, North Carolina

Five areas of the Warren County PCB Landfill in North Carolina, a site with both PCB and dioxin contamination, were sampled. Dioxin concentrations in the landfill soils range approximately from 475 to 700 pg/g, and PCB concentrations are greater than 100 parts per million (ppm). The Warren County PCB Landfill contains soil that was contaminated by the illegal spraying of waste transformer oil containing PCBs from over 210 miles of highway shoulders. Over 30,000 gallons of contaminated oil were disposed of in 14 North Carolina counties. The landfill is located on a 142-acre tract of land. The EPA permitted the landfill under the Toxic Substances Control Act. Between September and November 1982, approximately 40,000 cubic yards (equivalent to 60,000 tons) of PCB-contaminated soil were removed and hauled to the newly constructed landfill located in Warren County, North Carolina. The landfill is equipped with both polyvinyl chloride and clay caps and liners. It also has a dual leachate collection system. The material in the landfill is solely from the contaminated roadsides. The landfill was never operated as a commercial facility. The remedial action was funded by the EPA and the State of North Carolina. The site was deleted from the National Priorities List on March 7, 1986.

3.2.1.2 Tittabawassee River Flood Plain

The MDEQ sampled the Tittabawassee River flood plain soils from three sites in the flood plain. The source of the contamination was speculated to be attributed to legacy contamination from chemical manufacturing. Two 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 analysis 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. Two 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

Table 3-1. Summary of Environmental Sampling Locations

Sample Type	Sampling Location	Number of Samples	
		Submitted for Consideration	Included in Demonstration
Soil	Warren County, North Carolina	5	3
	Tittabawassee River, Michigan	6	3
	Midland, Michigan	6	4
	Winona Post, Missouri	6	3
	Solutia, West Virginia	6	3
Sediment	Newark Bay, New Jersey	6	4
	Raritan Bay, New Jersey	6	3
	Tittabawassee River, Michigan	6	3
	Saginaw River, Michigan	6	3
	Brunswick, Georgia	5	3
Total		***58***	***32***

within the park complex. Previous PCDD/F concentrations were from 300 to 3,400 pg/g, and total PCBs were in the low ppt range. The final two samples were collected from Department of Natural Resources (DNR)-owned property in Saginaw, which was formerly a farming area located almost at the end of the Tittabawassee River where it meets the Shiawassee River to form the Saginaw River. Previous PCDD/F concentrations ranged from 450 to 1,150 pg/g. Total PCBs were not previously analyzed, but concentrations were expected to be less than 1 ppm. The DNR property is approximately a 10-minute walk from where the demonstration was conducted at the Green Point ELC.

3.2.1.3 Midland, Michigan

Soil samples were collected by the MDEQ from various locations in Midland, Michigan. The soil type and nature of dioxin contamination are different in the Midland residential area than it is on the Tittabawassee River flood plain, but it is from the same suspected source (legacy contamination from chemical manufacturing). Samples were collected in various locations around Midland. Estimated TEQ concentrations ranged from 10 pg/g to 1,000 pg/g.

3.2.1.4 Winona Post

The Winona Post site in Winona, Missouri, was a Superfund cleanup of a wood treatment facility. 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 dioxin equivalents. Samples obtained for this study from this site were obtained from the treatment cell after these concentrations had been achieved.

3.2.1.5 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. The source of the 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 ppt to low parts per billion (ppb) range. No PCBs or PAHs were identified in the soil.

3.2.2 Sediment Sampling Sites

This section provides descriptions of each of the sediment sites that includes how the sites became contaminated and approximate dioxin concentrations, as well as the type and concentrations of other major constituents (such as PCBs, PCP, and PAHs). This information was provided from site owners/samples providers (e.g., the EPA, EPA contractors, and the MDEQ).

3.2.2.1 New York/New Jersey Harbors

Dredged materials from the New York and New Jersey harbors were provided as samples for the demonstration. The U.S. Army Corps of Engineers, New York District, and EPA Region 2 are responsible for managing dredged materials from the New York and New Jersey harbors. Dioxin levels affect the disposal options for dredged material. Dredged materials are naturally occurring bottom sediments, but some in this area have been contaminated with dioxins and other compounds by municipal or industrial wastes or by runoff from terrestrial sources such as urban areas or agricultural lands.

3.2.2.1.1 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 (TOC) was about 4%.

3.2.2.1.2 Raritan Bay

Surrounded by industry and residential discharges, Raritan Bay has dioxin contamination in the area, but it is not to the degree of 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 TOC values were similar to percentages in Newark Bay.

3.2.2.2 Tittabawassee River

The first Tittabawassee River location was approximately ¼-mile upstream of the Bob Caldwell Boat Launch in Midland, Michigan. The sediments are dark gray, fine sand with some silt. The estimated TEQ concentration was 260 pg/g; however, concentrations as high as 2,100 pg/g TEQ have been found in this area. The second site was on the Tittabawassee River approximately 100 yards downstream from old Smith's Crossing Bridge in Midland, Michigan. The sediment was brown and sandy with organic material. The estimated TEQ concentration was 870 pg/g; but, again, concentrations as high as 2,100 pg/g TEQ are possible in the area. The third site was on Tittabawassee River at the Emerson Park Golfside Boat Launch. The sediment was gray black silty sand, with many leaves and high organic matter. The estimated TEQ concentration was < 5 pg/g. The fourth site was on the Tittabawassee River adjacent to Imerman Park in Saginaw County across from the fishing dock. The sediment was sand with some silt. The estimated TEQ concentration was between 100 and 2,000 pg/g TEQ. The fifth site was on the Tittabawassee River approximately 1 mile downstream of Center Road Boat Launch in Saginaw Township. The sediment consisted of sand and gravel with some shells and not much organic matter. The estimated TEQ concentration was between 100 and 1,000 pg/g TEQ. The sixth site also was on the Tittabawassee River across from the Center Road Boat Launch. The sediment was fine sand with high organic matter. The estimated TEQ concentration was 1,000 pg/g TEQ. The source of the contamination was speculated to be attributed to legacy contamination from chemical manufacturing.

3.2.2.3 Saginaw River

Saginaw River samples were collected at six locations. The first sampling location was in the Saginaw River just downstream of Green Point Island. Samples were collected near the middle of the river in about 21 feet of water. The sample was granular with some organic material. The estimated TEQ concentration was 100 ppt. Another Saginaw River sample was taken upstream of Genesee Bridge on the right side of the river. The sample was a brown fine sand from about 15 feet of water. The estimated TEQ concentration was 100 ppt. The third location was in the Saginaw River downstream of the Saginaw wastewater treatment plant in about eight feet of water. The sample was gray silty clay with an unknown TEQ concentration. The fourth location was in the Saginaw River in about eight feet of water. The sample was a black sandy material. The estimated TEQ concentration for this location was unknown. The fifth location was downstream of a petroleum pipeline crossing upstream of the Detroit and Mackinaw railroad bridge crossing. This location was selected because of its proximity to a former PCB dredging location. The sediment sample consisted of dark black silt with some sand. The estimated TEQ concentration was unknown, but PCB concentrations are expected to be high. The sixth and final sampling location was near the mouth of the Saginaw River in about five feet of water. The sediment was a mix of fine black silt and layers of sand and shells. The estimated TEQ concentration for this location was also unknown.

3.2.2.4 Brunswick Wood Preserving Site

The Brunswick Wood Preserving Superfund site is located in Glynn County, Georgia, north of the city of Brunswick. The site was originally located in the city of Brunswick, but moved to its present location around 1958. The site is approximately 84 acres and is about two-thirds of a mile long. Burnett Creek, a tidally influenced stream, is located at the western corner of the site. At several points, most, if not all, of the drainage from the site flows into Burnett Creek. The site was first operated by American Creosote Company, which constructed the facility sometime between 1958 and 1960. The site was acquired by Escambia Treating Company in 1969 from Georgia Creosoting Company and the Brunswick Creosoting Company. In 1985, a corporate reorganization resulted in the purchase of the facility by the Brunswick Wood Preserving Company, which operated the site until it closed in early 1991. Each of the three major wood-treating operations was carried out at the facility: PCP, creosote, and chromium-copper-arsenic (CCA). The site was listed on the EPA's National Priorities List on April 1, 1997.

Sediment samples from the Brunswick Wood Preserving site in Brunswick, Georgia, were collected from six locations on the site, including areas thought to have lower (< 300 pg/g TEQ) and higher (> 10,000 pg/g TEQ) D/F concentrations. Due to the processes that occurred on this site, the samples also contain varying levels of PAHs and PCP, but they were not expected to contain PCBs.

Chapter 4
Demonstration Approach

This chapter discusses the demonstration objectives, sample collection, sample homogenization, and demonstration design.

4.1 Demonstration Objectives

The primary goal of the SITE MMT Program is to develop reliable performance and cost data on innovative, commercial-ready technologies. A SITE demonstration must provide detailed and reliable performance and cost data so that technology users have adequate information to make sound decisions regarding comparability to conventional methods. The demonstration had both primary and secondary objectives. Primary objectives were critical to the technology evaluation and required the use of quantitative results to draw conclusions regarding a technology's performance. Secondary objectives pertained to information that is useful to know about the technology but did not require the use of quantitative results to draw conclusions regarding a technology's performance.

The primary objectives for the demonstration of the participating technologies were as follows:

P1. Determine the accuracy.
P2. Determine the precision.
P3. Determine the comparability of the technology to EPA standard methods.
P4. Determine the estimated method detection limit (EMDL).
P5. Determine the frequency of false positive and false negative results.
P6. Evaluate the impact of matrix effects on technology performance.
P7. Estimate costs associated with the operation of the technology.

The secondary objectives for the demonstration of the participating technologies were as follows:

S1. Assess the skills and training required to properly operate the technology.
S2. Document health and safety aspects associated with the technology.
S3. Evaluate the portability of the technology.
S4. Determine the sample throughput.

Application of these objectives to the demonstration was addressed based on input from the Dioxin SITE Demonstration Panel members,[2] general user expectations of field measurement technologies, the time available to complete the demonstration, technology capabilities that the developers participating in the demonstration intend to highlight, and the historical experimental components of former SITE Program demonstrations to maintain consistency.

Note that this demonstration does not assess all parameters that can affect performance of the technologies in comparison to the reference methods (i.e., not all Ah-receptor inducing compounds have been characterized in the test samples, calibration of technologies results to HRMS results on site-by-site basis was not evaluated, etc.). However, the demonstration as outlined below was agreed upon by the Dioxin SITE Demonstration Panel members to provide a reasonable evaluation of the technologies.

4.2 Toxicity Equivalents

For risk assessment purposes, estimates of the toxicity of samples that contain a mixture of dioxin, furan, and PCB congeners are often expressed as TEQs. TEQs are calculated by multiplying the concentration of each congener with a toxicity equivalency factor (TEF), according to the equation:

$$TEQ = C_C * TEF$$

where C_C is the concentration of the congener. The TEF (see Table 4-1) provides an equivalency factor for each congener's toxicity relative to the toxicity of 2,3,7,8-TCDD. The TEFs used in this demonstration were determined by the World Health Organization (WHO) for mammalian species.[5] The total TEQ from dioxin and furans ($TEQ_{D/F}$) in a sample is calculated by adding up all of the TEQ values from the individual dioxin and furan congeners. The total TEQ contribution from PCBs (referred to as TEQ_{PCB}) is calculated by summing up the individual PCB TEQ values. The total TEQ in a sample is the sum of the $TEQ_{D/F}$ and TEQ_{PCB} values. TEQ concentrations for soils and sediments are typically reported in pg/g, which is equivalent to ppt.

Concentrations of dioxins, furans, and PCBs, represented as total TEQ concentration, provide a quantitative estimate of toxicity for all congeners expressed as if the mixture were a TEQ mass of 2,3,7,8-TCDD only. While the TEQ concept provides a way to estimate potential health or ecological effects, the limitations of this approach should be understood. The WHO report noted that the TEF indicates an order of magnitude estimate of the toxicity of a compound relative to 2,3,7,8-TCDD.[5] Therefore, the accuracy of the TEF factors could be affected by differences in species, in the functional responses elicited by the compounds, and in additive and nonadditive effects when the congeners are present in complex mixtures. The WHO report[5] concluded, however, that it is unlikely that a significant error would be observed due to these differences. The larger impact to the TEF concept is the presence of AhR binding compounds, such as PAHs (including naphthalenes, anthracenes, and fluorenes) and brominated and chloro/bromo-substituted analogues of PCDD/Fs that have not been assigned TEF values but which may

Table 4-1. World Health Organization Toxicity Equivalency Factor Values

Compound[a]	WHO TEF	Compound	WHO TEF
PCDDs		**PCDFs**	
2,3,7,8-TCDD	1	2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDD	1	1,2,3,7,8-PeCDF	0.05
		2,3,4,7,8-PeCDF	0.5
1,2,3,4,7,8-HxCDD	0.1	1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDD	0.1	1,2,3,7,8,9-HxCDF	0.1
1,2,3,7,8,9-HxCDD	0.1	1,2,3,6,7,8-HxCDF	0.1
		2,3,4,6,7,8-HxCDF	0.1
1,2,3,4,6,7,8-HpCDD	0.01	1,2,3,4,6,7,8-HpCDF	0.01
		1,2,3,4,7,8,9-HpCDF	0.01
OCDD	0.0001	OCDF	0.0001
Dioxin-like PCBs			
Coplanar		**mono-*ortho***	
3,3',4,4'-TCB (PCB 77)	0.0001	2,3,3',4,4'-PeCB (PCB 105)	0.0001
3,4,4',5-TCB (PCB 81)	0.0001	2,3,4,4',5-PeCB (PCB 114)	0.0005
3,3',4,4',5-PeCB (PCB 126)	0.1	2,3',4,4',5-PeCB (PCB 118)	0.0001
3,3',4,4',5,5'-HxCB (PCB 169)	0.01	2,3,4,4',5-PeCB (PCB 123)	0.0001
		2,3,3',4,4',5-HxCB (PCB 156)	0.0005
		2,3,3',4,4',5-HxCB (PCB 157)	0.0005
		2,3',4,4',5,5'-HxCB (PCB 167)	0.00001
		2,3,3',4,4'5,5'-HpCB (PCB 189)	0.0001

[a] T = Tetra, Pe = Penta, Hx = Hexa, Hp = Hepta, O = Octa, CDD = chlorinated dibenzo-*p*-dioxin, CDF = chlorinated dibenzofuran, CB = chlorinated biphenyl

contribute to the total TEQ. This potentially can result in an underestimation of TEQs in environmental samples using the TEF approach.[5]

This demonstration was designed with these limitations of the TEQ concept in mind. The samples chosen contained a variety of combinations of dioxins, furans, and PCBs and at a wide range of concentration levels. Some samples were high in analytes with better understood TEFs, while others were high in analytes with TEFs that have more uncertainty. Some were high in other AhR binding compounds such as PAHs, while still others were free of these possible TEQ contributing compounds. The purpose was to evaluate each of the technologies and assess the comparability of the $TEQ_{D/F}$ and TEQ_{PCB} values determined by the reference laboratory.

4.3 Overview of Demonstration Samples

The goal of the demonstration was to perform a detailed evaluation of the overall performance of each technology for use in the field or mobile environment. The demonstration objectives were centered around providing performance data that support action levels for dioxin at contaminated sites. The Centers for Disease Control's Agency for Toxic Substances and Disease Registry (ATSDR) has established a decision framework for sites that are contaminated with dioxin and dioxin-like compounds.[6] If samples are determined to have dioxin TEQ levels between 50 and 1,000 pg/g, the site should be further evaluated; action is recommended for levels above 1,000 pg/g (i.e., 1 ppb) TEQ. A mix of PE samples, environmentally contaminated ("real-world") samples, and extracts were evaluated that bracket the ATSDR guidance levels. Table 4-2 lists the primary and secondary performance objectives for this demonstration and which sample types were used in each evaluation. The PE samples were used primarily to determine the accuracy of the technology and consisted of purchased soil and sediment standard reference materials with certified concentrations of known contaminants and newly prepared spiked samples. The PE samples also were used to evaluate precision, comparability, EMDL, false positive/negative results, and matrix effects. Environmentally contaminated samples were collected from dioxin-contaminated sites around the country and were used to evaluate the precision, comparability, false positive/negative results, and matrix effects. Extracts, prepared in toluene, which was the solvent used by the reference laboratory, were used to evaluate precision, EMDL, and matrix effects. All samples were used to evaluate qualitative performance objectives such as technology cost, the required skill level of the operator, health and safety aspects, portability, and sample throughput. Table 4-3 shows the number of each sample type included in the experimental design. The following sections describe each sample type in greater detail.

4.3.1 PE Samples

PE standard reference materials are available through Cambridge Isotope Laboratories (CIL) (Andover, Massachusetts), LGC Promochem (United Kingdom), Wellington Laboratories (U.S. distributor TerraChem, Shawnee Mission, Kansas) the National Institute of Standards and Technology (NIST) (Gaithersburg, Maryland), and Environmental Resource Associates (ERA, Arvada, Colorado). All of these sources were utilized to obtain PE samples for use in this demonstration, and Table 4-4 summarizes the PE samples that were included. PE samples consisted of three types of samples: (1) reference materials (RMs) or certified samples, which included soil and/or sediment samples with certified concentrations of dioxin, furan, and/or PCBs; (2) spiked samples, which included a certified dioxin, furan, PCB, and PAH-clean matrix spiked with known levels of dioxin and/or other contaminants; and (3) blank samples that were certified to have levels of dioxins, furans, WHO PCBs, and PAHs that were non-detectable or were considerably lower than the detection capabilities of developer technologies. The PE samples were selected based on availability and on the correlation of the PE composition as it related to the environmental samples that were chosen for the demonstration (e.g., the PE sample had a similar congener pattern to one or more of the environmental sites).

Table 4-2. Distribution of Samples for the Evaluation of Performance Objectives

Performance Objective	Sample Type Used in Evaluation
P1: Accuracy	PE
P2: Precision	PE, environmental, extracts
P3: Comparability	PE, environmental, extracts
P4: EMDL	PE, extracts
P5: False positive/negative results	PE, environmental, extracts
P6: Matrix effects	PE, environmental, extracts
P7: Cost	PE, environmental, extracts
S1: Skill level of operator	PE, environmental, extracts
S2: Health and safety	PE, environmental, extracts
S3: Portability	PE, environmental, extracts
S4: Sample throughput	PE, environmental, extracts

Table 4-3. Number and Type of Samples Analyzed in the Demonstration

Sample Type	No. of Samples
PE	58
Environmental	128
Extracts	23
Total number of samples per technology	209

Table 4-4 indicates a correlation between the composition of the PE sample and the samples from the environmental sites, where applicable. The certified samples only required transfer from the original jar to the demonstration sample jar. The spiked samples were shipped to the characterization laboratory in bulk quantities so each had to be aliquoted in 50-g quantities. Additional details about each source of PE sample are provided in this section.

4.3.1.1 Cambridge Isotopes Laboratories

Two RMs were obtained from CIL for use in this demonstration. RM 5183 is a soil sample that was collected from a location in Texas with the intended purpose of serving as an uncontaminated soil for use as a spiking material. The soil was sieved to achieve uniform particle size and homogenized to within 5% using a disodium fluorescein indicator. Samples were then sterilized three times for 2 hours at 121°C and 15 pounds per square inch (psi). Analytical results indicated that the soil had low levels of D/F and PCBs.

RM 5184 is a heavily contaminated soil sample with relatively high levels of D/F and PCBs. According to the Certificate of Analysis (CoA), approximately 75 kg of contaminated sediment were obtained from an EPA Superfund site in Massachusetts that was known to contain considerable contamination from PCBs and other chemical pollutants. The sediment was sieved to achieve uniform particle size and homogenized to within 5% using a disodium fluorescein indicator. Samples were then sterilized three times for 2 hours at 121°C and 15 psi.

Both 5183 and 5184 are newly available RMs from CIL. For both RM 5183 and RM 5184, certified analytical values are provided for the D/F and the 12 WHO PCB congeners. The samples were included in an international interlaboratory study conducted by CIL and Cerilliant Corporation. More than 20 laboratories participated in analysis of the D/Fs; up to 20 laboratories participated in the analysis of the PCBs. Participating laboratories used a variety of sample preparation and analytical techniques.

Table 4-4. Summary of Performance Evaluation Samples

Sample Type ID	Source	PE Type	Product No.	Certified Concentration			Correlation to Environ. Sample Type ID[a]	No. of Replicates Per Sample
				$TEQ_{D/F}$ (pg/g)	TEQ_{PCB} (pg/g)	PAH (mg/kg)		
PE #1	CIL	Certified	RM 5183	3.9	5.0	0.18	6	7[b]
PE #2	LGC Promochem	Certified	CRM 529	6,583	424[c]	NA[d]	5	4
PE #3	Wellington	Certified	WMS-01	62	10.5	NA	6	7[b]
PE #4	CIL	Certified	RM 5184	171	941	27	2, 8, 9	4
PE #5	NIST	Certified	SRM 1944	251	41[c]	2.4[e]	3, 4	4
PE #6	ERA	Spiked	custom	11	NS[f]	<0.33	10	4
PE #7	ERA	Spiked	custom	33	NS	< 0.33	10	4
PE #8	ERA	Spiked	custom	NS	NS	61[g]	5, 7	4
PE #9	ERA	Spiked	custom	NS	11	< 0.33	1	4
PE #10	ERA	Spiked	custom	NS	1,121	< 0.33	1	4
PE #11	ERA	Spiked	custom	11	3,760[c]	< 0.33	1	4
PE #12	ERA	Organic, Semivolatile, Blank Soil	056 (lot 56011)	0.046	0.01	< 0.33	not applicable	8
Total Number of PE samples								***58***

[a] Environmental Sample IDs are provided in Table 4-5.
[b] Seven replicates were analyzed for EMDL evaluation.
[c] Little or no certified PCB data were available; mean of reference laboratory measurements was used.
[d] NA = no data available.
[e] Approximate concentration of 2-methyl naphthalene, acenaphthene, and fluorene, which were the only PAHs that were included in the analysis.
[f] NS = not spiked.
[g] Each of the 18 target PAHs was spiked at levels that ranged from 1 to 10 mg/kg. (See Section 5.2.3 for the list of 18 PAHs.)

4.3.1.2 LGC Promochem

Certified reference material (CRM) 529 was obtained from LGC Promochem. The following description is taken from the reference material report that accompanied CRM 529. The soil for CRM 529 was collected in Europe from a site where chloro-organic and other compounds had been in large-scale production for several decades, but where production had ceased more than five years before sampling. The site had been contaminated during long-term production of trichlorophenoxyacetic acid. An area of sandy soil was excavated to a depth of several meters. Several hundred kilograms of this mixed soil were air-dried at about 15°C for 3 months. After removal of stones and other foreign matter by sieving, the remaining material was sterilized in air at 120 °C for 2 hours, thoroughly mixed, and ground in an Alpine air jet mill to a particle size of < 63 micrometers (µm). The material was homogenized once more in a Turbula mixer and packaged in 50-g quantities. The final mean moisture content at the time of bottling was found to be 1.5%. According to the CoA, certified values are provided for five dioxin congeners, seven furan congeners, three chlorobenzene compounds, and three chlorophenol compounds. No PCBs were reported with certified values on the CoA, so the mean concentration determined by the reference laboratory was used as the certified value.

4.3.1.3 Wellington

PE sample WMS-01 was obtained from TerraChem, the U.S. distributor for Wellington, an Ontario-based company. As described in the CoA, WMS-01 is a homogeneous lake sediment that was naturally contaminated (and not fortified). The crude, untreated sediment used to prepare WMS-01 was collected from Lake Ontario. The sediment obtained was subsequently air-dried; crushed to break up agglomerates; air-dried again; and then sieved, milled, and re-sieved (100% < 75 µm). The sediment was then subsampled into 25-g aliquots. The demonstration samples for only the Wellington PE samples were 25 g rather than 50 g based on the package size available from Wellington. Certified

values for the 17 D/F congeners and the 12 WHO PCB congeners are provided on the CoA.

