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Demonstration and Quality Assurance Project Plan

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

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

Prepared by

Battelle Memorial Institute
505 King Avenue
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Concurrence Signatures

The primary purpose of the demonstration is to evaluate measurement technologies for dioxin in soil and sediment based on their performance and cost as compared to conventional, off-site laboratory analytical methods. The demonstration will take place under the sponsorship of the U.S. Environmental Protection Agency Superfund Innovative Technology Evaluation Program.

This document is intended to ensure that all aspects of the demonstration are documented and scientifically sound and that operational procedures are conducted in accordance with quality assurance and quality control specifications and health and safety regulations.

The signatures of the individuals specified below indicate their concurrence and agreement to operate in compliance with the procedures specified in this document.

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Notice

This document was prepared for the U.S. Environmental Protection Agency (EPA) Superfund Innovative Technology Evaluation Program under Contract No. 68-C-00-185. The document has met the EPA's requirements for peer and administrative review and has been approved for publication. Mention of corporation names, trade names, or commercial products does not constitute endorsement or recommendation for use.

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 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 (RCRA) 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 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 Monitoring and Measurement Technology Program is managed by the Office of Research and Development's Environmental Sciences Division in Las Vegas, Nevada.

Gary Foley, Ph