4.3.1.4 National Institute for Standards and Technology

Standard Reference Material® (SRM) 1944 was purchased through NIST. As described in the CoA, SRM 1944 is a mixture of marine sediment collected from six sites in the vicinity of New York Bay and Newark Bay in October 1994. Site selection was based on contaminant levels measured in previous samples from these sites and was intended to provide relatively high concentrations for a variety of chemical classes of contaminants. The sediment was collected using an epoxy-coated modified Van Veen-type grab sampler designed to sample the sediment to a depth of 10 centimeters. A total of approximately 2,100 kg of wet sediment was collected from the six sites. The sediment was freeze-dried, sieved (nominally 61 to 250 μm), homogenized in a cone blender, radiation sterilized, then packaged in 50-g quantities. Certified values are provided on the COA for the 17 D/F congeners, 30 PCB congeners, 24 PAHs, four chlorinated pesticides, 36 metals, and TOC. Since only three WHO PCBs were reported out of the 30 PCB congeners, the mean concentration of the reference laboratory measurements was used as the certified value so that the TEQ_{PCB} concentration would not be underestimated when compared to the developer technologies.

4.3.1.5 Environmental Resource Associates

ERA synthesized PE samples for this demonstration. ERA spiked blank, uncontaminated soil to pre-determined levels of D/Fs, PCBs, and/or PAHs. Spiked PE samples were prepared to include additional concentration ranges and compositions that were not covered with the commercially available certified materials. The organic semivolatile soil blank (ERA Product #056, Lot 56011) is a topsoil that was obtained from a nursery and processed according to ERA specifications by a geochemical laboratory. The particle size distribution of the soil was -20/+60 mesh. The soil was processed and blended with a sandy loam soil to create a blank soil with the following make-up: 4.1% clay, 4.5% silt, 91.2% sand, and 0.2% organic material. Initially, ERA was required to certify that the blank soil matrix to be used as the blank and for the preparation of the spiked PE samples was "clean" relative to the list of required target analytes. This was accomplished through a combination of ERA-conducted analyses (PAHs, pesticides, semivolatile organic compounds, Aroclors that are trade mixtures of PCB congeners) and subcontracted analytical verification (D/F and PCBs). The subcontracted analyses were performed by Alta Analytical Perspectives, LLC, in Wilmington, North Carolina. The Alta Analytical Certificate of Results and the ERA Certification sheets for the organic semivolatile soil blank indicated that trace levels of the octa-dioxins and several WHO PCB congeners were detected, but the total TEQ (combined D/F and PCBs) was less than 0.06 pg/g. The level of PAHs, pesticides, Aroclors, and semivolatile organic compounds in the soil was determined to be < 0.33 pg/g. The TEQ level was considerably below the detection capabilities of the participating technologies, so the organic semivolatile soil blank was considered adequately clean for use in this demonstration.

The manufacturing techniques that ERA used to prepare the PE samples for this demonstration were consistent with those used for typical semivolatile soil products by ERA. These techniques have been validated through hundreds of round robin performance test studies over ERA's more than 25 years in business. The D/F stock solutions used in the manufacture of these PE samples were purchases from CIL. The PCB and PAH stock solutions were purchased from ChemService. For each PE sample, a spiking concentrate was prepared by combining appropriate weight/volume aliquots of stock materials required for that PE sample. Typically, additional solvent was added to this concentrate to yield sufficient volume of solution, appropriate for the mass of soil to be spiked. Based on a soil mass of 1,600 g, the volume of spike concentrate was approximately 10 to 30 mL. For each PE sample, the blank soil matrix was weighed into a 2-liter (L) wide mouth glass jar, the spike concentrate was distributed onto the soil, and the soil was allowed to air-dry for 30 to 60 minutes. The PE samples were then capped and mixed in a rotary tumbler for 30 minutes. Each PE sample was certified as the concentration of target analytes present in the blank matrix, plus the amount added during manufacture, based on volumetric and gravimetric measurements. CoAs were provided by ERA for all six ERA-provided PE samples. The certified values provided by ERA were different from the commercially available certified samples since the data were not based on analytically

derived results. Further confirmation of the concentrations was conducted by the reference laboratory.

4.3.2 Environmental Samples

Handling of the environmental samples is described in this section. Note that once the environmental samples were collected, they were dried and homogenized as best as possible to eliminate variability introduced by sample homogeneity. As such, the effect of moisture on the sample analysis was not investigated.

4.3.2.1 Environmental Sample Collection

Samples were collected by the EPA, an EPA contractor, or the MDEQ and shipped to the characterization laboratory. When determining whether a soil or sediment site had appropriate dioxin contamination, a guideline concentration range of < 50 pg/g to 5,000 pg/g was used.

Once necessary approvals and sampling locations had been secured, sample containers were shipped to site personnel. Each site providing samples received 1-gallon containers [Environmental Sampling Supply, Oakland, California, Part number 3785-1051, wide-mouth, 128-ounce high-density polyethylene round packer] for collecting five or six samples.

Instructions for sample collection, as well as how the containers were to be labeled and returned, were included in a cover letter with the sample containers that were shipped to each site. Personnel collecting the samples were instructed to label two containers containing the same sample as "1 of 2" and "2 of 2" and to attach a description or label to each container with a description of the sample, including where the sample was collected and the estimated concentrations of dioxin and any other anticipated contamination (e.g., PCBs, PAHs, PCP). Final instructions to sample providers indicated that collected samples were to be shipped back to the characterization laboratory using the provided coolers. Federal Express labels that included an account number and the shipping address were enclosed in each shipment.

Sample providers also were asked to provide any information about the possible source of contamination or any historical data and other information, such as descriptions of the sites, for inclusion in the D/QAPP.(2)

4.3.2.2 Homogenization of Environmental Samples

If the material had very high moisture content, the jar contents were allowed to settle, and the water was poured off. Extremely wet material was poured through fine mesh nylon material to remove water. After water removal, the material was transferred to a Pyrex™ pan and mixed. After thorough mixing, an aliquot was stored in a pre-cleaned jar as a sample of "unhomogenized" material and was frozen.[1] The remaining bulk sample was mixed and folded bottom to top three times. This material was split equally among multiple pans. In each pan, the material was spread out to cover the entire bottom of the pan to an equal depth of approximately 0.5 inches. The pans were placed in an oven at 35ºC and held there until the samples were visibly dry. This process took from 24 to 72 hours, depending on the sample moisture. The trays were removed from the oven and allowed to rise to room temperature by sitting in a fume hood for approximately 2 hours. Approximately 500 g of material were put in a blender and blended for 2 minutes. The blender sides were scraped with a spatula and the sample blended for a second 2-minute period. The sample was sieved [USA Standard testing, No. 10, 2.00-millimeter (mm) opening] and the fine material placed in a tray. Rocks and particles that were retained on the sieve were placed in a pan. This process was repeated until all of the sediment or soil were blended and sieved. The blended and sieved sediment or soil in the tray was mixed well, and four aliquots of 100- to 300-g each were put into clean jars (short, wide-mouth 4-ounce, Environmental Sampling Supply, Oakland, California, Part number 0125-0055) to be used for the characterization analyses. The remaining sediment or soil was placed in a clean jar, and the particles that were retained on the sieve were disposed of. The jars of homogenized sediment and soil were stored frozen (approximately -20°C), unless the samples were being used over a period of several days, at which time they were temporarily stored at room temperature.

[1] Ideally, the samples would have been stored at 4° ± 2°C; but, due to the large volume of buckets and jars that needed to be stored, the most adequate available storage at the characterization laboratory was a walk-in freezer that was at approximately minus 20°C.

4.3.2.3 Selection of Environmental Samples

Once homogenized, the environmental samples were characterized for D/Fs [EPA Method 1613B[3]), PCBs, low-resolution mass spectrometry (LRMS) modified EPA Method 1668A[4], and 18 target PAHs [National Oceanic and Atmospheric Administration (NOAA) method[7]] to establish the basic composition of the samples. (Characterization analyses are described in Chapter 5.) Because the soil and sediment samples were dried and homogenized, they were indistinguishable. As such, the soil and sediment samples were jointly referred to as "environmental" samples, with no distinction made between soil or sediment, other than during the matrix effects evaluations, as described in Section 4.7.6. Environmental samples were selected for inclusion in the demonstration based on the preliminary characterization data. The number and type of samples from each sampling location included in the demonstration are presented in Table 4-5.

Four aliquots of the homogenized material and one aliquot of unhomogenized material were analyzed. Two criteria had to be met for the environmental sample to be considered for inclusion in the demonstration. The first criterion was that the relative standard deviation (RSD) of the total D/F TEQ values from the four aliquots had to be less than 20% for samples with total TEQ values > 50 pg/g; RSD values up to 30% were considered acceptable if the concentration was < 50 pg/g TEQ. The second criterion was that no single RSD for an individual congener could be greater than 30%. If both of these criteria were met, the sample met the homogenization criteria and was considered for inclusion in the demonstration. If either of these criteria was not met, options for the sample included (a) discarding it and not considering it for use in the demonstration, (b) reanalyzing it to determine if the data outside the homogenization criteria were due to analytical issues, or (c) rehomogenizing and reanalyzing it. Of these options, (a) and (b) were utilized, but (c) was not because an adequate number of environmental samples were selected using criteria (a) and (b). The average D/F concentration and RSDs for the homogenization analyses of environmental samples are shown in Table 4-5. The composition of two particular Saginaw River samples were of interest for inclusion in the demonstration because of their concentration and unique congener pattern, but the homogenization criteria were slightly exceeded (i.e., 28% and 34% RSD, for Saginaw River Sample #2 and Saginaw River Sample #3, respectively). Since multiple replicates of every sample were analyzed, those samples were included in the study because of their unique nature but are flagged as slightly exceeding the homogenization criteria. A correlation of environmental samples to PE samples, similar to that presented in Table 4-4, is presented in Table 4-5.

4.3.3 Extracts

A summary of the extract samples is provided in Table 4-6. The purpose of the extract samples was to evaluate detection and measurement performance independent of the sample extraction method. As shown in Table 4-6, two environmental samples (both sediments) were extracted using Soxhlet extraction with toluene. These extractions were performed by AXYS Analytical Services consistent with the procedures to extract the demonstration samples for reference analyses.[2] The environmental sample extracts represented a 10-g sediment sample extraction and were reported in pg/mL, which was calculated by the following equation:

$$\text{pg/mL} = \frac{(\text{pg/g samples}) \times (10\,\text{g aliquot})}{(300\ \text{mL extraction volume})} \times (30\ \text{DF})$$

where DF = dilution factor.

Total extract volume per 10-g aliquot was 300 mL, but the sample extracts were concentrated and provided to the developers as 10-mL extracts, so a 30x dilution factor is included. The extracts were not processed through any cleanup steps, but they were derived from sediment samples that also were included in the suite of environmental samples. All environmental sample extractions were prepared in the same solvent (toluene). The extract samples also included three toluene-spiked solutions that were not extractions of actual environmental samples. Because adequate homogenization at trace quantities was difficult to achieve, one set of extract samples was spiked at low levels (approximately 0.5 pg/mL of 2,3,7,8-TCDD) and used as part of the method detection limit (MDL) evaluation.

Table 4-5. Characterization and Homogenization Analysis Results for Environmental Samples

Sample Type ID	Environmental Site Location	Soil or Sediment	Sample No.	Average Total $TEQ_{D/F}$ Concentration (pg/g)	RSD (%)	No. of Replicates Per Sample	Correlation with PE Sample Type ID[a]
Env Site #1	Warren County, North Carolina	soil	1	274	11	4	9, 10, 11
			2	5,065	7	4	
			3	11,789	3	4	
Env Site #2	Tittabawassee River, Michigan	soil	1	42	23[b]	4	4
			2	435	5	4	
			3	808	10	4	
Env Site #3	Newark Bay, New Jersey	sediment	1	16	26[a]	4	5
			2	62	14	4	
			3	45	26[b]	4	
			4	32	6	4	
Env Site #4	Raritan Bay, New Jersey	sediment	1	12	2	4	5
			2	14	3	4	
			3	13	7	4	
Env Site #5	Winona Post, Missouri	soil	1	3,831	1	4	2, 8
			2	11,071	2	4	
			3	11,739	1	4	
Env Site #6	Tittabawassee River, Michigan	sediment	1	1	23[b]	4	1, 3
			2	55	7	4	
			3	16	26[b]	4	
Env Site #7	Brunswick, Georgia	sediment	1	69	8	4	8
			2	65	1	4	
			3	14,500	2	4	
Env Site #8	Saginaw River, Michigan	sediment	1	921	9	4	4
			2	1,083	28[c]	4	
			3	204	34[c]	4	
Env Site #9	Midland, Michigan	soil	1	239	5	4	4
			2	184	5	4	
			3	149	7	4	
			4	25	10	4	
Env Site #10	Solutia, West Virginia	soil	1	48	10	4	6, 7
			2	1,833	19	4	
			3	3,257	11	4	
Average RSD for all environmental samples used in demonstration						***11%***	
Total number of environmental samples						***128***	

[a] PE Sample IDs are provided in Table 4-4.

[b] RSD values up to 30% were allowed for samples where the characterization analyses determined concentration to be < 50 pg/g total $TEQ_{D/F}$.

[c] RSD value slightly exceeded the homogeneity criteria, but samples were included in the demonstration because they were samples of interest.

Table 4-6. Distribution of Extract Samples

Sample Type ID	Sample ID	Sample Description	No. of Replicates per Sample
Extract #1	Environmental #6, Sample #2	Soxhlet extraction in toluene; no cleanup	4
Extract #2	Environmental #7, Sample #1	Soxhlet extraction in toluene; no cleanup	4
Extract #3	Spike #1[a]	0.5 pg/mL 2,3,7,8-TCDD	7 [b]
Extract #4	Spike #2[a]	100 pg/mL 2,3,7,8-TCDD 1,000 pg/mL each WHO PCB (TEQ ~ 11)	4
Extract# 5	Spike#3[a]	10,000 pg/mL each WHO PCB (TEQ ~ 1,000)[c]	4
Total number of extracts			***23***

[a] Prepared in toluene.

[b] Seven replicates were analyzed for EMDL evaluation.

[c] This sample was spiked with only PCBs, but a low-level (approximately 0.3 pg/mL) 2,3,7,8-TCDD contamination was confirmed by the reference laboratory.

4.4 Sample Handling

In preparation for the demonstration, the bulk homogenized samples were split into jars for distribution. Each 4-ounce, amber, wide-mouth glass sample jar (Environmental Sampling Supply, Oakland, California, Part number 0125-0055) contained approximately 50 g of sample. Seven sets of samples were prepared for five developers, the reference laboratory, and one archived set. A minimum of four replicate splits of each sample was prepared for each participant, for a total of at least 28 aliquots prepared for each sample. The purchased PE samples (i.e., standard reference materials and spiked materials) were transferred from their original packaging to the jars to be used in the demonstration for the environmental samples, making the environmental and PE samples visually indistinguishable.

The samples were randomized in two ways. First, the order in which the filled jars were distributed was randomized. All jars had two labels. The label on the top of the jar was the analysis order and contained sample numbers 1 through 209. A second label placed on the side of the jar contained a coded identifier including a series of ten numbers coded to include the site, replicate, developer, and matrix. All samples believed to have at least one D/F or PCB congener greater than 10,000 pg/g were marked with an asterisk for safety purposes. This was consistent for both the developer and reference laboratory samples. The developer was given the option of knowing which environmental site the samples came from and whether the sample was a soil or sediment. Hybrizyme elected to just analyze the soil samples in the field, so soil and sediment samples were identified to Hybrizyme. As described in the D/QAPP, AXYS was informed of which environmental site that the samples came from so it could use congener profiles and dilution schemes determined during the pre-demonstration phase as a guide, along with the concentration range data that was provided in the D/QAPP. This information was supplied to the reference laboratory with the samples, along with which samples contained high (i.e., a sample with at least one congener with concentration > 120,000 pg/g) or ultrahigh (i.e., a sample with at least one congener with concentration > 1,200,000 pg/g) PCB levels. Using this information, AXYS regrouped the samples in batches so that, to the extent possible, samples from the same site would be analyzed within the same analytical batch. Because an analytical laboratory might know at least what site samples came from, and because it is reasonable from an analytical standpoint to group samples that might require similar dilution schemes and which have similar congener patterns in an analytical batch, this approach was an acceptable deviation from the original intention of having the samples run by the reference laboratory completely blind

and in the prescribed analytical order. Hybrizyme analyzed the samples in the prescribed order, but only analyzed the soils in the field (extracts and sediments were analyzed in its laboratories).

The environmental samples were stored at room temperature until homogenized. After homogenization and prior to distribution during the demonstration, the samples were stored in a walk-in freezer (approximately -20°C) at the characterization laboratory. At the demonstration site, the samples were stored at ambient temperature. After the demonstration analyses were completed, the samples were stored at the characterization laboratory in the walk-in freezer until the conclusion of the project.

4.5 Pre-Demonstration Study

Prior to the demonstration, pre-demonstration samples were sent to Hybrizyme for evaluation in its laboratory. The pre-demonstration study comprised 15 samples, including PE samples, environmental samples, and extracts. The samples selected for the pre-demonstration study covered a wide range of concentrations and included a representative of each environmental site analyzed during the demonstration.

The pre-demonstration study was conducted in two phases. In Phase 1, Hybrizyme was sent six soil/sediment samples with the corresponding D/F, PCB, and PAH characterization data to perform a self-evaluation of its technology. In Phase 2, seven additional soil/sediment samples and two extracts were sent to Hybrizyme for blind evaluation. AXYS analyzed all 15 pre-demonstration samples blindly. The Hybrizyme pre-demonstration results were paired with the AXYS results and returned to Hybrizyme so it could use the HRMS pre-demonstration sample data to refine the performance of its kit prior to participating in the field demonstration. Results for the pre-demonstration study can be found in the DER, which can be obtained by contacting the EPA program manager for this demonstration. The results confirmed that Hybrizyme was a viable candidate to continue in the demonstration process.

4.6 Execution of Field Demonstration

Hybrizyme arrived on-site on Saturday, April 24, and spent several hours that day and the next day (Sunday, April 25) setting up its trailer. The demonstration officially commenced on Monday, April 26 after 1.5 hours of safety and logistical training. During this meeting, the health and safety plan was reviewed to ensure participants understood the safety requirements for the demonstration. Logistics, such as how samples would be distributed and results reported, were also reviewed during this meeting. After the safety and site-specific training meeting and prior to samples being received by the developers, each trailer and mobile laboratory was surface wipe sampled on the floor to the entrance of the developer work area to establish the background level of D/F and PCB contamination. The wipe sampling procedure was followed as described in the D/QAPP. Following demobilization by the developers, all of the trailers and mobile laboratories were cleaned and surface-wipesampled. Analysis of the pre- and post-deployment wipe samples indicated that all trailers and mobile laboratories met the acceptable clearance criteria that were outlined in the D/QAPP. Only one fume hood had to be re-cleaned and re-sampled before receiving final clearance.

Ideally, all 209 demonstration samples would have been analyzed on-site, but sample throughput of some of the technologies participating in the demonstration would require three weeks or more in the field to analyze 209 samples. Consequently, it was decided, as reported in the D/QAPP, that the number of samples to be analyzed in the field by each developer would be determined at the discretion of the developer.

Hybrizyme received its first batch of samples by midmorning on April 26. Hybrizyme completed analysis of 110 soil samples in 4 working days (on April 29). It should be noted that the morning of April 28 was dedicated to a Visitor's Day, so minimal work on sample analyses was performed. The remaining 99 samples were completed by Hybrizyme in its laboratories and were reported on August 31. Hybrizyme was also offered the opportunity to reanalyze any samples before reporting final results. Hybrizyme reanalyzed all 110 soil samples that were analyzed in the field because refinements were made to the analytical procedure based on experience gained during the field demonstration. Only data generated using the refined method (i.e., all analyses performed in Hybrizyme's laboratory) were used in the evaluation of the technology. Hybrizyme

reported that the total analysis time once the method refinements were completed was one week.

4.7 Assessment of Primary and Secondary Objectives

The purpose of this section is to describe how the primary and secondary objectives are assessed, as presented in Chapters 6 and 7.

The Hybrizyme Corporation AhRC PCR™ Kit is a technology that reports the concentration of AhR binding compounds in a sample, and the units reported as AhRBU. At the time of the 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. It has been suggested that correlation between the Hybrizyme AhRBU results and HRMS TEQ could be established by first characterizing a site and calibrating the Hybrizyme results to HRMS TEQ results. This approach was not evaluated during this demonstration.

The developer's goal is a highly portable screening technology which can help to determine areas of greatest concern for cleanup at a site and can help to minimize the number of more expensive analyses needed for specific analytes. Given the current state of development of the Hybrizyme technology, the technology's results were compared to the HRMS results in terms of ranking sample concentrations from low to high, rather than comparing in a quantitative fashion. The results which were compared to Hybrizyme's results also included contributions from PAHs because PAHs are AhR binding compounds and are included in the Hybrizyme results. It should be noted that the suite of PAHs which were quantified in the samples may do not include all of the PAHs which are responsive to this kit.

4.7.1 Primary Objective P1: Accuracy

The determination of accuracy was based on ranking of the PE samples results from low to high AhR binding compounds (D/F + PCB + PAH) and comparing it to the rank order reported by Hybrizyme based on AhRBU. For the PE samples, the D/F, PCB, and PAH concentrations were summed from the concentrations reported on the certificate of analysis. Note that the PAH data for the PE samples was rather limited, and it is possible that additional contributions from PAHs were not included in the certified data. Ideally, the rankings would be identical.

4.7.2 Primary Objective P2: Precision

To evaluate precision, all samples (including PE, environmental, and extract samples) were analyzed in at least quadruplicate. Seven replicates of three different samples were analyzed to evaluate MDLs.

Precision was evaluated at both low and high concentration levels and across different matrices. The statistic used to evaluate precision was RSD. The equation used to calculate standard deviation (*SD*) between replicate measurements was:

$$SD = \left[\frac{1}{n-1} \sum_{k-1}^{n} \left(\overline{C}_k - \overline{C} \right)^2 \right]^{1/2}$$

where *SD* (in AhRBU) is the standard deviation and $\overline{C}$ (in AhRBU) is the average measurement.

The equation used to calculate RSD between replicate measurements was:

$$\text{RSD} = \left| \frac{\text{SD}}{\overline{\text{C}}} \right| \times 100\%$$

RSD, reported in percent, was calculated if detectable concentrations were reported for at least three replicates. The mean, median, minimum, and maximum RSD values are reported as an assessment of overall precision.

Low RSD values (< 20%) indicated high precision. For a given set of replicate samples, the RSD of results was compared with that of the laboratory reference method's results to determine whether the reference method is more precise than the technology or vice versa for a particular sample set. The mean RSD for all samples was calculated to determine an overall precision estimate.

4.7.3 Primary Objective P3: Comparability

Technology results reported by Hybrizyme Corporation as AhRBU in a sample and ordered from low to high were compared to the corresponding reference laboratory results generated from the sum of the reference laboratory HRMS data for D/F and PCB and the characterization PAH data (in nanogram [ng]/g) ordered from low to high. The comparability evaluation was only performed for the environmental samples. Ideally, the rankings would be identical.

4.7.4 Primary Objective P4: Estimated Method Detection Limit

The MDL calculation procedure described in the demonstration plan was 40 CFR Part 136, Appendix B, Revision 1.11. This procedure is based on an assumption that the replicates are homogeneous enough to allow proper measurement of the analytical precision and that the concentration is in the appropriate range for evaluation of the technology's sensitivity. For this evaluation, Hybrizyme analyzed seven aliquots each of a low-level PE soil, PE sediment, and a toluene-spiked extract. MDL-designated samples are indicated in Tables 4-4 and 4-6. The developer reported values for all of the replicates (i.e., no nondetect values were reported). Because the true detection limits of the technology were not known by the developer, it was not known if the sample concentrations selected for this evaluation were appropriate, so the evaluation was considered an EMDL. A Student's t-value and the standard deviation of seven replicates were used to calculate the EMDL in AhRBU is shown in the following equation:

$$EMDL = t_{(n-1,1-\infty=0.99)}(SD)$$

where $t_{(n-1,1-\infty=0.99)}$ = Student's t-value appropriate for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom. The lower the EMDL value, the more sensitive the technology is at detecting contamination.

4.7.5 Primary Objective P5: False Positive/False Negative Results

The tendency for the AhRC PCR™ Kit to return false positive results (e.g., results reported above a specified level for the field technology but below a specified level by the reference laboratory) and false negative results (e.g., results reported below a specified level for the field technology but above a specified level by the reference laboratory) was not evaluated since the Hybrizyme technology results are not directly comparable with HRMS results.

4.7.6 Primary Objective P6: Matrix Effects

The likelihood of matrix-dependent effects on performance was investigated by grouping the data by matrix type (i.e., soil, sediment, extract), by sample type (i.e., PE, environmental, and extract), and by varying levels of PAHs. Precision (RSD) data were summarized by soil, sediment, and extract (matrix type); by environmental, PE, and extract (sample type); and by PAH concentration. Analysis of variance (ANOVA) tests were performed to determine if there was a dependence on matrix type or sample type. Only the environmental samples were included in the matrix effect assessment based on PAH concentration, because only the environmental samples were analyzed for PAHs during the characterization analysis (described in Section 5.2.3). Some PAH data were available for the PE samples, but data were not available for all of the same analytes that were determined during the characterization analysis. The environmental samples were segregated into four ranges of total PAH concentrations: < 1,000 nanogram/g (ng/g), 1,000 to 10,000 ng/g, 10,000 to 100,000 ng/g, and > 100,000 ng/g. The precision (RSD) data were summarized for samples within these PAH concentration ranges. ANOVA tests were used to determine if the summary values for RSD were statistically different, indicating performance dependent upon PAH concentration.

This objective also evaluated if performance was affected by measurement location (i.e., in-field versus laboratory conducted measurements), although this is not a traditional matrix effect. However, the effect of measurement location was not tested because Hybrizyme re-ran the 110 samples that it analyzed during the field demonstration by a modified method in its laboratory, so all 209 samples were run in its laboratories.

This objective included an environmental site evaluation, where the comparability values from each of the 10 environmental sites were compared to see if the developer results were more or less comparable to the reference laboratory for a particular site. Since Hybrizyme did not produce a result that was directly comparable to HRMS reference data, this could not be evaluated. This objective also included an assessment of known interferences where the developer's reported results for PE samples were summarized for samples where the PE samples did not contain the target analyte. This parameter also could not be evaluated, since Hybrizyme's technology responded to all AhR binding compounds.

4.7.7 Primary Objective P7: Technology Costs

The full cost of each technology was documented and compared the cost to typical and actual costs for D/F and PCB reference analytical methods. Cost inputs included equipment, consumable materials, mobilization and demobilization, and labor. The evaluation of this objective is described in Chapter 8, Economic Analysis.

4.7.8 Secondary Objective S1: Skill Level of Operator

Based on observations during the field demonstration, the type of background and training required to properly operate the AhRC PCR™ Kit was assessed and documented. The skill required of an operator was also evaluated. The evaluation of this secondary objective also included user-friendliness of the technology.

4.7.9 Secondary Objective S2: Health and Safety Aspects

Health and safety issues, as well as the amount and type of hazardous and nonhazardous waste generated, were evaluated based on observer notes during the field demonstration. This also included an assessment of the personal protective equipment required to operate the technology.

4.7.10 Secondary Objective S3: Portability

Observers documented whether the AhRC PCR™ Kit could be readily transported to the field and how easy it was to operate in the field. This included an assessment of what infrastructure requirements were provided to Hybrizyme (e.g., a trailer), and an assessment of whether the infrastructure was adequate (or more than adequate) for the technology's operation. Limitations of operating the technology in the field are also discussed.

4.7.11 Secondary Objective S4: Sample Throughput

Sample throughput was measured based on the observer notes, which focused on the time-limiting steps of the procedures, as well as the documentation of sample custody. The number of hours Hybrizyme worked in the field was documented using attendance log sheets where Hybrizyme recorded the time they arrived and departed from the demonstration site. Time was removed for training and Visitor's Day activities. The number of operators involved in the sample analyses also was noted. Throughput of the developer technology was compared to that of the reference laboratory.

Chapter 5
Confirmatory Process

This chapter describes the characterization analyses and the process for selecting the reference methods and the reference laboratory.

5.1 Traditional Methods for Measurement of Dioxin and Dioxin-Like Compounds in Soil and Sediment

Traditional methods for analysis of dioxin and dioxin-like compounds involve extensive sample preparation and analysis using expensive instrumentation resulting in very accurate and high-quality, but costly, information. The ability to use traditional methods for high-volume sampling programs or screening of a contaminated site often is limited by budgetary constraints. The cost of these analyses can range approximately from $500 to $1,100 per sample per method, depending on the method selected, the level of QA/QC incorporated into the analyses, and the reporting requirements.

5.1.1 High-Resolution Mass Spectrometry

EPA Method 1613B[3] and SW846 Method 8290[8] are both appropriate for low and trace-level analysis of dioxins and furans in a variety of matrices. They involve matrix-specific extraction, analyte-specific cleanup, and HRGC/HRMS analysis. The main differences between the two methods are that EPA Method 1613B has an expanded calibration range and requires use of additional $^{13}C_{12}$-labeled internal standards resulting in more accurate identifications and quantitations. The calibration ranges for the HRMS methods based on a typical 10-g sample and 20-microliter (µL) final sample volume are presented in Table 5-1.

Table 5-1. Calibration Range of HRMS Dioxin/Furan Method

Compound	EPA Method 1613B	SW846 Method 8290
Tetra Compounds	1–400 pg/g	2–400 pg/g
Penta-Hepta Compounds	5–2,000 pg/g	5–1,000 pg/g
Octa Compounds	10–4,000 pg/g	10–2,000 pg/g

5.1.2 Low-Resolution Mass Spectrometry

SW846 Method 8280 is appropriate for determining dioxins and furans in samples with relatively high concentrations, such as still bottoms, fuel oils, sludges, fly ash, and contaminated soils and waters. This method involves matrix specific extraction, analyte-specific cleanup, and HRGC/LRMS analysis. The calibration ranges in Table 5-2 are based on a typical 10-g sample size and 100-µL final volume.

Table 5-2. Calibration Range of LRMS Dioxin/Furan Method

Compound	SW846 Method 8280
Tetra-Penta Compounds	1,000–20,000 pg/g
Hexa-Hepta Compounds	2,500–50,000 pg/g
Octa Compounds	5,000–100,000 pg/g

5.1.3 PCB Methods

There are more options for analysis of dioxin-like compounds such as PCBs. EPA Method 1668A[4] is for low- and trace-level analysis of PCBs. It involves matrix-specific extraction, analyte-specific cleanup, and HRGC/HRMS analysis. This method provides very accurate determination of the WHO-designated dioxin-like PCBs and can be used to determine all 209 PCB congeners. Not all PCBs are determined individually with this method because some are determined as sets of coeluting congeners. The calibration range for PCBs based on a typical 10-g sample and 20-µL final sample volume is from 0.4 to 4,000 pg/g. PCBs also can be determined as specific congeners by GC/LRMS or as Aroclors[1] by GC/electron capture detection.

5.1.4 Reference Method Selection

Three EPA analytical methods for the quantification of dioxins and furans were available: Method 1613B, Method 8290, and Method 8280. Method 8280 is a LRMS method that does not have adequate sensitivity (i.e., the detection limits reported by the developers are less than that of the LRMS method). Methods 1613B and 8290 are HRMS methods with lower detection limits. Method 1613B includes more labeled internal standards than Method 8290, which affords more accurate congener quantification. Therefore, it was determined that Method 1613B best met the needs of the demonstration, and it was selected as the D/F reference method. Reference data of equal quality needed to be generated to determine the PCB contribution to the TEQ, since risk assessment is often based on TEQ values that are not class-specific. As such, the complementary HRMS method for PCB TEQ determinations, Method 1668A,[4] was selected as the reference method for PCBs. Total $TEQ_{D/F}$ concentrations were generated by Method 1613B, and total TEQ_{PCB} concentrations were generated by Method 1668A. These data were summed to derive a total TEQ value for each sample.

5.2 Characterization of Environmental Samples

All of the homogenized environmental samples were analyzed by the Battelle characterization laboratory to determine which would be included in the demonstration. The environmental samples were characterized for the 17 D/Fs by Method 1613B, the 12 WHO PCBs by LRMS-modified Method 1668A, and 18 target PAHs by the NOAA Status and Trends GC/Mass Spectrometry (MS) method.[7]

5.2.1 Dioxins and Furans

Four aliquots of homogenized material and one unhomogenized (i.e., "as received") aliquot were prepared and analyzed for seventeen 2,3,7,8-substituted dioxins and furans following procedures in EPA Method 1613B. The homogenized and unhomogenized aliquots were each approximately 200 g. Depending on the anticipated levels of dioxins from preliminary information received from each sampling location, approximately 1 to 10 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. One method blank and one laboratory control spike were processed with the batch of material from each site. 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. Extracts were spiked with $^{13}C_{12}$-labeled recovery standards and concentrated to a final volume of 20 to 50 µL. Dilution and reanalysis of the extracts were performed if high levels of a particular congener were observed in the initial analysis; however, extracts were not rigorously evaluated to ensure that all peaks were below the peak area of the highest calibration standard.

Each extract was analyzed by high-resolution GC/HRMS in the selected ion monitoring (SIM) mode at a resolution of 10,000 or greater. A DB-5 column was used for analysis of the seventeen 2,3,7,8-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. Using a DB5 column, 2,3,7,8-TCDF is not separated from other non2,3,7,8-TCDF isomers. However, since the primary objective was to determine adequacy of homogenization and not congener quantification, it was determined that sufficient information on precision could be obtained with the DB5

analysis of 2,3,7,8-TCDF and no second column confirmation of 2,3,7,8-TCDF was performed. PCDD/F data were reported as both concentration (pg/g dry) and TEQs (pg TEQ/g dry).

5.2.2 PCBs

One aliquot of material from each sampling location was prepared and analyzed for the 12 WHO-designated dioxin-like PCBs by GC/LRMS. The LRMS PCB analysis method is based on key components of the PCB congener analysis approach described in EPA Method 1668A and the PCB homologue approach described in EPA Method 680. Up to 30 g of sample were spiked with surrogates and extracted with methylene chloride using shaker table techniques. The mass of sample extracted was determined based on information supplied to the laboratory regarding possible contaminant concentrations. The extract was dried over anhydrous sodium sulfate and concentrated. Extracts were processed through alumina column cleanup, followed by high-performance liquid chromatography/gel permeation chromatography (HPLC/GPC). Additionally, sulfur was removed using activated granular copper. The post-HPLC extract was concentrated and fortified with recovery internal standards. Extracts were concentrated to a final volume between 500 µL and 1 mL, depending on the anticipated concentration of PCBs in the sample, as reported by the sample providers. PCB congeners and PCB homologues were separated via capillary GC on a DB5-XLB column and identified and quantified using electron ionization MS. This method provides specific procedures for the identification and measurement of the selected PCBs in SIM mode.

5.2.3 PAHs

One aliquot of material from each sampling location was analyzed for PAHs. The 18 target PAHs included:

- naphthalene
- 2-methylnaphthalene
- 2-chloronaphthalene
- acenaphthylene
- acenaphthene
- fluorene
- phenanthrene
- anthracene
- fluoranthene
- pyrene
- benzo(a)anthracene
- chrysene
- benzo(b)fluoranthene
- benzo(k)fluoranthene
- benzo(a)pyrene
- indeno(1,2,3-cd)pyrene
- dibenzo(a,h)anthracene
- benzo(g,h,i)perylene.

The method for the identification and quantification of PAH in sediment and soil extracts by GC/MS was based on the NOAA Status and Trends method[7] and, therefore, certain criteria (i.e., initial calibrations and daily verifications) are different from those defined in traditional EPA Methods 625 and 8270C. Up to 30 g of sample were spiked with surrogates and extracted using methylene chloride using shaker table techniques. The mass of sample extracted was determined based on information supplied to the characterization laboratory regarding possible contaminant concentrations. The extract was dried over anhydrous sodium sulfate and concentrated. The extract was processed through an alumina cleanup column followed by HPLC/GPC. The post-HPLC extract was concentrated and fortified with recovery internal standards. Extracts were concentrated between 500 µL and 1 mL, depending on the anticipated concentration of PCBs in the sample, as reported by the sample providers. PAHs were separated by capillary GC on a DB-5, 60-m column and were identified and quantified using electron impact MS. Extracts were analyzed in the SIM mode to achieve the lowest possible detection limits.

5.3 Reference Laboratory Selection

Based on a preliminary evaluation of performance and credibility, ten laboratories were contacted and were sent a questionnaire geared toward understanding the capabilities of the laboratories, their experience with analyzing dioxin samples for EPA, and their ability to meet the needs of this demonstration. Two laboratories were selected for the next phase of the selection process and were sent three blind audit samples. Each laboratory went through a daylong audit that included a technical systems audit and a quality systems audit. At each laboratory, the audit consisted of a short opening conference; a full day of observation of laboratory procedures, records, interviews with laboratory staff; and a brief closing meeting. Auditors submitted followup

questions to each laboratory to address gaps in the observations.

Criteria for final selection were based on the observations of the auditors, the performance on the audit samples, and cost. From this process, it was determined that AXYS Analytical Services (Sidney, British Columbia, Canada) would best meet the needs of this demonstration.

5.4 Reference Laboratory Sample Preparation and Analytical Methods

AXYS Analytical Services received all 209 samples on April 27, 2004. To report final data, AXYS submitted 14 D/F and 14 PCB data packages from June 11 to December 20, 2004. The following sections briefly describe the reference methods performed by AXYS.

5.4.1 Dioxin/Furan Analysis

All procedures were carried out according to protocols as described in AXYS Summary Method Doc MSU-018 Rev 2 18-Mar-2004 [AXYS detailed Standard Operating Procedure (SOP) MLA-017 Rev 9 May-2004], which is based on EPA Method 1613B. AXYS modifications to the method are summarized in the D/QAPP.[2] Briefly, samples were spiked with a suite of isotopically labeled surrogate standards prior to extraction, solvent extracted, and cleaned up through a series of chromatographic columns that included silica, Florisil, carbon/Celite, and alumina columns. The extract was concentrated and spiked with an isotopically labeled recovery (internal) standard. Analysis was performed using an HRMS coupled to an HRGC equipped with a DB-5 capillary chromatography column [60 meters (m), 0.25-mm internal diameter (i.d.), 0.1-µm film thickness]. A second column, DB-225 (30 m, 0.25-mm i.d., 0.15-µm film thickness), was used for confirmation of 2,3,7,8-TCDF identification. Samples that were known to contain extremely high levels of PCDD/F were extracted without the addition of the surrogate standard, split, then spiked with the isotopically labeled surrogate standard prior to cleanup. This approach allowed extraction of the method-specified 10-g sample volume, and subsequent sufficient dilution that high level analytes were brought within the instrument calibrated linear range. While this approach induces some uncertainty because the actual recovery of analytes from the extraction process is unknown, it was decided by the demonstration panel that in general analyte recovery through the extraction procedures are known to be quite good and that the uncertainty introduced by this approach would be less than the uncertainty introduced by other approaches such as extracting a significantly smaller sample size.

5.4.2 PCB Analysis

The method was carried out in accordance with the protocols described in AXYS Summary Method Doc MSU-020 Rev 3 24-Mar-2004 (AXYS detailed SOP MLA-010 Rev 5 Sep-2003), which is based on EPA Method 1668A, with changes through August 20, 2003. AXYS modifications to the method are summarized in the D/QAPP. Briefly, samples were spiked with isotopically labeled surrogate standards, solvent extracted, and cleaned up on a series of chromatographic columns that included silica, Florisil, alumina, and carbon/Celite columns. The final extract was spiked with isotopically labeled recovery (internal) standards prior to instrumental analysis. The extract was analyzed by HRMS coupled to an HRGC equipped with a DB-1 chromatography column (30 m, 0.25-mm i.d., 0.25-µm film thickness). Because only the WHO-designated dioxin-like PCBs were being analyzed for this program and in order to better eliminate interferences, all samples were analyzed using the DB-1 column, which is an optional confirmatory column in Method 1668A rather than the standard SPB Octyl column. Samples that were known to contain extremely high levels of PCBs were extracted without the addition of the surrogate standard, split, then spiked with the isotopically labeled surrogate standard prior to cleanup. This approach allowed extraction of the method-specified 10-g sample volume, and subsequent sufficient dilution that high level analytes were brought within the instrument calibrated linear range. While this approach induces some uncertainty because the actual recovery of analytes from the extraction process is unknown, it was decided by the demonstration panel that in general analyte recovery through the extraction procedures are known to be quite good and that the uncertainty introduced by this approach would be less than the uncertainty introduced by other approaches such as extracting a significantly smaller sample size.

5.4.3 TEQ Calculations

For the reference laboratory data, D/F and PCB congener concentrations were converted to TEQ and subsequently summed to determine total TEQ, using the TEFs established by WHO in 1998 (see Table 4-1).[5] Detection limits were reported as sample-specific detection limits (SDLs). SDLs were determined from 2.5 times the noise in the chromatogram for D/F and 3.0 times the noise for PCBs, converted to an area, and then converted to a concentration using the same calculation procedure as for detected peaks. Any value that met all quantification criteria (> SDL and isotope ratio) was reported as a concentration. A "J" flag was applied to any reported value between the SDL and the lowest level calibration. The concentration of any detected congener that did not meet all quantification criteria (such as isotope ratio or peak shape) were reported but given a "K" flag to indicate estimated maximum possible concentration (EMPC).[8] TEQs were reported in two ways to cover the range of possible TEQ values:

(1) All nondetect and EMPC values were assigned a zero concentration in the TEQ calculation.

(2) Nondetects were assigned a concentration of one-half the SDL. EMPCs were assigned a value equal to the EMPC.

In both cases, any total TEQ value that had 10% contribution or more from J-flagged or K-flagged data was flagged as J or K (or both) as appropriate.

TEQs were calculated both ways for all samples. For $TEQ_{D/F}$, 63% of the samples had the same TEQ value based on the two different calculation methods, and the average relative percent difference (RPD) was 8% (median = 0%). For TEQ_{PCB}, 65% of the samples had the same TEQ value based on the two different calculation methods, and the average RPD was 9% (median = 0%). Because overall there were little differences between the two calculation methods, as presented in Appendix D, TEQ values calculated by option #1 were used in comparison with the developer technologies. On a case-by-case basis, developer results were compared to TEQs calculated by option #2 above, but no significant differences in comparability results were observed so no additional data analysis results using these TEQ values were presented.

Chapter 6
Assessment of Reference Method Data Quality

Ensuring reference method data quality is of paramount importance to accurately assessing and evaluating each of the innovative technologies. To ensure that the reference method has generated accurate, defensible data, a quality systems/technical audit of the reference laboratory was performed during analysis of demonstration samples after the first batch of demonstration sample analyses was complete. The quality systems/technical audit evaluated implementation of the demonstration plan. In addition, a full data package was prepared by the reference laboratory for each sample batch for both dioxin and dioxin-like PCB analyses. Each data package was reviewed by both a QA specialist and technical personnel with expertise in the reference methods for agreement with the reference method as described in the demonstration plan. Any issues identified during the quality systems/technical audit and the data package reviews were addressed by the reference laboratory prior to acceptance of the data. In this section, the reference laboratory performance on the QC parameters is evaluated. In addition, the reference data were statistically evaluated for the demonstration primary objectives of accuracy and precision.

6.1 QA Audits

A quality systems/technical audit was conducted at the reference laboratory, AXYS Analytical Services, Ltd., by Battelle auditors on May 26, 2004, during the analysis of demonstration samples. The purpose of the audit was to verify AXYS compliance with its internal quality system and the D/QAPP.[2] The scope specifically included a review of dioxin and PCB congener sample processing, analysis, and data reduction; sample receipt, handling, and tracking; supporting laboratory systems; and followup to observations and findings identified during the independent laboratory assessment conducted by Battelle on February 11, 2004, prior to contract award. Checklists were prepared to guide the audit, which consisted of a review of laboratory records and documents, staff interviews, and direct observation.

The AXYS quality system is documented in a comprehensive QA/QC manual and detailed SOPs. No major problems or issues were noted during the audit. Two findings were identified, one related to a backlog of unfiled custody records and the other related to the need for performance criteria for the DB-1 column used for the analysis of PCB congeners by HRMS. Both issues were addressed satisfactorily by AXYS after the audit. One laboratory practice that required procedural modification was identified: the laboratory did not subject all QC samples to the most rigorous cleanup procedures that might be required for individual samples within a batch. The AXYS management team agreed that this procedure was incorrect. As corrective action, the QA manager provided written instructions regarding cleanup of the QC samples to the staff, and the laboratory manager conducted follow up discussion with the staff. Other isolated issues noted by the auditors did not reflect systemic problems and were typical of analytical laboratories (e.g., occasional documentation lapses or an untrackable balance weight).

The audit confirmed that the laboratory procedures conformed to the SOPs and D/QAPP and that the quality system was implemented effectively. Samples were processed and analyzed according to the laboratory SOPs and D/QAPP using the Soxhlet Dean Stark extraction method. No substantial deviations were noted. The audit verified the traceability of samples within the laboratory, as well as the traceability of standards, reagents, and solvents used in preparation, and that the purity and reliability of the latter materials were demonstrated through documented quality checks. In addition, the audit confirmed that analytical instruments and equipment were maintained and

calibrated according to manufacturers' specifications and laboratory SOPs. Analytical staff members were knowledgeable in their areas of expertise. QC samples were processed and analyzed with each batch of authentic samples as specified by the D/QAPP. QA/QC procedures were implemented effectively, and corrective action was taken to address specific QC failures. Data verification, reporting, and validation procedures were found to be rigorous and sufficient to ensure the accuracy of the reported data. The auditors concluded that AXYS is in compliance with the D/QAPP and its SOPs, and that the data generated at the laboratory are of sufficient and known quality to be used as a reference method for this project.

In addition, each data package was reviewed by both a QA specialist and technical personnel with expertise in the reference methods for agreement with the reference method as described in the demonstration plan. Checklists were prepared to guide the data package review. This review included an evaluation of data package documentation such as chain-of-custody (COC) and record completeness, adherence to method prescribed holding times and storage conditions, standard spiking concentrations, initial and continuing calibrations meeting established criteria, GC column performance, HRMS instrument resolution, method blanks, lab control spikes (ongoing precision and recovery samples), sample duplicates, internal standard recovery, transcription of raw data into the final data spreadsheets, calculation of TEQs, and data flag accuracy. Any issues identified during the data package reviews were addressed by the reference laboratory prior to acceptance of the data. All of the audit reports and responses are included in the DER.

6.2 QC Results

Each data package was reviewed for agreement with the reference method as described in the demonstration plan. This section summarizes the evaluation of the reference method quality control data.

6.2.1 Holding Times and Storage Conditions

All demonstration samples were stored frozen (< -10°C) upon receipt and were analyzed within the method holding time of one year.

6.2.2 Chain of Custody

All sample identifications were tracked from sample login to preparation of record sheets, to instrument analysis sheets, to the final report summary sheets and found to be consistent throughout. One COC with an incomplete signature and one discrepancy in date of receipt between the COC and sample login were identified during the Battelle audit and were corrected before the data packages with these affected items were accepted as final.

6.2.3 Standard Concentrations

The concentration of all calibration and spiking standards was verified.

6.2.4 Initial and Continuing Calibration

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 with one minor exception for D/F sample batch WG13551, which contained five samples from Environmental Site #1 (North Carolina) and 12 samples from Environmental Site #5 (Winona Post). On one analysis day, a high-level sample analyzed just prior to the ending calibration verification caused the verification to fail. In this instance, the verification was repeated just outside of the 12-hour period. The repeat calibration verification met the acceptance criteria and was considered to show acceptable instrument performance in the preceding analytical period; therefore, the data were accepted.

Continuing calibration results were within the criteria stated in Table 9-2 (D/F) and Table 9-4 (PCB) of the D/QAPP, with one exception. For PCB sample batch WG12108, which contained nine samples from Environmental Site #3 (Newark Bay) and 12 samples from Environmental Site #4 (Raritan Bay), isotopically labeled PCB 169 was above the acceptable range during one calibration verification on May 15, 2004. The acceptance range included in the D/QAPP is tighter than the acceptance range in Method 1668A Table 6. Because the result for labeled PCB 169 was within the Method 1668A acceptance limits, the data were accepted.

The minimum signal-to-noise criteria for analytes in the calibration verification solution were met in all instances.

6.2.5 Column Performance and Instrument Resolution

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. In PCB sample batch WG13554, which contained five PE samples and 15 extract samples, on one analysis day (September 17, 2004), the ending resolution documentation was conducted at 12 hours and 54 minutes. However, as this resolution documentation met all criteria, it was considered representative of acceptable instrument performance during the analytical period, and the data were accepted.

6.2.6 Method Blanks

Method blanks were analyzed with each sample batch to verify that laboratory procedures did not introduce significant contamination. A summary of the method blank data is presented in Appendix C. There were many instances for both D/F and PCB data where analyte concentrations in the method blank exceeded the target criteria in the D/QAPP. Samples from this demonstration, which had very high D/F and PCB concentrations, contributed to the difficulty in achieving method blank criteria in spite of steps the reference laboratory took to minimize contamination (such as proofing the glassware before use in each analytical batch). In many instances, the concentrations of D/F and PCBs in the samples exceeded 20 times the concentrations in the blanks. For all instances, the sample results were unaffected because the method blank TEQ concentration was compared to the sample TEQ concentrations to ensure that background contamination did not significantly impact sample results.

6.2.7 Internal Standard Recovery

Internal standard recoveries were generally within the D/QAPP criteria. D/QAPP criteria were tighter than the standard EPA method criteria; in instances where internal standard recoveries were outside of the D/QAPP criteria, but within the standard EPA method criteria, results were accepted. In several instances, the dioxin cleanup standard recoveries were affected by interferences. As the cleanup standard is not used for quantification of native analytes, these data were accepted. Any samples affected by internal standard recoveries outside of the D/QAPP and outside of the EPA method criteria were evaluated for possible impact on total TEQ and for comparability with replicates processed during the program before being accepted.

6.2.8 Laboratory Control Spikes

One laboratory control spike (ongoing precision and recovery sample), which consisted of native analytes spiked into a reference matrix (sand), was processed with each analytical batch to assess accuracy. Recovery of spiked analytes was within the D/QAPP criteria in Table 9-2 for all analytes in all laboratory control spike samples.

6.2.9 Sample Batch Duplicates

A summary of the duplicate data is presented in Appendix C. One sample was prepared in duplicate in most sample batches; four batches were reported without a duplicate. Three of 14 dioxin sample batches and 5 of 14 PCB sample batches did not meet criteria of <20% RPD between duplicates. Data where duplicates did not meet D/QAPP criteria were evaluated on an individual basis.

6.3 Evaluation of Primary Objective P1: Accuracy

Accuracy was assessed through the analysis of PE samples consisting of certified standard reference materials, certified spikes, and certified blanks. A summary of reference method percent recovery (R) values is presented in Table 6-1. The R values are presented for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ. The minimum, maximum, mean, and median R values are presented for each set of TEQ results. The reference method values were in best agreement with the certified values for the TEQ_{PCB} results, with a mean R value of 96%. The mean R values for $TEQ_{D/F}$ and total TEQ were 125% and 94%, respectively. The mean and median R values for the TEQ_{PCB} and total TEQ were identical. The mean and median R values for $TEQ_{D/F}$ were not similar and were largely influenced by the $TEQ_{D/F}$ recovery for ERA Aroclor of 324%. The ERA Aroclor-certified $TEQ_{D/F}$ values were based on TCDD and TCDF only,

Table 6-1. Objective P1 Accuracy - Percent Recovery

PE Sample ID	PE Sample Description	% Recovery					
		TEQ_{PCB}		$TEQ_{D/F}$		Total TEQ	
1	Cambridge 5183	81		111		94	
2	LCG CRM-529	100		106		106	
3	Wellington WMS–01	93		106		105	
4	Cambridge 5184	120		106		118	
5	NIST 1944	102		91		93	
6	ERA TCDD 10	NA		79		79	
7	ERA TCDD 30	NA		77		77	
8	ERA PAH	NA		NA		NA	
9	ERA PCB 100	96		NA		95	
10	ERA PCB 10000	95		NA		95	
11	ERA Aroclor	82		324		83	
12	ERA Blank	NA		NA		NA	
All PE Samples		NUMBER	8	NUMBER	8	NUMBER	10
		MIN	81	MIN	77	MIN	77
		MAX	120	MAX	324	MAX	118
		MEDIAN	96	MEDIAN	106	MEDIAN	94
		MEAN	96	MEAN	125	MEAN	94

NA = not applicable.

whereas the reference method $TEQ_{D/F}$ values were based on contributions from all 2,3,7,8-substituted D/F analytes. The R values presented in Table 6-1 indicate that the reference method reported data that were on average between 94 and 125% of the certified values of the PE samples.

The effect of known interferences on reference method TEQs is listed in Table 6-2. D/F and PCB TEQs were not affected by PAH as evidenced through the analysis of ERA PAH reference material. D/F and PCB TEQs were not affected by each other as evidenced by spikes that contained only one set of analytes having negligible influence on the TEQ of the other analyte set.

6.4 Evaluation of Primary Objective P2: Precision

The 209 samples included in the demonstration consisted of replicates of 49 discrete samples. There were four replicates of each sample except for PE sample Cambridge 5183 (7 replicates), ERA blank reference material (8 replicates), Wellington WMS-01 standard reference material (7 replicates), and 0.5 pg/mL 2,3,7,8-TCDD extract (7 replicates). Reference method data were obtained for all 209 samples; however, $TEQ_{D/F}$ and total TEQ data for samples Ref 197 (ERA PCB 100) and Ref 202 (LCG CRM-529) were omitted as outliers as it appeared that these two samples were switched

Table 6-2. Evaluation of Interferences

PE Material with Known Interference	Mean TEQ (pg/g)
ERA PAH	0.195 (D/F + PCB)
ERA PCB 100	0.073 (D/F)
ERA PCB 10,000	0.220 (D/F)
ERA TCDD 10	0.025 (PCB)
ERA TCDD 30	0.036 (PCB)

during preparation after observing results of the replicates and evaluating the congener profiles of these two samples.

A summary of the reference method replicate RSD values is presented in Tables 6-3a and 6-3b. The RSD values are presented for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ in Table 6-3a, and a summary by sample type is presented in Table 6-3b, along with the minimum R value, the maximum R value, and the mean R value for each set of TEQ results and sample types. In terms of sample type, the reference method had the most precise data for the environmental sample $TEQ_{D/F}$ results, with a mean RSD value of 12%. This was followed closely by environmental sample TEQ_{PCB} and total TEQ results, which both had mean RSDs of 13%. In terms of TEQ values, the reference method had the most precise data for the total TEQ values, with a mean overall RSD of 13%. Overall RSD values ranged from 1% to 119%. Precision was significantly worse for certified blanks and blank samples (e.g., samples that contained spikes of only one analyte set and were blank for the other analytes) as might be expected due to the very low levels detected in these samples.

6.5 Comparability to Characterization Data

To assess comparability, reference laboratory D/F data for environmental samples were plotted against the characterization data that was generated by Battelle prior to the demonstration. Characterization data were obtained as part of the process to verify homogenization of candidate soil and sediment samples as described in Chapter 5 and reported in Table 4-5. It should be noted that second column confirmations of 2,3,7,8-TCDF results were not performed during characterization analyses; therefore, characterization TEQs are biased high for samples where a large concentrations of non2,3,7,8-TCDF coeluted with 2,3,7,8-TCDF on the DB-5 column. Characterization samples also were not rigorously evaluated to ensure that high concentration extracts were diluted sufficiently so that all peak areas were less than the peak areas of the highest calibration standard. In spite of these differences between reference and characterization analyses, the results had fairly high correlation ($R^2 = 0.9899$) as demonstrated in Figure 6-1.

Table 6-3a. Objective P2 Precision - Relative Standard Deviation

Sample Type	Sample ID	RSD for TEQ_{PCB} (%)	RSD for $TEQ_{D/F}$ (%)	RSD for Total TEQ (%)
Environmental	Brunswick #1	8	6	6
	Brunswick #2	3	16	16
	Brunswick #3	5	8	8
	Midland #1	4	9	9
	Midland #2	10	6	6
	Midland #3	4	6	6
	Midland #4	77	9	10
	NC PCB Site #1	21	15	20
	NC PCB Site #2	21	2	21
	NC PCB Site #3	25	12	24
	Newark Bay #1	7	28	25
	Newark Bay #2	2	22	20
	Newark Bay #3	6	6	6
	Newark Bay #4	1	12	11
	Raritan Bay #1	6	5	4
	Raritan Bay #2	3	2	1
	Raritan Bay #3	3	5	4
	Saginaw River #1	8	25	23
	Saginaw River #2	7	19	18
	Saginaw River #3	60	19	19
	Solutia #1	36	13	13
	Solutia #2	4	7	7
	Solutia #3	11	5	5
	Titta. River Soil #1	7	6	5
	Titta. River Soil #2	9	10	10

Sample Type	Sample ID	RSD for TEQ_{PCB} (%)	RSD for $TEQ_{D/F}$ (%)	RSD for Total TEQ (%)
	Titta. River Soil #3	12	26	26
	Titta. River Sed #1	19	27	26
	Titta. River Sed #2	14	37	37
	Titta. River Sed #3	13	9	8
	Winona Post #1	13	2	2
	Winona Post #2	4	9	9
	Winona Post #3	9	4	4
Extract	Envir Extract #1	71	50	50
	Envir Extract #2	83	2	2
	Spike #1	119	6	9
	Spike #2	1	5	3
	Spike #3	4	13	4
PE	Cambridge 5183	7	19	9
	Cambridge 5184	3	4	2
	ERA Aroclor	44	6	43
	ERA Blank	62	65	61
	ERA PAH	83	27	30
	ERA PCB 100	4	65[a]	3
	ERA PCB 10000	7	91	7
	ERA TCDD 10	60	5	5
	ERA TCDD 30	39	6	6
	LCG CRM-529	14	2[a]	1
	NIST 1944	4	9	7
	Wellington WMS-01	5	3	3

[a] Does not include sample excluded due to sample preparation error.

Table 6-3b. Objective P2 Precision - Relative Standard Deviation (By Sample Type)

	RSD for TEQ_{PCB} (%)					RSD for $TEQ_{D/F}$ (%)					RSD for Total TEQ (%)				
Sample Type	N	MIN	MAX	MED	MEAN	N	MIN	MAX	MED	MEAN	N	MIN	MAX	MED	MEAN
Environmental	32	1	77	8	13	32	2	37	9	12	32	1	37	10	13
Extract	5	1	119	71	56	5	2	50	6	15	5	2	50	4	14
PE	12	3	83	11	28	12	2	91	7	25	12	1	61	7	15
Overall	49	1	119	8	21	49	2	91	9	16	49	1	61	8	13

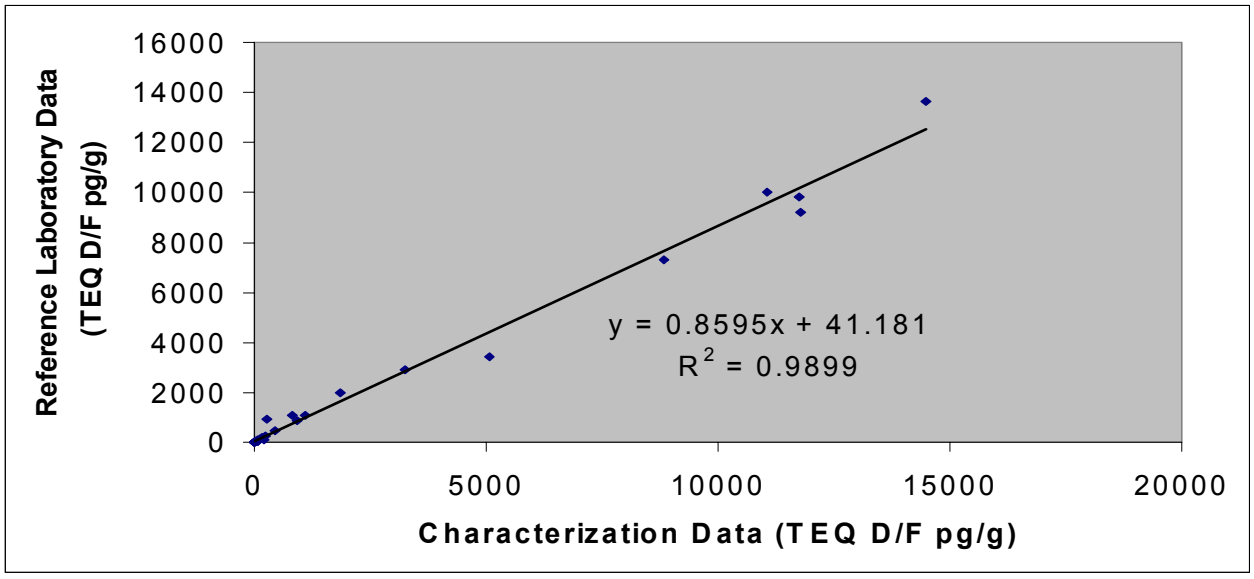

Figure 6-1. Comparison of reference laboratory and characterization D/F data for environmental samples.

6.6 Performance Summary

This section provides a performance summary of the reference method by summarizing the evaluation of the applicable primary objectives of this demonstration (accuracy, precision, and cost) in Table 6-4. A total of 209 samples was analyzed for seventeen 2,3,7,8-substituted D/F and 12 PCBs over an eight-month time frame (April 27 to December 20, 2004). Valid results were obtained for all 209 PCB analyses, while 207 valid results were obtained for D/F. The D/F and total TEQ results for samples Ref 197 (ERA PCB 100) and Ref 202 (LCG CRM-529) were omitted as outliers because it appeared that these two samples were switched during preparation after observing results of the replicates and evaluating the congener profiles of these two samples. The demonstration sample set provided particular challenges to the reference laboratory in that there was a considerable range of sample concentrations for D/F and PCB. This caused some difficulty in striving for low MDLs in the presence of high-level samples. The range of concentrations in the demonstration sample set also required the laboratory to modify standard procedures, which contributed to increased cost and turnaround time delay. For example, an automated sample cleanup system could not be used due to carryover from high-level samples; instead, more labor-intensive manual cleanup procedures were used; glassware required extra cleaning and proofing before being reused; cleanup columns sometimes became overloaded from interferences and high-level samples, causing low recoveries so that samples had to be re-extracted or cleanup fractions had to be analyzed for the lost analytes; and method blanks often showed trace levels of contamination, triggering the repeat of low-level samples.

Because the reference method was not to be altered significantly for this demonstration, the reference laboratory was limited in its ability to adapt the trace-level analysis to higher level samples. In spite of these challenges, the quality of the data generated met the project goals. The main effect of the difficulties associated with these samples was on schedule and cost.

Table 6-4. Reference Method Performance Summary - Primary Objectives

Objective	Performance			
	Statistic	**TEQ_{PCB}**	**$TEQ_{D/F}$**	**Total TEQ**
P1: Accuracy	Number of data points	8	8	10
	Median Recovery (%)	96	106	94
	Mean Recovery (%)	96	125	94
P2: Precision	Number of data points	49	49	49
	Median RSD (%)	8	9	8
	Mean RSD (%)	21	16	13
P7: Cost	209 samples were analyzed for 17 D/F and 12 PCBs. Total cost was $398,029. D/F cost was $213,580 ($1,022 per sample) and PCB cost was $184,449 ($883 per sample).			

Chapter 7
Performance of Hybrizyme Corporation AhRC PCR™ Kit

7.1 Evaluation of AhRC PCR™ Kit Performance

The Hybrizyme Corporation AhRC PCR™ Kit is a technology that reports the concentration of AhR binding compounds in units reported as AhRBU. At the time of the 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 is a highly portable screening technology that can help determine areas of greatest concern for cleanup at a site and can help minimize the number of more expensive analyses needed for specific analytes. It has been suggested that correlation between the Hybrizyme AhRBU results and HRMS TEQ results could be established by first characterizing a site and calibrating the Hybrizyme results to HRMS results. This approach was not evaluated during this demonstration. Since the technology measures an actual biological response, it is possible that the technology may give a better representation of the true toxicity of a sample or site from a risk assessment standpoint.

The following sections describe the performance of the AhRC PCR™ Kit , according to the primary objectives for this demonstration. The developer and reference laboratory data are presented in Appendix D. The statistical methods used to evaluate the primary objectives are described in Section 4.7. Detailed data evaluation records can be found in the DER.

7.1.1 Evaluation of Primary Objective P1: Accuracy

Based on the current state of development of the Hybrizyme technology, the technology's results were compared to the HRMS results in terms of ranking sample concentrations from low to high, rather than comparing to HRMS TEQ in a quantitative fashion. The determination of accuracy was based on ranking of the PE samples results from low to high concentration and comparing it to the rank order reported by Hybrizyme based on AhRBU. For the PE samples, the D/F, PCB, and PAH concentrations were summed from the concentrations reported on the certificate of analysis. Note that the PAH data for the PE samples was rather limited, and it is possible that there were additional PAH compounds present in the PE samples that were not included in the certified data.

Table 7-1 compares the Hybrizyme AhRC PCR™ Kit ranking of the PE samples in average AhRBU concentration from low to high to the low to high ranking by total concentration (D/F+PCB+PAH) determined from the certified data. The Wellington and LCG CRM 529 PE samples were excluded from this evaluation because PAH data were not available on the certificates of analysis. The Hybrizyme ranking was identical to the certified concentrations for one of the 10 PE samples (Cambridge 5184). Of the four highest sample concentrations according to the certified values (NIST 1944, ERA Aroclor, Cambridge 5184, and ERA PAH), Hybrizyme's data ranked three of the samples (NIST 1944, Cambridge 5184, and ERA PAH) as having the highest concentrations. The ERA Aroclor sample, which was spiked with Aroclor 1254, was ranked as the next lowest concentration by Hybrizyme and the next to highest concentration by the certified data.

Table 7-1. Objective P1 Accuracy - Ranking of PE Samples According to the Concentration of AhR Binding Compound

PE Sample ID	PE Sample Description	Low to High Ranking	
		Hybrizyme AhRBU Results	Certified Results (D/F+PCB+PAH)
1	Cambridge 5183	5	6
2	LCG CRM-529	not included	
3	Wellington WMS-01	not included	
4	Cambridge 5184	9	9
5	NIST 1944	10	7
6	ERA TCDD 10	4	3
7	ERA TCDD 30	7	4
8	ERA PAH	8	10
9	ERA PCB 100	3	2
10	ERA PCB 10000	1	5
11	ERA Aroclor	2	8
12	ERA Blank	6	1

7.1.2 Evaluation of Primary Objective P2: Precision

A summary of the Hybrizyme AhRC PCR™ RSD values is presented in Tables 7-2a and 7-2b. A minimum of three and maximum of four replicate results were used to calculate RSD. The RSD values are presented for AhRBU in Table 7-2a, and a summary by sample type is presented in Table 7-2b, with the minimum RSD value, the maximum RSD value, the median RSD value, and the mean RSD value presented. Low RSD values (< 20 %) indicate high precision. The Hybrizyme AhRC PCR™ Kit values had the most precise data for the extract analysis, with a mean RSD of 14%. The overall mean RSD was 25%, with values ranging from 2% to 111%. The median overall RSD value was 19% and in good agreement with the mean, indicating that the precision values were symmetrically distributed.

7.1.3 Evaluation of Primary Objective P3: Comparability

Given the state of development of the Hybrizyme technology, the technology's results were compared to the HRMS results in terms of ranking sample concentrations of AhR-binding compounds from low to high, rather than comparing to HRMS TEQ in a quantitative fashion. PAH data from the characterization were added to the HRMS D/F and PCB data generated during the demonstration because PAHs are AhR binding compounds and are included in the Hybrizyme results. It should be noted that the suite of PAHs which were quantified in the samples were a selected target list for this demonstration and likely do not include all of the PAHs which are responsive to this kit.

Table 7-3 compares the Hybrizyme low to high ranking of the environmental samples to the reference laboratory low to high ranking. The environmental samples were the only samples included in this evaluation because the PAH data were consistently generated for each of the environmental sites. The environmental samples were ordered in terms of the sample numbers provided in Table 4-5. For this evaluation, the environmental samples were ranked with the samples from each site only, rather than ranking all of the environmental sites in one ordering, because the Hybrizyme technology is intended to rank samples within a particular site.

This evaluation demonstrated that the Hybrizyme technology was able to rank the samples from low to high concentration within an environmental site fairly consistently with the reference laboratory based on average total concentration data. Table 7-4 summarizes the environmental sample ranking comparisons. For seven of the 10 environmental sites (70%), Hybrizyme's ranking was identical to the reference laboratory's ranking. If samples that are close in average HRMS concentration and are indistinguishable when uncertainties are considered, the Hybrizyme rankings

Table 7-2a. Objective P2 Precision - Relative Standard Deviation

Sample Type	Sample ID	RSD (%)[a]
Environmental	Brunswick #1	15
	Brunswick #2	16
	Brunswick #3	13
	Midland #1	16
	Midland #2	2
	Midland #3	14
	Midland #4	18
	NC PCB Site #1	20
	NC PCB Site #2	8
	NC PCB Site #3	17
	Newark Bay #1	30
	Newark Bay #2	28
	Newark Bay #3	8
	Newark Bay #4	14
	Raritan Bay #1	4
	Raritan Bay #2	29
	Raritan Bay #3	24
	Saginaw River #1	25
	Saginaw River #2	8
	Saginaw River #3	6
	Solutia #1	20
	Solutia #2	31
	Solutia #3	45
	Titta. River Soil #1	16
	Titta. River Soil #2	19
	Titta. River Soil #3	15
	Titta. River Sed #1	20
	Titta. River Sed #2	33
	Titta. River Sed #3	29
	Winona Post #1	19
	Winona Post #2	12
	Winona Post #3	21
Extracts	Envir. Extract #1	16
	Envir. Extract #2	11
	Spike #1	NA
	Spike #2	13
	Spike #3	16
PE	Cambridge 5183	35
	Cambridge 5184	10
	ERA Aroclor	30
	ERA Blank	83
	ERA PAH	25
	ERA PCB 100	46
	ERA PCB 10000	37
	ERA TCDD 10	91
	ERA TCDD 30	111
	LCG CRM-529	12
	NIST 1944	26
	Wellington WMS-01	19

NA = not applicable; all values reported as nondetects.

[a] Three or four replicate results were used to calculate the RSD values.

Table 7-2b. Objective P2 Precision - Relative Standard Deviation (By Sample Type)

Statistic	RSD (%)				
	N	MIN	MAX	MEDIAN	MEAN
Environmental	32	2	45	17	19
Extract	4	11	16	14	14
PE	12	10	111	33	44
Overall	48	2	111	19	25

Table 7-3. Objective P3 - Comparability by Ranking Environmental Samples from Low to High Concentration Within an Environmental Site

Environmental Site	Hybrizyme Ranking by Average AhRBU[a]	Reference Laboratory HRMS Ranking by Average Total Concentration (ng/g) [a]	Did Ranking Agree?
Brunswick, GA	2 1 3	2 1 3	Yes
Midland, MI	4 1 3 2	4 1 3 2	Yes
Warren County, NC	1 2 3	1 2 3	Yes
Newark Bay, NJ	1 4 2 3	1 2 4 3	No
Raritan Bay, NJ	1 2 3	1 3 2	No
Saginaw River, MI	3 2 1	3 2 1	Yes
Solutia, WV	1 3 2	1 3 2	Yes
Tittabawassee River, MI (soil)	3 2 1	3 1 2	No
Tittabawassee River, MI (sediment)	3 1 2	3 1 2	Yes
Winona Post, MO	1 2 3	1 2 3	Yes

[a] Ranking of sample numbers within a site from low to high. See Table 4-5 for sample numbers.

Table 7-4. Objective P3 - Summary of Environmental Sample Ranking Comparisons

Evaluation Parameter	Performance	
	Number	Percentage
Hybrizyme ranking agreed with reference laboratory within the environmental site	7 out of 10 environmental sites	70
Hybrizyme ranking when uncertainty around reference laboratory values was considered	9 out of 10 environmental sites	90
Hybrizyme individual rankings which agreed with the reference laboratory	26 out of 32 individual rankings	81

agree with the reference laboratory's ranking for nine of 10 sites (90%). On an individual ranking basis, the Hybrizyme and reference laboratory rankings agreed 81% of the time (26 of 32 rankings).

7.1.4 Evaluation of Primary Objective P4: Estimated Method Detection Limit

The EMDL of the Hybrizyme AhRC PCR™ Kit was determined using Cambridge 5183 and Wellington WMS-01. Extract Spike #1 was prepared with 0.5 pg/mL of 2,3,7,8-TCDD only, which was reported as not detected by Hybrizyme for all seven replicates. As shown in Table 7-5, the Hybrizyme AhRC PCR™ Kit EMDL was determined to be 71 AhRBU for the Cambridge 5183. The calculated EMDL for Wellington WMS-01 sample (576 AhRBU) was considerably higher, due to the higher concentration of analytes in the sample, so it was not included in the evaluation of EMDL. Hybrizyme noted that their estimated detection limits were on the order of 100 AhRBU, so the calculated EMDL appears to be a reasonable estimation.

Table 7-5. Objective P4 - Estimated Method Detection Limit

Statistic	Cambridge 5183
Degrees of Freedom	6
Standard Deviation (AhRBU)	23
EMDL (AhRBU)	71

7.1.5 Evaluation of Primary Objective P5: False Positive/False Negative Results

This parameter was not evaluated. See Section 4.7.5 for further explanation.

7.1.6 Evaluation of Primary Objective P6: Matrix Effects

Six types of potential matrix effects were investigated: (1) measurement location (field vs. laboratory), (2) matrix type (soil vs. sediment vs. extract), (3) sample type (PE vs. environmental vs. extract), (4) PAH concentration, (5) environmental sites, and (6) known interferences. A summary of the matrix effects is provided in the bullets below, followed by a detailed discussion:

- Measurement location: not evaluated (all results generated in the laboratory)
- Matrix type: none (according to statistical evaluation of mean RSD values)
- Sample type: Significant effect
- PAH concentration: none
- Environmental site: not evaluated since AhRBU results weren't directly comparable to TEQ
- Known interferences: not evaluated since assay reacts to AhR binding compounds

In Table 7-6, precision summary values are presented by matrix type. A one-way ANOVA model was used to test the effect of soil vs. sediment vs. extract on RSD. These tests showed no significant effect on mean RSD although the range of RSD values was much greater for soil than for sediment and extracts. In Table 7-7, precision summary values are presented by PAH concentrations for environmental samples only. A one-way ANOVA model was used to test the effect of PAH

Table 7-6. Objective P6 - Matrix Effects Using RSD as a Description of Precision by Matrix Type

Matrix Type	RSD for AhRBU (%)				
	N	MIN	MAX	MEAN	MEDIAN
Soil	26	2	111	30	20
Sediment	18	4	33	19	19
Extract	4	11	16	14	14
Overall	48	2	111	25	19

Table 7-7. Objective P6 - Matrix Effects Using RSD as a Description of Precision by PAH Concentration Levels (Environmental Samples Only)

PAH Concentration Level (ng/g)	RSD for AhRBU (%)				
	N	MIN	MAX	MED	MEAN
> 100,000	3	8	17	13	13
10,000-100,000	4	12	21	17	17
1,000-10,000	16	2	45	20	18
< 1,000	9	6	33	19	19
Overall (Environmental Samples Only)	32	2	45	19	17

concentration on RSD. These tests showed no effect. The summary of RSD values segregated by sample type is presented in Table 7-2b. A one-way ANOVA model was used to test the effect of sample type (PE vs. environmental vs. extract) on RSD. These tests showed a significant effect on RSD. This effect is visually noticeable, as the environmental (mean 19%) and extract (mean 14%) RSD values were half as much as the PE RSD (mean 44%). An evaluation of effect on performance based on measurement location was not performed because all sample results used in this evaluation were performed in Hybrizyme's laboratory. The comparability to the HRMS values for the assessment of environmental sites was not performed because the Hybrizyme results are not quantitatively comparable to the HRMS TEQ data.

7.1.7 Evaluation of Primary Objective P7: Technology Costs

Evaluation of this objective is fully described in Chapter 8, Economic Analysis.

7.2 Observer Report: Evaluation of Secondary Objectives

The toxicity of a compound can be indicated by how strongly it binds to the AhR. Therefore, in a sample extract, how well the extract activates the AhR gives an indication of the toxicity of compounds in the extract. Dioxins and furans are known to activate the AhR. The AhRC PCR™ test kit detects compounds that activate the AhR in a sample extract in the following manner: toxic components in a sample extract activate the AhR, the activated AhR binds to a DNA-probe, and the AhR bound DNA probe is amplified and measured by PCR. The AhR specificity to dioxin-like compounds is similar to the TEQ approach for estimating toxicity of a mixture of dioxin-like compounds, i.e., 2,3,7,8-TCDD is strongly bound to the AhR, OCDD is significantly less strongly bound. Therefore, this assay can assess the toxicity of a sample in a manner similar to the TEQ approach of traditional dioxin and furan analysis. However, it should be noted that the AhR response to various dioxin-like compounds, while similar, is not identical to the TEF used to determine TEQ, and AhR activity can be induced by compounds other than the dioxins and furans. While the AhR activity can be calibrated to TCDD to generate a TEQ value, a highly accurate TEQ value is not the goal of this technology due to the differences in AhR activity vs. TEFs and the responses induced by other toxic compounds. At the time of the 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 a highly accurate TEQ. The developer's goal is a highly portable screening technology that can help to determine areas of greatest concern for cleanup at a site and can help to minimize the number of more expensive analyses needed for specific analytes.

The following activities were observed during the demonstration: sample weighing and extraction, addition of extract to the activation solution, plate washing, addition of primer/probe solution, and instrument read-out of results. The sample extraction procedure was not included in the demonstration plan; otherwise, instructions in the demonstration plan were generally followed. The developer intends to add more specific sample extraction instructions to the kit instructions. Note that the extraction procedure observed during the demonstration was not the procedure used to generate the data submitted for this evaluation. Hybrizyme refined its extraction procedure after the field demonstration and reanalyzed all samples with its new procedure in its laboratory. The refined procedure is described in Section 2.3. Hybrizyme had no knowledge of reference sample results at this point in the process, but they requested to reanalyze the samples because they were not satisfied with their data based on their quality control procedures. Hybrizyme's request was granted to ensure that the most representative data would be evaluated during this demonstration. The refined analytical procedure and field demonstration procedure were very similar, differing only in the type and quantity of reagents and chemicals that were used. The type of activities (extraction by sonication, clean-up, PCR assay) were the same in both procedures.

7.2.1 Evaluation of Secondary Objective S1: Skill Level of Operator

During the demonstration, sample weighing and extraction activities were carried out by Dr. Terry Nestrick, who has a Ph.D. in chemistry and 26 years of experience at Dow Chemical Co. in the analytical laboratories located in Midland, Michigan. His primary experience was trace and ultra-trace determination of organic species in environmental, product, and process matrices specializing in dioxins/furans. Dr. Randy Allen processed the extracts with the AhRC PCR™ kit and performed the data collection and reduction. Dr. Allen has a Ph.D. in molecular biology/biochemistry and 17 years of experience in developing environmental bioassays.

For successful operation of this technology, Hybrizyme recommends that users have a minimum of a high school degree, have good work skills, and be trainable. Based on observation of the technology, the recommended level of experience and education seemed reasonable. Decent laboratory skills, respect for safety, and having reasonable attention to detail would also be useful attributes for successful technology operation.

Information needs to be added to instructions on how to extract samples. Otherwise, instructions for kit use were reasonably clear. A day of training would help to ensure that someone who already possessed good laboratory skills would know how to properly use the kit. For the most part, this technology seemed very forgiving. Temperatures were not critical as long as there were no extremes, samples needed to be weighed only to 0.1 g (as a screening tool, it is more important for the weights to be consistent between samples than to be highly accurate), and times for shaking and incubation were not critical. There are several places where the technology can be stopped and stored (e.g., after sonication, when exchanged to hexane, when loaded onto the strips and ready to analyze, etc.). The activation solution and the primer/probe require freezing and the capture reagent and PCR wash concentrate require refrigeration; otherwise, the reagents can be kept at room temperature. Only 5 µL of the 200-µL sample extract is used for analysis so there is plenty of extract available should repeat analyses be warranted. The assay is relatively quick (~5 hours from sample weigh-out to first read of results) and so repeat analyses can be performed without significant time delay. In addition to purchasing the kits, samples can be sent to the developer for processing in the developer's laboratory. The developer will also consider coming to the client's laboratory or the field. There are no particular health or safety concerns with this technology beyond what is common for laboratory sample processing.

7.2.2 Evaluation of Secondary Objective S2: Health and Safety Aspects

A complete inventory of the waste generated was performed after processing 110 samples by Hybrizyme.

None of the containers were verified as full. Note that this summary does not include the samples which were analyzed in the Hybrizyme laboratories.

(1) One 5-gallon container marked "low concentration" with 80 polypropylene weigh pans; 80 plastic weigh spoons; 160 polypropylene vial caps; four 10-mL glass pipettes; and 90 glass vials (0.5 mL).

(2) One 5-gallon container marked "high concentration" with 31 polypropylene weigh pans; 31 plastic weigh spoons; 62 polypropylene vial caps; and 31 glass vials (0.5 mL).

(3) One 5-gallon container with 2 L buffer salts.

(4) Three 5-gallon containers containing among them: 168 glass vials (42 mL) each filled with 2 g of soil plus 10 mL of 50% HCl; 168 used 10-mL glass pipettes; 120 vials with 10 mL of 50% HCl and 800 µL of hexane; 168 pipette tips; and 96 glass test tubes with 150 µL MeOH.

The reader should be advised that, although no difficulties were encountered during this project, difficulties could arise with disposal of dioxin-contaminated waste.

7.2.3 Evaluation of Secondary Objective S3: Portability

The developer intends for this technology to become a highly portable field kit. However, as used in the demonstration, this technology required at a minimum a trailer to protect from the environment and to house equipment such as a centrifuge, sonicator, and the PCR thermocycler analyzer. Electricity was also a necessity. The developer ultimately intends for this technology to be usable in a minimally controlled environment and is even considering having an option of leasing out miniaturized equipment that will make the technology more field friendly. As tested in the demonstration, this technology required approximately 2 hours of setup to get the trailer ready to begin analyses. Operation in a fume hood is not a critical requirement for this technology because there are no lengthy solvent extractions or cleanup steps with particular ventilation requirements.

7.2.4 Evaluation of Secondary Objective S4: Throughput

Sample throughput during the demonstration was approximately 28 samples per day. A total of 110 samples was processed in the field during four days. Out of the four days, several hours of the first day were not active working hours due to start-up meetings for the program, and several hours were lost on the third day for participation in Visitor's Day. Two people processed the samples. One person focused on the sample weighing and extraction, and the second person focused on analyzing the extracts.

According to the developer, if operating in a production mode, 40 to 60 samples could be processed in one day with two people operating the technology. Forty samples would appear to be the more reasonable/ comfortable goal. One person would focus on the sample weighing and extraction and the second person would focus on the analysis of the extracts. The developer felt this throughput goal would be about the same regardless of whether the operators were experienced or novice kit users. If looking for a rapid turnaround on a specific set of samples (i.e., a small number, fewer than 10 samples), the quickest results could be delivered on such a set in about 5 hours. It takes roughly 2 to 3 hours to get through the sample weighing and extraction and 2 to 3 hours to get the extract prepared and analyzed. Based on the observations of this technology in the field, two trained, but not necessarily highly experienced people could reasonably process 30 to 40 samples per day. This pace would likely be sustainable for several days. Sample processing would proceed as quickly in the field as in the lab; however, analysis may be somewhat more rapid in a laboratory setting due to more comfortable conditions. Laboratory analysis instrumentation is set up to read an entire 96-well plate at once, whereas a field portable unit is only able to read 16 wells at a time. Each kit contains materials to process one 96-well plate. The number of samples processed with each plate will vary based on the number of wells used for calibration and quality control standards and whether the samples are analyzed in duplicate.

7.2.5 Miscellaneous Observer Notes

Hybrizyme is a U.S.-based company. Its test kit comes with a set of instructions with reasonable details. At the

time of the demonstration, the instructions were going to be revised with additional information on sample extraction procedures implemented as a result of this demonstration. Hybrizyme provides phone support to its customers during business hours. Training by Hybrizyme staff is available and quotes for this service can be provided. According to Hybrizyme, effective training could be accomplished in one day. Hybrizyme did not have training videos at the time of the demonstration; however, this option may be available in the future.

This kit comes with supplies to process one 96-well plate. At this time, kits are available off-the-shelf for orders of less than 10 kits. Orders of more than 10 kits are filled within a two-week period. The kit includes activation solution, capture strips, assay buffer, capture reagent, primer/probe, PCR wash concentrate, glass vials (rack with 96 vials), and a set of instructions. The user needs to supply a mini-balance and supplies for weighing out samples (such as weighing boats and spatulas or disposable spoons), vials to extract the sample (such as 4-dram, polytetrafluoroethylene-lined vials), methanol, hydrochloric acid, distilled water, a multichannel pipettor, a sample shaker, a centrifuge, an incubator, a dioxin standard in methanol (commercially available from vendors such as CIL), and a thermocycler. The developer notes that if a user did not have a thermocycler, sample preparation could be stopped at the stage of drying the strips immediately prior to analysis and the strips sent to Hybrizyme for the final instrument read-out. Also, leasing field portable analysis equipment may become an option from this developer in the future. Most of the equipment and materials necessary to use this technology are common laboratory items. The developer notes that the PCR analysis is clinical in origin making replacement parts easily accessible since most hospitals use this type of equipment and could be contacted if there were emergency equipment needs in the field. This technology has been developed for use with both laboratory-based and field-based PCR systems.

At the time of the demonstration, the developer was still refining recommendations for quality control samples to be processed with a sample batch. For the demonstration, one blank and one sample extracted in duplicate were prepared with each set of 20 test samples. Future recommendations may also include a lab control spike with each sample batch. For this technology, the need for confirmation or verification of results by conventional HRMS methods depends on project-specific goals and action levels and may need to be applied on a project-specific basis.

Chapter 8
Economic Analysis

During the demonstration, the AhRC PCR™ kit and the reference laboratory analytical methods were each used to perform more than 200 analyses of dioxin-contaminated samples, including samples with a variety of distinguishing characteristics such as high levels of polychlorinated biphenyls and PAHs. The purpose of the economic analysis was to estimate the total cost of generating results by using the AhRC PCR™ kit and then comparing this cost to the reference method. This cost estimate also is provided so that potential users can understand the costs involved with using this technology.

This chapter provides information on the issues and assumptions involved in the economic analysis (Section 8.1), discusses the costs associated with using the AhRC PCR™ kit (Section 8.2), discusses the costs associated with using the reference method (Section 8.3), and presents a comparison of the economic analysis results for the AhRC PCR™ kit and the reference laboratory (Section 8.4).

8.1 Issues and Assumptions

Several factors affect sample measurement costs. Wherever possible in this chapter, these factors are identified in such a way that decision-makers can independently complete a project-specific economic analysis. The following five cost categories were included in the economic analysis for the demonstration: capital equipment, supplies, support equipment, labor, and investigation-derived waste (IDW) disposal. The issues and assumptions associated with these categories and the costs not included in the analysis are briefly discussed below. The issues and assumptions discussed below only apply to the AhRC PCR™ kit unless otherwise stated.

8.1.1 Capital Equipment Cost

The capital equipment cost was the cost associated with the purchase of the AhRC PCR™ kit. Components of the AhRC PCR™ kit are presented in detail in Chapters 2 and 7. Hybrizyme offers a lease option for the PCR thermocycler, but the thermocycler can also be purchased. The purchase price information was obtained from a standard price list provided by Hybrizyme.

8.1.2 Cost of Supplies

The cost of supplies was estimated based on the supplies required to analyze all demonstration samples using the AhRC PCR™ kit that were not included in the capital equipment cost category. Examples of such supplies include filters, cleanup columns, gas cylinders, solvents, and distilled water. The supplies that Hybrizyme used during the demonstration fall into two general categories: consumable (or expendable) and reusable. Examples of expendable supplies utilized by Hybrizyme during the demonstration include methanol, pipette tips, and extraction vials. Examples of reusable supplies include a PCR thermocycler, microplate shaker and washer, ultrasonic bath, centrifuge, and pipettors. It should be noted that this type of equipment may or may not be already owned by a potential AhRC PCR™ kit user; however, for this economic analysis, an assumption was made that the user does not possess these items.

The purchase price of these supplies was either obtained from a standard price list provided by Hybrizyme, or it was estimated based on price quotes from independent sources.

8.1.3 Support Equipment Cost

This section details the equipment used at the demonstration such as the construction trailer, fume

hood, and laptop computer required by the technology. Costs for these items will be reported per actual costs for the demonstration.

8.1.4 Labor Cost

The labor cost was estimated based on the time required for work space setup, sample preparation, sample analysis, and reporting. For the demonstration, developers reported results by submitting a COC/result form. The measurement of the time required for Hybrizyme to complete all analyses (74 labor-hours) was estimated by the sign-in log sheets that recorded the time the Hybrizyme operators were on-site. Time was removed for site-specific training activities and Visitor's Day. Time estimates were rounded to the nearest hour.

During the demonstration, the skill level required for the operators to complete analyses and report results was evaluated. As stated in Section 7.2.1, based on the field observations, the recommended skill level for operation of this technology includes a minimum of a high school degree, have good work skills, and be trainable. Decent laboratory skills, respect for safety, and having reasonable attention to detail would also be useful attributes for successful technology operation. This information was corroborated by Hybrizyme.

Education levels of the actual field operators included Ph.D. degrees for both operators. For the economic analysis, costs were estimated using both actual and projected necessary skill levels for operators.

8.1.5 Investigation-Derived Waste Disposal Cost

During the demonstration, Hybrizyme was provided with 5-gallon containers for collecting wastes generated during the demonstration. Sample by-products such as used samples, aqueous and solvent-based effluents generated from analytical processes, and used glassware were disposed of in the containers. The total cost to dispose of these wastes generated during the demonstration is included in the economic analysis. Items such as coffee cups, food waste, and office waste were disposed of in regular public refuse containers and were not included as IDW and, therefore, not discussed in this economic analysis.

8.1.6 Costs Not Included

Items whose costs were not included in the economic analysis are identified below along with a rationale for the exclusion of each.

Electricity. During the demonstration, some of the capital equipment was operated using AC power. The costs associated with providing the power supply were not included in the economic analysis as it is difficult to estimate the electricity used solely by the Hybrizyme technology. The total cost for electricity usage over the 10-day demonstration was $288. With seven mobile labs/trailers and miscellaneous equipment being operated continuously during the course of the demonstration, the cost of Hybrizyme electricity usage would be no more than $41. There was significantly more cost (approximately $13,000) to install an electrical board and additional power at the demonstration site, but this was a function of the demonstration site and not the responsibility of the individual developers, so this cost was not included in the economic analysis.

Oversight of Demonstration Activities. A typical user of the AhRC PCR™ kit would not be required to pay for customer oversight of sample analysis. The EPA, the MDEQ, and Battelle representatives were present during the field demonstration, but costs for oversight were not included in the economic analysis because these activities were project-specific. For these same reasons, cost for auditing activities (i.e., technical systems audits at the reference laboratory and during the field demonstration) were also not included.

Travel and Per Diem for Operators. Operators may be available locally. Because the availability of operators is primarily a function of the location of the project site, travel and per diem costs for operators were not included in the economic analysis.

Sample Collection and Management. Costs for sample collection and management activities, including sample homogenization and labeling, were not included in the economic analysis because these activities were project-specific and were not dependent upon the selected reference method or developer technology. Additionally, sample shipping, COC activities, preservation of

samples, and distribution of samples were specific requirements of this project that applied to all developer technologies and may vary from site to site. None of these costs were included in the economic analysis.

Shipping. Costs for (1) shipping equipment and supplies to the demonstration site and (2) sample coolers to the reference laboratory were not included in the economic analysis because such costs vary depending on the shipping distance and the service used (for example, a courier or overnight shipping versus economy shipping).

Items Costing Less Than $10. The cost of inexpensive items was not included in the economic analysis when the estimated cost was less than $10. Items where it is estimated that the cost was less than $10 included:

- Distilled water
- Personal protective equipment
- Waste containers
- Lab stools

8.2 AhRC PCR™ Kit Costs

This section presents information on the individual costs of capital equipment, supplies, support equipment, labor, and IDW disposal for the AhRC PCR™ kit as well as a summary of these costs. Additionally, Table 8-1 summarizes the AhRC PCR™ kit costs. As described in Section 4.6, Hybrizyme analyzed 110 samples during the field demonstration and then re-analyzed the 110 samples in its laboratory in addition to the 99 samples remaining in the total 209 demonstration samples. It is important to note that costs estimated in this section are based on actual costs to analyze the 110 samples during the field demonstration. Cost estimates for analyzing the entire set of 209 demonstration samples were then determined based on the field demonstration costs.

8.2.1 Capital Equipment Cost

The capital equipment cost was the cost associated with the purchase of the technology in order to perform sample preparation and analysis. Capital equipment includes the kit itself and purchase of a real-time PCR thermocycler ($40,000). For the purposes of this cost estimate, a lease price of $1,200 per week was used because it is common practice for Hybrizyme to lease the PCR thermocycler to customers. The lease of the thermocycler also includes lease of a laptop computer, microplate shaker, microplate washer, ultrasonic bath, centrifuge, all pipettors, and bottle top dispensers. The AhRC PCR™ kit can be purchased from Hybrizyme for $2,350. One kit contains enough supplies for 30-60 samples to be analyzed. During the field demonstration, Hybrizyme utilized three AhRC PCR™ kits for approximately four days to analyze 110 samples. It is estimated that four additional kits were used in Hybrizyme's laboratory to complete the sample analyses. It is possible that fewer kits could be used for 209 sample analyses since 30-60 samples can be analyzed with each kit, but this reflects the number of kits used during this demonstration.

8.2.2 Cost of Supplies

The supplies that Hybrizyme used during the demonstration fall into two general categories: expendable or reusable. Table 8-1 lists all expendable supplies that Hybrizyme used during the demonstration and the corresponding costs. The cost of each item was rounded to the nearest $1. Expendable supplies are ones that are consumed during the preparation or analysis. Reusable costs are items that must be used during the analysis but ones that can be repeatedly reused. Resuable supplies (microplate shaker, microplate washer, ultrasonic bath, centrifuge, pipettors, and bottle top dispensers) are included as part of the lease of the PCR thermocycler, so these items were not costed separately. If the resuable items were purchased, the approximate cost would be $13,600. The total cost of the supplies employed by Hybrizyme during the demonstration was $676. Supplies have to be purchased from a retail vendor of laboratory supplies. Reusable items listed in Table 8-1 can be substituted with other models that operate under the same specifications; thereby, modifying the cost of supplies to the potential kit user.

8.2.3 Support Equipment Cost

Hybrizyme analyzed demonstration samples in a 32-foot construction trailer equipped with a fume hood. The rental cost for the construction trailer for use during sample extraction and sample analysis was $1,919. The minimum rental rate for the construction trailer was one month. Hybrizyme only used the mobile laboratory for four days. Since weekly or daily rental rates for the mobile lab were not an option, the entire cost is reported. As determined by the observers, a trailer with a fume hood is necessary for operation of this technology in the field, although the developer hopes to make the technology infrastructure less dependent in the future. A

Table 8-1. Cost Summary

Item	Quantity Used During Field Demo		Unit Cost ($)	Itemized Cost[a] ($)	
				110 Samples	209 Samples
Capital Equipment					
Purchase of AhRC PCR™ Kit	2	kits	2,350	4,700	17,045[b]
PCR Real-time Thermocycler and Reusable Supplies (one-week lease)	1	unit	1,200	1,200	2,400
Supplies					
Expendable[c]					
Extraction Cocktail (Acetic acid/Hexane/Acetone)	2	unit	40	80	152
Methanol	2	unit	1	2	4
Pipette Tips (Filter 10)	2	unit	7	14	27
Pipette Tips (Filter 200)	2	unit	5	10	19
Pipette Tips (Non-Filter 200)	6	unit	3	18	34
Pipette Tips (Non-Filter 1000)	2	unit	3	6	11
Extraction Vials (I-Chem 100, 40 mL)	2	unit	110	220	418
Extract Vials (16x100 mm)	2	unit	3	6	11
Support Equipment					
Construction Trailer	1	unit	1,919	1,919	1,919
Fume Hood	1	unit	1,100	1,100	1,100
Labor					
Operator	74	labor hours	80[d]	5,920	11,248
IDW Disposal[e]	1	unit	334	334	635
Total Cost				**$15,529**	**$35,023**

[a] Itemized costs were rounded to the nearest $1.

[b] It is possible that fewer kits could be used for 209 sample analyses, since the number of samples that can be analyzed per kit is 30-60 samples, but this is the number used in this demonstration.

[c] Hybrizyme is preferred vendor of all expendable supplies except the extraction and extract vials.

[d] Labor rate for field technicians to operate technology rather than research scientists was $50.75 an hour, $3,756 for 110 samples and $7,135 for 209 samples.

[e] Further discussion about waste generated during demonstration can be found in Chapter 7.

laptop computer is necessary for efficiently operating this technology, but it was not costed separately because it is included in the thermocycler lease.

8.2.4 Labor Cost

As described in Section 8.1.4, 74 labor-hours were spent in the field to analyze 110 samples. An hourly rate of $32.10 was used for a research scientist performing. sample extractions and sample analysis, and a multiplication factor of 2.5 was applied to labor costs in order to account for overhead costs.[9] Based on this hourly rate and multiplication factor, a labor rate of $5,920 was determined for the analysis of the 110 samples during the field demonstration. It was estimated that the labor cost for the total 209 samples was $11,248. Based on observation, it is anticipated that lower-cost field technicians, with proper training and skill levels, could have analyzed the samples in a similar amount of time. As such, the labor rate for the analysis of 110 samples during the field demonstration could have been as low as $3,756 (hourly rate of $20.30 with 2.5 multiplication factor for 74 labor-hours), and $7,135 for all 209 demonstration samples.

8.2.5 Investigation-Derived Waste Disposal Cost

As discussed in Chapter 7, Hybrizyme was provided with 5-gallon containers for collecting wastes generated during the demonstration. Chapter 7 discusses the type and amount of waste generated by the technology during the field demonstration in more detail.

During the demonstration, Hybrizyme analyzed 110 samples. The total cost to dispose of the waste generated for these samples was $334. The cost to dispose of waste for all 209 samples is estimated at $635.

8.2.6 Summary of AhRC PCR™ Kit Costs

The total cost for performing the AhRBU analyses using the AhRC PCR™ kit was $35,023. The analyses were performed for 58 soil and sediment PE samples, 128 soil and sediment environmental samples, and 23 extracts. When Hybrizyme performed multiple dilutions or reanalyses for a sample, these were not included in the number of samples analyzed.

The total cost of $35,023 for analyzing the demonstration samples under the AhRC PCR™ kit included $19,445 for capital equipment; $676 for supplies; $3,019 for support equipment; $11,248 for labor; and $635 for IDW disposal. Of these five costs, the largest cost was for the capital equipment (56% of the total cost).

8.3 Reference Method Costs

This section presents the costs associated with the reference method used to analyze the 209 demonstration samples for dioxin and dioxin-like PCBs. Typical costs of these analyses can range from $800 to $1,100 per sample, depending on the method selected, the level of quality assurance/quality control incorporated into the analyses, and reporting requirements. The reference laboratory utilized EPA Method 1613B for D/F analysis and EPA Method 1668A for coplanar PCB analysis for all soil and sediment samples for comparison with the CALUX® system. The reference method costs were calculated using cost information from the reference laboratory invoices.

Table 8-2 summarizes the projected and actual reference method costs. At the start of the demonstration, the reference laboratory's projected cost per sample was $785 for D/F analysis and $885 for PCB analysis. This cost covered the preparation and analysis of the demonstration samples, required method QC samples, electronic data deliverable, and the data package for each. The actual cost for the 209 demonstration analyses was $213,580 for D/F and $184,449 for PCBs, and a total of $398,029. This was higher than the projected ($321,380) due to reanalysis, re-extractions, dilutions and additional cleanups that were above the 30% repeats allowable by the original quote. The turnaround time by the reference laboratory for reporting all 209 samples was approximately eight months (171 business days). The quoted turnaround time was three months.

8.4 Comparison of Economic Analysis Results

The total costs for the AhRC PCR™ kit to analyze all 209 demonstration samples ($35,023) and the reference method ($398,029) are listed in Tables 8-1 and 8-2, respectively. The total cost for the AhRC PCR™ kit purchase was $363,006 less than the reference method. After the demonstration, Hybrizyme refined its sample preparation method, analyzed all 209 samples in its

Table 8-2. Reference Method Cost Summary

Analyses Performed	Number of Samples Analyzed	Cost per sample Quotation ($)	Itemized Cost ($)	
			Quotation[a]	Actual
D/F, EPA Method 1613B, GC/HRMS	23 extracts	735	16,905	213,580
	186 soil/sediment	785	146,010	
WHO PCBs EPA Method 1668A, GC/HRMS	23 extracts	685	15,755	184,449
	186 soil/sediment	735	136,710	
1668 Optional Carbon Column DB1	40	150	6,000	
Total Cost	209 samples		**321,380**	**398,029**

[a] Price includes up to 30% of samples requiring additional work of some kind (dilutions or extra cleanup). Greater than that would require additional work with further charges associated to them ($150 to $180 per sample per procedure).

laboratory, and reported results. The developer reported that the total analysis time was approximately one week once the method refinements were completed. By comparison, the reference laboratory took eight months to analyze all 209 samples.

Use of the AhRC PCR™ kit in the field will likely produce additional cost savings because the results will be available within a few hours of sample collection; therefore, critical decisions regarding sampling and analysis can be made in the field, resulting in a more complete data set. Additional possible advantages to using field technologies include reduction of multiple crew and equipment mobilization-demobilization cycles to a single cycle, dramatically increased spatial resolution mapping for higher statistical confidence, leading to reduced insurance costs and reduced disposal costs, and compression of total project time to reduce administrative overhead. However, these savings cannot be accurately estimated and thus were not included in the economic analysis. Project-specific costs associated with the use of the technology, such as the cost for HRMS confirmation analyses and training costs to be proficient in the use of the technology, were also not accounted for in this analysis.

The Hybrizyme AhRC PCR™ kit is a screening method that reports the amount of AhR binding compounds in the sample unlike the reference method which reports TEQ results and concentrations for individual congeners. The AhRC PCR™ kit provided data which resulted in ranking samples from low to high concentration of AhR-binding compounds identically to ranking generated by reference laboratory data 70-90% of the time. Additionally, the AhRC PCR™ kit can provide AhRBU results on-site at significant cost and time savings compared to the reference laboratory.

Chapter 9
Technology Performance Summary

The purpose of this chapter is to provide a performance summary of the Hybrizyme Corporation AhRC PCR™ Kit by summarizing the evaluation of the primary and secondary objectives of this demonstration in Tables 9-1 and 9-2, respectively. Detailed information about these evaluations, including a complete evaluation of the reference laboratory data, can be found in previous sections of this report.

At the time of the 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 is a highly portable screening technology which can help to determine areas of greatest concern for cleanup at a site and can help to minimize the number of more expensive analyses needed for specific analytes. It has been suggested that correlation between the Hybrizyme AhRBU results and HRMS results could be established by first characterizing a site and calibrating the Hybrizyme results to HRMS results. This approach was not evaluated during this demonstration. Since the technology measures an actual biological response, it is possible that the technology may give a better representation of the true toxicity from a risk assessment standpoint.

The data generated and evaluated during this demonstration showed that the Hybrizyme technology could be used as an effective tool to rank sample concentrations from low to high within a particular environmental site, particularly considering that both the cost ($35,023 vs. $398,029) and the time (less than two weeks vs. eight months) to analyze the 209 demonstration samples were significantly less than that of the reference laboratory.

Table 9-1. Hybrizyme Corporation AhRC PCR™ Kit Performance Summary - Primary Objectives

Objective	Performance	
P1: Accuracy: Ranking versus PE certified values	• The Hybrizyme ranking was identical to the certified concentrations for one of the 10 PE samples (Cambridge 5184). • Of the four highest sample concentrations according to the certified values (NIST 1944, ERA Aroclor, Cambridge 5184, and ERA PAH), Hybrizyme's data ranked three of the samples as having the highest concentrations (NIST 1944, Cambridge 5184, and ERA PAH). • The ERA Aroclor sample, which was spiked with Aroclor 1254, was ranked as the next lowest concentration by Hybrizyme and the next to highest concentration by the certified data.	
P2: Precision	Number of data points	48
	Median RSD (%)	19
	Mean RSD (%)	25
P3: Comparability: Ranking versus reference laboratory average total concentration for environmental samples	• Hybrizyme ranking agreed with reference laboratory within the environmental site for 7 of the 10 sites (70%) • Hybrizyme ranking agreed with the reference laboratory for 9 of the 10 sites when uncertainty around reference laboratory values was considered (90%) • Hybrizyme individual rankings agreed with the reference laboratory for 26 of the 32 individual rankings (81%)	
P4: Estimated method detection limit	EMDL = 71 AhRBU	
P5: False Positive/False Negative Rate	not evaluated	
P6: Matrix Effects	• Measurement location: not evaluated (all results generated in the laboratory) • Matrix type: none • Sample type: Significant effect • PAH concentration: none • Environmental site: not evaluated since AhRBU results weren't directly comparable to TEQ • Known interferences: not evaluated since assay reacts to AhR binding compounds	
P7: Cost	110 samples during field demonstration: $15,529 Projected if all 209 demonstration samples were analyzed in field: $35,023	

Table 9-2. Hybrizyme Corporation AhRC PCR™ Kit Performance Summary - Secondary Objectives

Objective	Performance
S1: Skill level of Operator	Based on observation during the field demonstration, the recommended skill level for operation of this technology includes a minimum of a high school degree, having good work skills, and being trainable. Decent laboratory skills, respect for safety, and having reasonable attention to detail would also be useful attributes for successful technology operation.
S2: Health and Safety Aspects	The majority of this technology's waste was generated during the sample extraction. While hydrochloric acid was used for this demonstration, the process normally uses hexane, acetic acid, acetone, and, optionally, sulfuric acid. A fume hood is recommended for solvent extraction.
S3: Portability	As used in the demonstration, this technology required at a minimum a trailer to protect from the environment and to house some equipment such as a centrifuge, sonicator, and the PCR thermocycler analyzer. Electricity was also a necessity, so a trailer with a fume hood would be the minimum required for successful field operation. The developer intends for this technology ultimately to be usable in a minimally controlled environment.
S4: Sample Throughput	During the field demonstration, 110 samples were processed by Hybrizyme, equating to a sample throughput rate of 28 samples per day. This was accomplished in about four full working days (74 labor-hours), with two operators (one doing sample preparation and one performing the analysis) performing the work. Hybrizyme reported that, once the method refinements were completed, the total analysis time in its laboratory to complete the sample analysis and repeat analysis of the 110 samples that were analyzed during the field demonstration was one week.

Chapter 10
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Appendix A
SITE Monitoring and Measurement Technology Program
Verification Statement

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Office of Research and Development
Washington, DC 20460

SITE Monitoring and Measurement Technology Program Verification Statement

TECHNOLOGY TYPE:	**Ah Receptor - Polymerase Chain Reaction**
APPLICATION:	**MEASUREMENT OF DIOXIN AND DIOXIN-LIKE COMPOUNDS**
TECHNOLOGY NAME:	**AhRC PCR™ Kit**
COMPANY:	**Hybrizyme Corporation**
ADDRESS:	**Suite G-70** **2801 Blue Ridge Road** **Raleigh, North Carolina 27607**
PHONE:	**(919) 783-9595**
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VERIFICATION PROGRAM DESCRIPTION

The U.S. Environmental Protection Agency (EPA) created the Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) Program to facilitate deployment of innovative technologies through performance verification and information dissemination. The goal of this program is to further environmental protection by substantially accelerating the acceptance and use of improved and cost-effective technologies. The program assists and informs those involved in designing, distributing, permitting, and purchasing environmental technologies. This document summarizes results of a demonstration of the Hybrizyme Corporation AhRC PCR™ Kit.

PROGRAM OPERATION

Under the SITE MMT Program, with the full participation of the technology developers, the EPA evaluates and documents the performance of innovative technologies by developing demonstration plans, conducting field tests, collecting and analyzing demonstration data, and preparing reports. The technologies are evaluated under rigorous quality assurance protocols to produce well-documented data of known quality. The EPA's National Exposure Research Laboratory, which demonstrates field sampling, monitoring, and measurement technologies, selected Battelle as the verification organization to assist in field testing technologies for measuring dioxin and dioxin-like compounds in soil and sediment.

DEMONSTRATION DESCRIPTION

The demonstration of technologies for the measurement of dioxin and dioxin-like compounds was conducted at the Green Point Environmental Learning Center in Saginaw, Michigan, from April 26 to May 5, 2004. The primary objectives for the demonstration were as follows:

P1. Determine the accuracy.
P2. Determine the precision.
P3. Determine the comparability of the technology to EPA standard methods.
P4. Determine the estimated method detection limit (EMDL).
P5. Determine the frequency of false positive and false negative results.
P6. Evaluate the impact of matrix effects on technology performance.
P7. Estimate costs associated with the operation of the technology.

The secondary objectives for the demonstration were as follows:

S1. Assess the skills and training required to properly operate the technology.
S2. Document health and safety aspects associated with the technology.
S3. Evaluate the portability of the technology.
S4. Determine the sample throughput.

A total of 209 samples was analyzed by each technology, including a mix of performance evaluation (PE) samples, environmentally contaminated samples, and extracts. Hybrizyme analyzed 110 samples in the field, but refined its method after the demonstration and analyzed all 209 samples in its laboratory. The PE samples were used primarily to determine the accuracy of the technology and consisted of purchased reference materials with certified concentrations. The PE samples also were used to evaluate precision, comparability, EMDL, false positive/negative results, and matrix effects. Dioxin-contaminated samples from Warren County, North Carolina; the Saginaw River, Michigan; Tittabawassee River, Michigan; Midland, Michigan; Winona Post, Missouri; Nitro, West Virginia; Newark Bay, New Jersey; Raritan Bay, New Jersey; and Brunswick, Georgia were used to evaluate precision, comparability, false positive/negative results, and matrix effects. Extracts prepared in toluene were used to evaluate precision, EMDL, and matrix effects. All samples were used to evaluate qualitative performance objectives such as technology cost, the required skill level of the operator, health and safety aspects, portability, and sample throughput. AXYS Analytical Services (Sidney, British Columbia) was contracted to perform the reference analyses by high-resolution mass spectrometry (HRMS) (EPA Method 1613B and EPA Method 1668A). The purpose of the verification statement is to provide a summary of the demonstration and its results; detailed information is available in *Technologies for Monitoring and Measurement of Dioxin and Dioxin-like Compounds in Soil and Sediment — Hybrizyme Corporation AhRC PCR™ Kit* (EPA/540/R-05/005).

TECHNOLOGY DESCRIPTION

The technology description and operating procedure below are based on information provided by Hybrizyme Corporation.

The Hybrizyme AhRC PCR™ kit detects molecules in a test sample that bind to the aryl hydrocarbon receptor (AhR). The AhR mediates most, if not all, of the harmful effects associated with exposure to 2,3,7,8-substituted dioxin/furan (D/F). How tightly or loosely these compounds bind to the AhR is one of the determining factors of their toxicity. The AhR also binds certain coplanar polychlorinated biphenyls and carcinogenic polynuclear aromatic hydrocarbons (PAHs), such as benzo-[a]-pyrene. Sample cleanup procedures can be employed so that all or a subset of these AhR-reactive compounds are detected by the assay. Sample results are reported in Ah-receptor binding units (AhRBU).

Samples are prepared using an extraction method designed for speed and simplicity while maintaining sample concentration. Two grams of each sample are placed in a vial, and an extraction cocktail is added. The samples are extracted using an ultrasonic bath followed by brief centrifugation to remove solids. Water is added to the sample, forcing a small amount of hexane to partition from the extraction cocktail. A portion of the hexane is removed and dried for analysis. Depending on the condition of the sample being analyzed, an acid wash may be added as an additional step. The dried sample extracts are suspended in methanol for analysis by the AhRC PCR™ Kit.

Extracts in methanol are added to glass vials, followed by the addition of activation solution containing the AhR and dioxin-responsive element (DRE) probe and incubated at room temperature for one hour. This reaction mix is transferred from the glass vials to capture strips using a multichannel pipettor, and the capture strips are incubated at room temperature for an additional 30 minutes. During this time, the AhR/DRE-probe complexes are trapped onto the wells of the capture strip. The capture strips are washed to remove free DRE-probe, and a polymerase chain reaction (PCR) master mix is added. The strips are placed in a real-time thermocycler, and the amount of the DRE-probe is measured. The signal generated from the DRE-probe is directly proportional to the amount of dioxin in the samples.

VERIFICATION OF PERFORMANCE

At the time of the 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 toxicity equivalent (TEQ). The developer's goal is a highly portable screening technology that can help to determine areas of greatest concern for cleanup at a site and can help to minimize the number of more expensive analyses needed for specific analytes. It has been suggested that correlation between the Hybrizyme AhRBU results and HRMS results could be established by first characterizing a site and calibrating the Hybrizyme results to HRMS results. This approach was not evaluated during this demonstration. Since the technology measures an actual biological response, it is possible that the technology may give a better representation of the true toxicity from a risk assessment standpoint.

Accuracy: The determination of accuracy was based on ranking of the PE samples results from low to high concentration and comparing it to the rank order reported by Hybrizyme based on AhRBU. The Hybrizyme ranking was identical to the certified concentrations for one of the 10 PE samples (Cambridge 5184). Of the four highest sample concentrations according to the certified values, Hybrizyme's data ranked three of the samples as having the highest concentrations. One PE sample that was spiked with Aroclor 1254 was ranked as the next lowest concentration by Hybrizyme and the next to highest concentration by the certified data.

Precision: Replicates were incorporated for all samples (PE, environmental, and extracts) included in the 209 samples analyzed in the demonstration. Three samples had seven replicates in the experimental design, one sample had eight replicates, and all other samples had four replicates. Precision was determined by calculating the standard deviation of the replicates, dividing by the average concentration of the replicates, and multiplying by 100%. Ideal relative standard deviation (RSD) values are less than 20%. The overall RSD values were 25% (mean), 19% (median), 2% (minimum), and 111% (maximum).

Comparability: The reference laboratory average total cocnentration results were compared to Hybrizyme's AhRBU results including contributions from PAHs because PAHs are AhR binding compounds and are included in the Hybrizyme results. For this evaluation, the environmental samples were ranked with the samples from that site only, rather than ranking all of the environmental sites in one ordering, because the Hybrizyme technology is intended to rank samples within a particular site. This evaluation demonstrated that the Hybrizyme technology was able to rank the samples from low to high concentration within an environmental site fairly consistently with the reference laboratory based on average total concentration data. For seven of the 10 environmental sites (70%), Hybrizyme's ranking was identical to the reference laboratory's ranking. For samples that were close in average HRMS concentration and were indistinguishable when uncertainties were considered, the Hybrizyme rankings agreed with the reference laboratory's ranking for nine of 10 sites (90%). On an individual ranking basis, the Hybrizyme and reference laboratory rankings agreed 81% of the time (26 of 32 rankings). These data suggest that the Hybrizyme technology could be an effective screening tool for ranking samples within an environmental site from low to high concentration.

Estimated method detection limit: EMDL was calculated according to the procedure described in 40 CRF Part 136, Appendix B, Revision 1.11. Lower EMDL values indicate better sensitivity. The calculated EMDL was 71 AhRBU.

False positive/negative results: This parameter was not evaluated for this technology because quantitative comparisons to HRMS results are not appropriate.

Matrix effects: The likelihood of matrix-dependent effects on performance was investigated by evaluating results in a variety of ways. No significant effect was observed for the reproducibility of Hybrizyme results by matrix type (soil, sediment, and extract) or by PAH concentration, but a significant effect was observed for sample type (PE vs. environmental vs. extract).

Cost: The full cost of the technology was documented and compared to the cost of the reference analyses. The total cost for the Hybrizyme kit to analyze all 209 samples was $35,023. The total cost for the reference laboratory to analyze all 209 samples by EPA Method 1613B and EPA Method 1668A was $398,029. The total cost for the use of the Hybrizyme kit was $363,006 less than the reference method.

Skills and training required: Based on observation during the field demonstration, the recommended skill level for operation of this technology includes a minimum of a high school degree, having good work skills, and being trainable. Decent laboratory skills, respect for safety, and having reasonable attention to detail would also be useful attributes for successful technology operation.

Health and safety aspects: The majority of this technology's waste was generated during the sample extraction. While hydrochloric acid was used for this demonstration, the process normally uses hexane, acetic acid, acetone, and, optionally, sulfuric acid. A fume hood is recommended for solvent extraction.

Portability: As used in the demonstration, this technology required at a minimum a trailer to protect from the environment and to house equipment such as a centrifuge, sonicator, and the PCR thermocycler analyzer. Electricity was also a necessity, so a trailer with a fume hood would be the minimum required for successful field operation. The developer intends for this technology ultimately to be useable in a minimally controlled environment.

Sample throughput: During the field demonstration, 110 samples were processed by Hybrizyme, equating to a sample throughput rate of 28 samples per day. This was accomplished in about four full working days (74 labor-hours), with two operators (one doing sample preparation and one performing the analysis) performing the work. After the demonstration, Hybrizyme refined its sample preparation method, analyzed all 209 samples in its laboratory. Hybrizyme reported that the total analysis time for the samples was approximately one week once the method refinements were completed. By comparison, the reference laboratory took eight months to analyze all 209 samples.

NOTICE: Verifications are based on an evaluation of technology performance under specific, predetermined criteria and the appropriate quality assurance procedures. The EPA makes no expressed or implied warranties as to the performance of the technology and does not certify that a technology will always operate as verified. The end user is solely responsible for complying with any and all applicable federal, state, and local requirements.

Appendix B
Supplemental Information Supplied by the Developer

The purpose of this section is for the developer to provide additional information about the technology. This can include updates/changes/modifications planned for the technology or which have occurred since the technology was tested. The developers can also use this section to comment and expand on the findings of the report.

Information was provided by the developer and does not necesarily reflect the opinion of the EPA.

Hybrizyme Comments

Versatility of the AhRC PCR assay system

The AhRC PCR assay can be used in a variety of modes depending on the sample preparation steps employed. Typically, the more labor intensive the cleanup the more specific the test results reported. For example, the AhRC PCR assay has been accepted in Japan as a TEQ method that can be used in lieu of HRGC-HRMS for fly ash analysis (see below). Minimal sample cleanup was used in this study to maximize field portability, ease-of-use, and speed. The assay detects toxicants other than dioxins and furans including carcinogenic PAHs and, as such, reports in arbitrarily defined Ah-receptor binding units (AhRBUs).

Straightforward sample cleanup in combination with the ease-of-use of the AhRC PCR assay makes the method rapid, inexpensive, and precise (something that few, if any, trace organic analytical technologies can claim). This method provides a tool to quickly rank samples at a given site according to their reactivity with the Ah receptor. Because the assay may recognize additional hazardous compounds other than dioxins and furans the technology is best used in an environmentally site-specific manner. Environmental samples from a site with known concentrations of targeted contaminants can be used to calibrate the response of the AhRC PCR assay providing a quantitative measurement (please call for additional information).

Generating a Concentration Map

The ability to rapidly generate relative concentration data makes it feasible to produce a concentration map of a large environmental site in a reasonably short period of time. With such a concentration map in hand, the most relevant collected matrices can be subjected to more sophisticated techniques capable of accurately identifying the contaminants present. By eliminating the need to use sophisticated and expensive techniques on all of the collected samples, many of which typically contain low to non-detectable levels, one expedites the production of useful data in all areas of a given study. As a result, site personnel can begin remediation efforts faster saving both time and money.

The ability for two field technicians to determine site boundaries beyond which there is no measurable contamination using a field executable methodology capable of producing up to 40 to 60 data points per day is also beyond the current state of the art.

A Sample Pre-Screening Procedure for the HRGC-HRMS Laboratory

The method also has a pre-screening application in the typical laboratory performing HRGC-HRMS analyses of extended sets of environmental samples. In such a role, the assay system is used by experienced laboratory personnel to eliminate all samples demonstrating low to negligible response at a rate of perhaps greater than 60 samples per day in a fully equipped laboratory setting. Simultaneously, this data would also permit organizing the remainder of the samples from lowest to highest concentration of AhR reactive molecules. As such, when they are finally processed for HRGC-HRMS examination, the laboratory will not spend inordinate amounts of time examining “non-detects” nor will they accidentally cross-contaminate their laboratory equipment by processing samples containing elevated levels of the expected analytes.

The described benefits for using the Hybrizyme Method to pre-screen large sample sets as a regular function of sample processing and analysis in the HRGC-HRMS laboratory will likely decrease overall time and expense for these critical analyses. Data quality will also improve while permitting a much greater fraction of usable information to be generated on the first pass through the system. Reanalysis due to circumstances

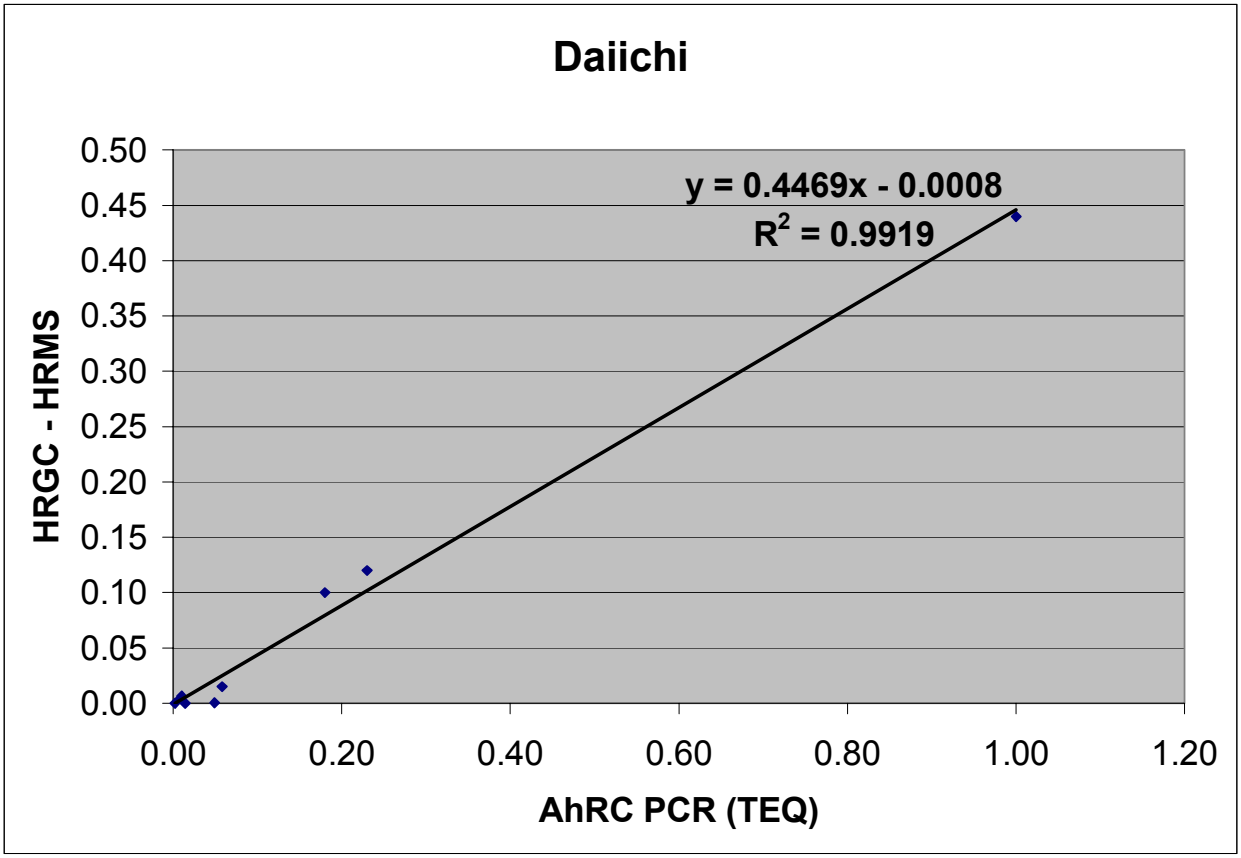
arising from cross-contamination caused by the presence of only a few very high concentration samples in a batch of otherwise low to negligible concentration samples will ultimately become a non-issue.

EQ Analysis

The AhRC PCR assay kit has been validated for use in Japan. Both Daiichi Fine Chemicals and the National Institute for Environmental Studies (NIES) analyzed the samples below for the Japanese Ministry of the Environment using AhRC PCR. Daiichi and NIES generated R^2 values of 0.99 and 0.92, respectively. A similar study was presented at DIOXIN 2004 comparing a number of quantitative bioassays. The authors indicated that the AhRC PCR demonstrated superior reproducibility over other bioassays tested (Ota, S. *t al*., Comparison of Various Bioassays for Dioxins Measurements in Fuel gas, Fly ash and Bottom ash. *Organohalogen Compounds*, Vol. 66, pp 682-689, 2004; http://dioxin2004.abstract-management.de/pdf/p528.pdf). Hybrizyme, in collaboration with Daiichi, is in the process of developing a similar method for use in the US for quantitative TEQ analysis.

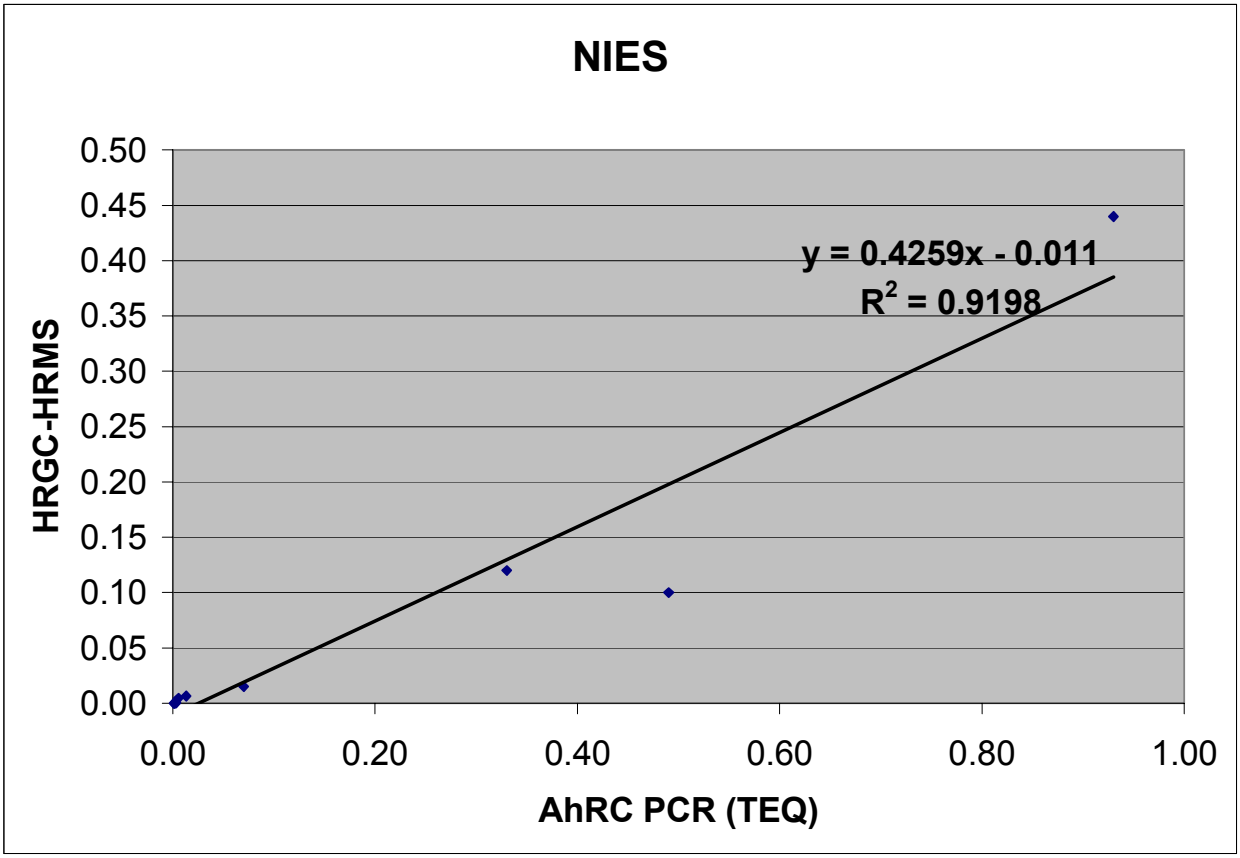
Daiichi Fine Chemical Co. LTD.

		AhR PCR	GC/MS	
		Conversion TEQ	TEQ	Unit
Sample 2A	Ex. Gas	0.18	0.10	ng - TEQ /m^3N
Sample 2B	Ex. Gas	0.23	0.12	ng - TEQ /m^3N
Sample 2C	Ex. Gas	0.058	0.015	ng - TEQ /m^3N
Sample 2D	Fly Ash	0.00089	0.0046	ng - TEQ /g
Sample 2E	Fly Ash	0.014	0.000056	ng - TEQ /g
Sample 2F	Fly Ash	1.0	0.44	ng - TEQ /g
Sample 2G	Bottom Ash	0.049	0.000034	ng - TEQ /g
Sample 2H	Bottom Ash	0.0022	0.000035	ng - TEQ /g
Sample 2I	Bottom Ash	0.010	0.0067	ng - TEQ /g



National Institute for Environmental Studies

		AhR PCR	GC/MS	
		Conversion TEQ	TEQ	Unit
Sample 2A	Ex. Gas	0.49	0.10	ng - TEQ /m^3N
Sample 2B	Ex. Gas	0.33	0.12	ng - TEQ /m^3N
Sample 2C	Ex. Gas	0.070	0.015	ng - TEQ /m^3N
Sample 2D	Fly Ash	0.0055	0.0046	ng - TEQ /g
Sample 2E	Fly Ash	0.0017	0.000056	ng - TEQ /g
Sample 2F	Fly Ash	0.93	0.44	ng - TEQ /g
Sample 2G	Bottom Ash	0.030	0.000034	ng - TEQ /g
Sample 2H	Bottom Ash	0.0010	0.000035	ng - TEQ /g
Sample 2I	Bottom Ash	0.013	0.0067	ng - TEQ /g



Appendix C
Reference Laboratory Method Blank and Duplicate Results Summary

Table C-1. Summary of Method Blank Performance

Sample Batch Number	Criteria Met	Method Blank TEQ[a] (pg/g)	Sample TEQ Range[a] (pg/g)	Comments
D/F WG12107	Y	0.000812	26.1–74.1 (Newark Bay) 9.93–13.3 (Raritan Bay)	
D/F WG12148	N	0.133	13.5–50.4 (Newark Bay) 49.5–15,200 (Brunswick)	Many samples had concentrations >20x blank. Few that didn't were not significantly affected on a total TEQ basis.
D/F WG12264	N	0.0437	1.0–94.1 (Titta. River sediment) 0.237–6,900 (PE)	Most samples had concentrations >20x blank. Low level Tittabawassee River sediment samples L6749-2 (Ref 48[b]), -9 (Ref 130), -10 (Ref 183), and -12 (Ref 207) were evaluated based on their replication within the demonstration analyses and comparison to characterization results and considered unaffected by method blank exceedances. Low level PE samples L6760-1 (Ref 25), –3 (Ref 28), and -4 (Ref 29) were D/F blanks with resulting TEQs sufficiently low enough to still be distinguished as blank samples.
D/F WG12534	N	0.610	25.3–7,100 (PE)	Sample concentrations > 20x blank.
D/F WG12641	N	0.0475	31–269 (Midland) 72.8 (Brunswick) 123 (Titta. River sediment) 0.159–7,690 (PE)	All but PE sample Ref 177 (0.159 TEQ) had significantly higher total TEQ than blank. Ref 177 was confirmed by running in another batch and results, which agreed within 18%. Additionally, Ref 177 was compared to its replicates within the program and considered acceptable.
D/F WG12737	N	0.348	25.7–192 (Midland) 35.2–1,300 (Titta. River soil)	Sample concentrations >20x blank.
D/F WG12804	N	0.0153	3.89–188 (PE)	A few analytes higher than criteria but no significant contribution to total TEQ.
D/F WG13547	N	0.0553	57.5–3,000 (Nitro) 37.9 (North Carolina) 122 (Saginaw River) 26.4– 222 (Midland)	Several analytes exceeded criteria, but blank total TEQ contribution to sample is relatively small.

Sample Batch Number	Criteria Met	Method Blank TEQ[a] (pg/g)	Sample TEQ Range[a] (pg/g)	Comments
D/F WG13548	N	0.0114	99.6–99.7 (Saginaw River) 32.9–36.4 (North Carolina) 0.268–100 (Extracts)	Several analytes exceeded criteria. In general, the blank contribution to total TEQ was negligible and in those cases results were accepted. Several low-level extract samples were evaluated as follows: Extract Spike #1 samples L6754-4 (Ref 4), -8 (Ref 8), -10 (Ref 10), -14 (Ref 14), -19 (Ref 19), -22 (Ref 22), and -23 (Ref 23) were known TCDD spikes at 0.5 pg/mL. Results were compared to the known spiked TEQ and considered unaffected by blank contribution to TEQ. Extract Spike #3 samples L6754-1 (Ref 1), -7 (Ref 7), -12 (Ref 12), and -15 (Ref 15) were PCB spikes and not expected to contain D/F. These spikes consistently contained a D/F TEQ of ~0.3. However, this came from a consistent ~0.3 pg/mL of TCDD detected in these extracts that was confirmed as a low-level TCDD contamination by AXYS. Since TCDD was not present in the lab blank, these results were accepted as unaffected by any blank contribution to TEQ.
D/F WG13549	N	0.0925	2,160–3,080 (Nitro) 146–1,320 (Saginaw River) 788–8,410 (North Carolina)	Many analytes exceeded limits, but the blank contribution to total TEQ is small relative to sample TEQs.
D/F WG13551	N	2.40	1,100–10,800 (North Carolina) 7,160–11,300 (Winona Post)	Many analytes exceeded limits, but the blank contribution to total TEQ is small relative to sample TEQs.
D/F WG13552	Y	0.000969	0.0386–9.28 (PE) 25.8 (Midland)	
D/F WG13984	N	0.0154	0.524–24.8 (PE) 10.4 (Raritan Bay) 53.1–444 (Extracts)	Blank contribution to total TEQ was negligible except for PE samples L7179-7 (Ref 94), -8 (Ref 96), -11 (Ref 108), -12 (Ref 109), -17 (Ref 132), and L7182-6 (Ref 150). All but L7179-8 were certified blanks. L7179-8 was a PAH spike with no D/F TEQ expected. The TEQs of these samples were considered sufficiently low enough to still be distinguished as blank samples and were accepted.
D/F WG14274	N	0.0434	2,800 (Nitro) 35.5–8,320 (North Carolina) 0.0530–5.93 (PE)	Sample TEQs were large enough to be unaffected by the blank TEQ except for four PE samples L7179-4 (Ref 85), -16 (Ref 124) and L7182-12 (Ref 169) and -14 (Ref 184). These PE samples were either certified blanks or PCB spikes with no expected D/F TEQ. Resulting TEQs for these samples were considered low enough to be distinguished as blank samples and were accepted.

Sample Batch Number	Criteria Met	Method Blank TEQ[a] (pg/g)	Sample TEQ Range[a] (pg/g)	Comments
PCB WG12108	N	0.000137	2.63–5.19 (Newark Bay) 2.04–2.82 (Raritan Bay)	PCB 77 slightly high, but all samples >20x blank levels.
PCB WG12147	Y	0.000	1.21–5.06 (Newark Bay) 0.104–0.330 (Brunswick)	
PCB WG12265	Y	0.0000584	0.132–0.369 (Brunswick) 0.034–0.649 (Titta. River sediment) 0.00277–1,030 (PE)	
PCB WG12457	N	0.000208	4.20–1,020 (PE)	PCB 77 slightly high. Did not report any samples where PCB 77 was <10x blank. No significant effect on total TEQ.
PCB WG12687	N	0.0183	0.974–2.73 (Midland) 10.3–1,180 (PE)	PCB 77 and 156 high, but all samples >20x blank levels.
PCB WG12834	N	0.000405	0.0157–62.4 (Saginaw River) 0.181–0.203 (Brunswick) 0.986–7.57 (Titta. River Soil)	PCB 77 slightly high. Does not affect total TEQ.
PCB WG12835	N	0.000125	0.822–2.06 (Winona Post)	PCB 77 slightly high. Sample TEQs much greater than blank TEQ.
PCB WG12836	N	0.0499	1,060–904,000 (North Carolina)	PCBs 77, 123, 126, 156, 167, and 118 high, but most samples significantly > 20x blank levels.
PCB WG13008	N	0.0221	2.38–3.15 (Midland) 1.03–8.37 (Titta. River soil) 41.0–1,140 (PE)	PCBs 77 and 118 high, but all samples >20x blank levels.
PCB WG13256	Y	0.000102	0.00385–0.051 (PE)	
PCB WG13257	Y	0.000251	0.253–0.318 (Midland) 0.135–2.08 (Extracts) 3.53–9.62 (PE) 1.14–1.33 (Titta. River Soil)	
PCB WG13258	Y	0.000301	0.163–37.0 (Nitro) 29.8–73.6 (Saginaw River) 40.1–42.1 (PE)	
PCB WG13554	N	0.0000900	0.000103–1,080 (Extracts) 435–1,160 (PE)	PCB 77 slightly high. Does not affect total TEQ.
PCB WG14109	N	0.000288	0.388–0.452 (Nitro) 0.0467 (Saginaw River) 0.654–1.87 (Winona Post) 0.00300–0.0420 (PE)	PCB 77 high. PE certified blanks Ref 85, Ref 85 duplicate, and Ref 108 were the only samples where PCB 77 was not >20x blank. TEQs for these certified blanks were considered low enough to be distinguished as blank samples and were accepted.

[a] All nondetect and EMPC values were assigned a zero concentration for the TEQ calculation.
[b] "Ref XX" is a reference laboratory sample ID number.

Table C-2. Sample Batch Duplicate Summary

Sample Batch Number	Criteria Met	Duplicate RPD[a] (%)	Comments
D/F WG12107	N	23	L6744-5, Ref 100 Newark Bay Because this was above the 20% criteria, an additional aliquot of this sample was prepared. Results for the additional aliquot were within 11% RPD from the original results; therefore, this duplicate result was accepted.
D/F WG12148	Y	2.1	L6744-9, Ref 122 Newark Bay
D/F WG12264	Y	1.2	L6760-2, Ref 27 PE
D/F WG12534	Y	5.7	L6760-14, Ref 55 PE
D/F WG12641	Y	4.6	L6747-1, Ref 32 Midland
D/F WG12737	Y	14	L6750-3, Ref 78 Tittabawassee River Soil
D/F WG12804	N	none	The duplicate processed with this batch was to be repeated due to some analytes being <20x blank level. However, it was reprocessed as a single sample and not a duplicate. Samples in this set were accepted based on their agreement with other replicates within the demonstration program.
D/F WG13547	Y	16	L7163-1, Ref 26 Nitro
D/F WG13548	Y	5.9	L6751-14, Ref 83 North Carolina
D/F WG13549	Y	3.6	L6751-7, Ref 135 North Carolina
D/F WG13551	Y	0.0	L6751-1, Ref 42 North Carolina
D/F WG13552	Y	20 (on U=1/2 DL basis[b])	L7179-3, Ref 74 PE. Fails on a U=0 DL basis due to presence of "K" flagged analytes in one replicate. When compared on U-1/2 DL basis where "K" concentrations are included in the TEQ calculation, the duplicate passed.
D/F WG13984	Y	3.4	L7179-14, Ref 113 PE
D/F WG14274	N	54	L7179-16, Ref 124 PE This was a PCB PE sample and contained only trace levels of D/F. Replicate precision is affected because D/F content is so low. This is not expected to indicate any problems with precision within this sample set. Samples in this set were accepted based on their agreement with other replicates within the demonstration program.
PCB WG12108	N	22	L6744-2, Ref 49 Newark Bay This result is only slightly above the acceptance criteria of 20%. The variability was influenced by 25% RPD for PCB126 (which has the highest TEF of the PCBs and, therefore, a larger influence on total TEQ). The slight exceedance in duplicate criteria was not considered to have any significant impact on the data reported in this sample batch. All samples in this set were also evaluated based on their agreement with other replicates within the demonstration program and deemed to be acceptable.

Sample Batch Number	Criteria Met	Duplicate RPD[a] (%)	Comments
PCB WG12147	N	none	L6748-9, Ref 129 Brunswick The duplicate sample for this batch required reprocessing. When reprocessed, it was not prepared in duplicate. Samples in this set were accepted based on the RPD of site replicates that were processed within the batch (RPDs <10%).
PCB WG12265	Y	2.5	L6760-5, Ref 35 PE
PCB WG12457	N	none	L6760-16, Ref 62 PE This duplicate set was to be repeated due to low internal standard recovery. When repeated, it was not prepared in duplicate. Data for this set was accepted because all samples in the set were PE samples. These PE samples met accuracy criteria and reproducibility criteria to other replicates of the same PE material processed within the demonstration.
PCB WG12687	Y	4.3	L6762-12, Ref 169 PE
PCB WG12834	Y	4.2	L6750-8, Ref 164 Tittabawassee River Soil
PCB WG12835	N	none	Duplicate sample repeated in WG13258. Results reported with that sample set. Three sets of sample replicates within this batch were also compared and found to have <13.5% RPD showing acceptable precision with this sample set.
PCB WG12836	Y	2.6	L6751-6, Ref 126 North Carolina
PCB WG13008	Y	5.1	L6750-6, Ref 121 Tittabawassee River Soil
PCB WG13256	Y	1.7 (on U=1/2 DL basis)	L6761-3, Ref 74 PE. Fails on a U=0 DL basis due to presence of "K" flagged analytes in one replicate. When compared on U=1/2 DL basis where "K" concentrations are included in the TEQ calculation, the duplicate passed.
PCB WG13257	Y	15	L7187-5, Ref 92 Tittabawassee River Soil
PCB WG13258	Y	19	L6743-2, Ref 36 Nitro
PCB WG13554	Y	12	L6762-1, Ref 202 PE
PCB WG14109	N	85 (on U=1/2 DL basis)	L7179-4, PE. Fails based on both U=0 and U=1/2 DL. This was a blank PE sample and contained only trace levels of PCBs. Replicate precision is affected because the PCB content is so low. This is not expected to indicate any problems with precision within this sample set. Samples in this set were accepted based on their agreement with other replicates within the demonstration program.

[a] Nondetects were assigned a concentration of zero unless otherwise noted and are referred to as U=0 DL values.

[b] U=1/2 DL indicates that nondetects were assigned a concentration equal to one-half the SDL and EMPC concentrations were assigned a value equal to the EMPC.

Appendix D
Summary of Developer and Reference Laboratory Data

Appendix D. Hybrizyme and Reference Laboratory One-to-One Matching[a]

Sample Type	Sample Number	Measurement Location[b]	Sample Description	REP	Developer[c]	Reference Laboratory[d]		
					AhRBU	$TEQ_{D/F}$ (pg/g)	Total TEQ (pg/g)[e]	Total Concentration (ng/g)[f]
Environmental	HYB 163	Laboratory	Brunswick #1	1	1154	67.2	67.51	22513
Environmental	HYB 194	Laboratory	Brunswick #1	2	895	71.6	71.94	22513
Environmental	HYB 93	Laboratory	Brunswick #1	3	1249	61.7	62.07	22513
Environmental	HYB 203	Laboratory	Brunswick #1	4	977	67.8	68.11	22513
Environmental	HYB 116	Laboratory	Brunswick #2	1	658	49.5	49.63	9818
Environmental	HYB 72	Laboratory	Brunswick #2	2	567	72.8	72.93	9818
Environmental	HYB 135	Laboratory	Brunswick #2	3	812	56	56.13	9818
Environmental	HYB 104	Laboratory	Brunswick #2	4	778	60.4	60.52	9818
Environmental	HYB 52	Laboratory	Brunswick #3	1	165375	12600	12600.19	1210432
Environmental	HYB 197	Laboratory	Brunswick #3	2	156894	15200	15200.18	1210432
Environmental	HYB 94	Laboratory	Brunswick #3	3	209582	13100	13100.20	1210432
Environmental	HYB 151	Laboratory	Brunswick #3	4	190299	13600	13600.18	1210432
Environmental	HYB 44	Laboratory	Midland #1	1	371	222	224.59	1150
Environmental	HYB 119	Laboratory	Midland #1	2	457	241	243.73	1150
Environmental	HYB 102	Laboratory	Midland #1	3	548	269	271.5	1150
Environmental	HYB 170	Laboratory	Midland #1	4	493	268	270.53	1150
Environmental	HYB 34	Laboratory	Midland #2	1	1416	208	210.7	2531
Environmental	HYB 134	Laboratory	Midland #2	2	1363	179	181.81	2531
Environmental	HYB 124	Laboratory	Midland #2	3	1389	197	199.48	2531
Environmental	HYB 175	Laboratory	Midland #2	4	1429	192	195.15	2531
Environmental	HYB 155	Laboratory	Midland #3	1	1119	185	187.28	1821
Environmental	HYB 86	Laboratory	Midland #3	2	1372	174	176.17	1821
Environmental	HYB 168	Laboratory	Midland #3	3	1422	176	178.23	1821
Environmental	HYB 48	Laboratory	Midland #3	4	1089	161	163.38	1821
Environmental	HYB 68	Laboratory	Midland #4	1	72	25.7	25.95	147
Environmental	HYB 80	Laboratory	Midland #4	2	89.3	26.4	26.72	147
Environmental	HYB 71	Laboratory	Midland #4	3	109	31	31.97	147
Environmental	HYB 157	Laboratory	Midland #4	4	104	25.8	26.063	147
Environmental	HYB 199	Laboratory	NC PCB Site #1	1	18473	788	53788	41960
Environmental	HYB 49	Laboratory	NC PCB Site #1	2	15485	1100	66400	41960
Environmental	HYB 202	Laboratory	NC PCB Site #1	3	20356	852	81352	41960
Environmental	HYB 59	Laboratory	NC PCB Site #1	4	25156	906	86006	41960
Environmental	HYB 178	Laboratory	NC PCB Site #2	1	115702	3400	314400	340394
Environmental	HYB 110	Laboratory	NC PCB Site #2	2	96224	3300	308300	340394
Environmental	HYB 146	Laboratory	NC PCB Site #2	3	114690	3430	213430	340394
Environmental	HYB 121	Laboratory	NC PCB Site #2	4	112695	3490	364490	340394

Sample Type	Sample Number	Measurement Location[b]	Sample Description	REP	Developer[c]	Reference Laboratory[d]		
					AhRBU	$TEQ_{D/F}$ (pg/g)	Total TEQ (pg/g)[e]	Total Concentration (ng/g)[f]
Environmental	HYB 89	Laboratory	NC PCB Site #3	1	221536	8320	856320	869927
Environmental	HYB 81	Laboratory	NC PCB Site #3	2	233517	8410	626410	869927
Environmental	HYB 43	Laboratory	NC PCB Site #3	3	314727	9360	542360	869927
Environmental	HYB 63	Laboratory	NC PCB Site #3	4	235576	10800	914800	869927
Environmental	HYB 128	Laboratory	Newark Bay #1	1	1930	23	24.22	2348
Environmental	HYB 130	Laboratory	Newark Bay #1	2	1525	14	15.44	2348
Environmental	HYB 142	Laboratory	Newark Bay #1	3	2986	14.5	15.89	2348
Environmental	HYB 112	Laboratory	Newark Bay #1	4	2824	13.5	14.84	2348
Environmental	HYB 141	Laboratory	Newark Bay #2	1	5486	50.6	55.61	4046
Environmental	HYB 140	Laboratory	Newark Bay #2	2	3299	47.4	52.59	4046
Environmental	HYB 164	Laboratory	Newark Bay #2	3	3046	74.1	79.24	4046
Environmental	HYB 165	Laboratory	Newark Bay #2	4	3869	50.4	55.49	4046
Environmental	HYB 91	Laboratory	Newark Bay #3	1	4318	38.9	43.51	8030
Environmental	HYB 118	Laboratory	Newark Bay #3	2	5015	44.9	49.94	8030
Environmental	HYB 162	Laboratory	Newark Bay #3	3	4630	40.2	44.7	8030
Environmental	HYB 47	Laboratory	Newark Bay #3	4	4191	41.9	46.93	8030
Environmental	HYB 132	Laboratory	Newark Bay #4	1	2640	33.6	36.33	4906
Environmental	HYB 37	Laboratory	Newark Bay #4	2	3454	26.1	28.75	4906
Environmental	HYB 182	Laboratory	Newark Bay #4	3	3694	27.6	30.32	4906
Environmental	HYB 159	Laboratory	Newark Bay #4	4	3493	26.8	29.5	4906
Environmental	HYB 145	Laboratory	Raritan Bay #1	1	5698	10.2	12.53	3850
Environmental	HYB 40	Laboratory	Raritan Bay #1	2	5278	10.3	12.36	3850
Environmental	HYB 35	Laboratory	Raritan Bay #1	3	5278	10.4	12.75	3850
Environmental	HYB 139	Laboratory	Raritan Bay #1	4	5536	11.4	13.65	3850
Environmental	HYB 183	Laboratory	Raritan Bay #2	1	6964	13.3	16	7376
Environmental	HYB 50	Laboratory	Raritan Bay #2	2	4573	13.1	15.77	7376
Environmental	HYB 136	Laboratory	Raritan Bay #2	3	4706	12.8	15.48	7376
Environmental	HYB 190	Laboratory	Raritan Bay #2	4	8116	13	15.85	7376
Environmental	HYB 166	Laboratory	Raritan Bay #3	1	5977	10.4	12.83	4465
Environmental	HYB 113	Laboratory	Raritan Bay #3	2	8930	11.1	13.53	4465
Environmental	HYB 147	Laboratory	Raritan Bay #3	3	6832	10.6	12.9	4465
Environmental	HYB 161	Laboratory	Raritan Bay #3	4	5129	9.93	12.26	4465
Environmental	HYB 171	Laboratory	Saginaw River #1	1	4666	1050	1112.4	6188
Environmental	HYB 186	Laboratory	Saginaw River #1	2	8848	683	756.6	6188
Environmental	HYB 67	Laboratory	Saginaw River #1	3	8069	1070	1139.9	6188
Environmental	HYB 87	Laboratory	Saginaw River #1	4	7473	694	757.7	6188
Environmental	HYB 201	Laboratory	Saginaw River #2	1	4048	1110	1140.6	5474
Environmental	HYB 58	Laboratory	Saginaw River #2	2	3872	953	984	5474

Sample Type	Sample Number	Measurement Location[b]	Sample Description	REP	Developer[c]	Reference Laboratory[d]		
					AhRBU	$TEQ_{D/F}$ (pg/g)	Total TEQ (pg/g)[e]	Total Concentration (ng/g)[f]
Environmental	HYB 200	Laboratory	Saginaw River #2	3	3329	1320	1346.7	5474
Environmental	HYB 189	Laboratory	Saginaw River #2	4	3872	864	893.8	5474
Environmental	HYB 174	Laboratory	Saginaw River #3	1	129	99.7	99.72	770
Environmental	HYB 30	Laboratory	Saginaw River #3	2	126	146	146.02	770
Environmental	HYB 126	Laboratory	Saginaw River #3	3	113	122	122.05	770
Environmental	HYB 51	Laboratory	Saginaw River #3	4	122	99.6	99.62	770
Environmental	HYB 193	Laboratory	Solutia #1	1	154	57.5	57.95	436
Environmental	HYB 152	Laboratory	Solutia #1	2	149	76.9	77.06	436
Environmental	HYB 154	Laboratory	Solutia #1	3	121	62	62.39	436
Environmental	HYB 106	Laboratory	Solutia #1	4	197	61.6	61.99	436
Environmental	HYB 46	Laboratory	Solutia #2	1	873	2090	2107.6	2789
Environmental	HYB 198	Laboratory	Solutia #2	2	464	1950	1968.8	2789
Environmental	HYB 96	Laboratory	Solutia #2	3	899	1860	1879.2	2789
Environmental	HYB 90	Laboratory	Solutia #2	4	1058	2160	2178.5	2789
Environmental	HYB 187	Laboratory	Solutia #3	1	790	2810	2839.7	1365
Environmental	HYB 38	Laboratory	Solutia #3	2	329	2800	2836.9	1365
Environmental	HYB 185	Laboratory	Solutia #3	3	430	3000	3037	1365
Environmental	HYB 148	Laboratory	Solutia #3	4	346	3080	3111.5	1365
Environmental	HYB 73	Laboratory	Titta. River Soil #1	1	501	35	42.32	613
Environmental	HYB 41	Laboratory	Titta. River Soil #1	2	630	35.2	43.46	613
Environmental	HYB 53	Laboratory	Titta. River Soil #1	3	728	40	47.57	613
Environmental	HYB 120	Laboratory	Titta. River Soil #1	4	567	35.8	44.17	613
Environmental	HYB 208	Laboratory	Titta. River Soil #2	1	354	420	420.99	831
Environmental	HYB 78	Laboratory	Titta. River Soil #2	2	518	450	451.2	831
Environmental	HYB 97	Laboratory	Titta. River Soil #2	3	357	523	524.03	831
Environmental	HYB 60	Laboratory	Titta. River Soil #2	4	428	506	507.06	831
Environmental	HYB 123	Laboratory	Titta. River Soil #3	1	110	1050	1051.26	268
Environmental	HYB 61	Laboratory	Titta. River Soil #3	2	106	676	677.16	268
Environmental	HYB 184	Laboratory	Titta. River Soil #3	3	144	1220	1221.54	268
Environmental	HYB 56	Laboratory	Titta. River Soil #3	4	138	1300	1301.33	268
Environmental	HYB 99	Laboratory	Titta. River Sed #1	1	206	1.05	1.10	271
Environmental	HYB 108	Laboratory	Titta. River Sed #1	2	236	1.11	1.14	271
Environmental	HYB 33	Laboratory	Titta. River Sed #1	3	213	1	1.04	271
Environmental	HYB 24	Laboratory	Titta. River Sed #1	4	144	1.7	1.74	271
Environmental	HYB 153	Laboratory	Titta. River Sed #2	1	185	52.8	53.45	693
Environmental	HYB 133	Laboratory	Titta. River Sed #2	2	305	123	123.71	693
Environmental	HYB 158	Laboratory	Titta. River Sed #2	3	139	66.1	66.67	693
Environmental	HYB 64	Laboratory	Titta. River Sed #2	4	241	94.1	94.62	693

Sample Type	Sample Number	Measurement Location[b]	Sample Description	REP	Developer[c]	Reference Laboratory[d]		
					AhRBU	$TEQ_{D/F}$ (pg/g)	Total TEQ (pg/g)[e]	Total Concentration (ng/g)[f]
Environmental	HYB 88	Laboratory	Titta. River Sed #3	1	74	13	13.07	77
Environmental	HYB 149	Laboratory	Titta. River Sed #3	2	105	11.2	11.30	77
Environmental	HYB 101	Laboratory	Titta. River Sed #3	3	55	12.7	12.78	77
Environmental	HYB 137	Laboratory	Titta. River Sed #3	4	103	13.8	13.89	77
Environmental	HYB 42	Laboratory	Winona Post #1	1	42535	7290	7290.65	31343
Environmental	HYB 27	Laboratory	Winona Post #1	2	30739	7370	7370.90	31343
Environmental	HYB 156	Laboratory	Winona Post #1	3	34759	7450	7450.83	31343
Environmental	HYB 143	Laboratory	Winona Post #1	4	27185	7160	7160.82	31343
Environmental	HYB 195	Laboratory	Winona Post #2	1	79297	9720	9721.2	53038
Environmental	HYB 84	Laboratory	Winona Post #2	2	64166	9770	9771.3	53038
Environmental	HYB 179	Laboratory	Winona Post #2	3	67060	9200	9201.32	53038
Environmental	HYB 75	Laboratory	Winona Post #2	4	82873	11300	11301.28	53038
Environmental	HYB 65	Laboratory	Winona Post #3	1	57214	10300	10301.68	53819
Environmental	HYB 181	Laboratory	Winona Post #3	2	86611	9770	9771.87	53819
Environmental	HYB 205	Laboratory	Winona Post #3	3	68254	9320	9321.8	53819
Environmental	HYB 177	Laboratory	Winona Post #3	4	90517	9870	9872.06	53819
Extract	HYB 13	Laboratory	Envir. Extract #1	1	1408	175	175.63	NA
Extract	HYB 18	Laboratory	Envir. Extract #1	2	1603	444	444.67	NA
Extract	HYB 22	Laboratory	Envir. Extract #1	3	2041	176	176.64	NA
Extract	HYB 12	Laboratory	Envir. Extract #1	4	1895	439	441.08	NA
Extract	HYB 4	Laboratory	Envir. Extract #2	1	2480	55.3	56.04	NA
Extract	HYB 11	Laboratory	Envir. Extract #2	2	2260	53.3	53.44	NA
Extract	HYB 17	Laboratory	Envir. Extract #2	3	2550	53.1	53.40	NA
Extract	HYB 7	Laboratory	Envir. Extract #2	4	2959	53.6	53.77	NA
Extract	HYB 5	Laboratory	Spike #1	1	ND	0.504	0.568	NA
Extract	HYB 8	Laboratory	Spike #1	2	ND	0.509	0.509	NA
Extract	HYB 21	Laboratory	Spike #1	3	ND	0.537	0.537	NA
Extract	HYB 14	Laboratory	Spike #1	4	ND	0.524	0.552	NA
Extract	HYB 9	Laboratory	Spike #1	5	ND	0.585	0.641	NA
Extract	HYB 2	Laboratory	Spike #1	6	ND	0.576	0.583	NA
Extract	HYB 10	Laboratory	Spike #1	7	ND	0.52	0.659	NA
Extract	HYB 19	Laboratory	Spike #2	1	1.77	91.6	204.6	NA
Extract	HYB 15	Laboratory	Spike #2	2	2.25	91.8	204.8	NA
Extract	HYB 1	Laboratory	Spike #2	3	2.31	89.1	200.1	NA
Extract	HYB 20	Laboratory	Spike #2	4	2.43	100	213	NA
Extract	HYB 6	Laboratory	Spike #3	1	10.83	0.324	1060.32	NA
Extract	HYB 3	Laboratory	Spike #3	2	9.62	0.348	1080.35	NA
Extract	HYB 23	Laboratory	Spike #3	3	13.2	0.363	1060.36	NA

Sample Type	Sample Number	Measurement Location[b]	Sample Description	REP	Developer[c]	Reference Laboratory[d]		
					AhRBU	$TEQ_{D/F}$ (pg/g)	Total TEQ (pg/g)[e]	Total Concentration (ng/g)[f]
Extract	HYB 16	Laboratory	Spike #3	4	9.62	0.268	990.27	NA
Performance	HYB 172	Laboratory	Cambridge 5183	1	82.5	4.78	8.59	193
Performance	HYB 191	Laboratory	Cambridge 5183	2	80.9	4.08	8.41	193
Performance	HYB 127	Laboratory	Cambridge 5183	3	46.8	4.06	8.26	193
Performance	HYB 160	Laboratory	Cambridge 5183	4	93.1	3.56	7.8	193
Performance	HYB 76	Laboratory	Cambridge 5183	5	33.1	3.89	8.14	193
Performance	HYB 176	Laboratory	Cambridge 5183	6	46.4	5.93	9.79	193
Performance	HYB 114	Laboratory	Cambridge 5183	7	72.1	3.89	7.42	193
Performance	HYB 85	Laboratory	Cambridge 5184	1	18355	187	1267	31879
Performance	HYB 207	Laboratory	Cambridge 5184	2	16597	188	1308	31879
Performance	HYB 169	Laboratory	Cambridge 5184	3	19570	173	1313	31879
Performance	HYB 77	Laboratory	Cambridge 5184	4	15710	180	1340	31879
Performance	HYB 196	Laboratory	ERA Aroclor	1	24.4	36.4	1096.4	18893
Performance	HYB 54	Laboratory	ERA Aroclor	2	52	32.9	3722.9	18893
Performance	HYB 100	Laboratory	ERA Aroclor	3	52.2	37.9	3827.9	18893
Performance	HYB 25	Laboratory	ERA Aroclor	4	44.9	35.5	3835.5	18893
Performance	HYB 29	Laboratory	ERA Blank	1	23.2	0.0942	0.119	0.1
Performance	HYB 79	Laboratory	ERA Blank	2	44.1	0.0728	0.077	0.1
Performance	HYB 95	Laboratory	ERA Blank	3	49.6	0.237	0.240	0.1
Performance	HYB 122	Laboratory	ERA Blank	4	218	0.307	0.349	0.1
Performance	HYB 144	Laboratory	ERA Blank	5	40.3	0.113	0.136	0.1
Performance	HYB 83	Laboratory	ERA Blank	6	110	0.0524	0.072	0.1
Performance	HYB 206	Laboratory	ERA Blank	7	41.3	0.211	0.244	0.1
Performance	HYB 109	Laboratory	ERA Blank	8	85.7	0.0692	0.092	0.1
Performance	HYB 188	Laboratory	ERA PAH	1	2788	0.159	0.184	61170
Performance	HYB 82	Laboratory	ERA PAH	2	1661	0.141	0.145	61170
Performance	HYB 69	Laboratory	ERA PAH	3	2112	0.161	0.165	61170
Performance	HYB 39	Laboratory	ERA PAH	4	1739	0.248	0.274	61170
Performance	HYB 209	Laboratory	ERA PCB 100	1	36.2	0.0386	10.64	1
Performance	HYB 62	Laboratory	ERA PCB 100	2	32	NA[g]	NA[g]	1
Performance	HYB 28	Laboratory	ERA PCB 100	3	77	0.053	10.65	1
Performance	HYB 74	Laboratory	ERA PCB 100	4	38.2	0.127	10.08	1
Performance	HYB 150	Laboratory	ERA PCB 10000	1	33.8	0.204	1030.20	120
Performance	HYB 204	Laboratory	ERA PCB 10000	2	56.9	0.507	1030.51	120
Performance	HYB 66	Laboratory	ERA PCB 10000	3	32.3	0.105	1180.11	120
Performance	HYB 36	Laboratory	ERA PCB 10000	4	25.5	0.0628	1020.06	120
Performance	HYB 103	Laboratory	ERA TCDD 10	1	21.7	8.69	8.70	20
Performance	HYB 45	Laboratory	ERA TCDD 10	2	118	9.28	9.29	20

Sample Type	Sample Number	Measurement Location[b]	Sample Description	REP	Developer[c]	Reference Laboratory[d]		
					AhRBU	$TEQ_{D/F}$ (pg/g)	Total TEQ (pg/g)[e]	Total Concentration (ng/g)[f]
Performance	HYB 107	Laboratory	ERA TCDD 10	3	33.1	8.44	8.50	20
Performance	HYB 173	Laboratory	ERA TCDD 10	4	27.6	8.2	8.24	20
Performance	HYB 98	Laboratory	ERA TCDD 30	1	26.5	27.4	27.45	60
Performance	HYB 105	Laboratory	ERA TCDD 30	2	211	25.3	25.32	60
Performance	HYB 125	Laboratory	ERA TCDD 30	3	31.4	24.8	24.84	60
Performance	HYB 111	Laboratory	ERA TCDD 30	4	49.1	23.9	23.94	60
Performance	HYB 92	Laboratory	LCG CRM-529	1	702	NA[g]	NA[g]	NA
Performance	HYB 180	Laboratory	LCG CRM-529	2	867	6930	7335	NA
Performance	HYB 32	Laboratory	LCG CRM-529	3	671	6900	7398	NA
Performance	HYB 117	Laboratory	LCG CRM-529	4	813	7190	7546	NA
Performance	HYB 57	Laboratory	NIST 1944	1	38927	237	277.1	2481
Performance	HYB 70	Laboratory	NIST 1944	2	41758	206	249.7	2481
Performance	HYB 131	Laboratory	NIST 1944	3	65899	252	294.1	2481
Performance	HYB 167	Laboratory	NIST 1944	4	59314	219	260	2481
Performance	HYB 138	Laboratory	Wellington WMS-01	1	902	68	78.6	NA
Performance	HYB 115	Laboratory	Wellington WMS-01	2	1017	65.7	75.1	NA
Performance	HYB 192	Laboratory	Wellington WMS-01	3	777	61.9	71.52	NA
Performance	HYB 55	Laboratory	Wellington WMS-01	4	997	66.1	75.17	NA
Performance	HYB 26	Laboratory	Wellington WMS-01	5	754	68	78.3	NA
Performance	HYB 129	Laboratory	Wellington WMS-01	6	1292	65.7	75.32	NA
Performance	HYB 31	Laboratory	Wellington WMS-01	7	1048	65.4	75.08	NA

[a] Due to the state of development of the Hybrizyme technology, which is intended to rank samples by concentration of AhR binding compounds, the Hybrizyme results were not compared to HRMS TEQ values in this report. The HRMS TEQ data are provided in this appendix for convenient reference and document consistency.

[b] 110 samples were analyzed in the field by Hybrizyme, but the sample results were repeated and reported from laboratory analysis.

[c] Data listed exactly as reported by the developer.

[d] Qualifier flags (e.g., J and K flags) included in the raw data have been removed for the purposes of statistical analysis.

[e] Data calculated by summing TEQ_{PCB} and $TEQ_{D/F}$.

[f] Total concentration values for environmental samples are the sum of reference laboratory total D/F concentration, reference laboratory total PCB concentration and PAH concentration. Total concentration values for PE samples were calculated using certified data. Samples where PAH data were not available are listed as "NA."

[g] Reference laboratory data was discarded due to laboratory sample preparation error.

ND = nondetect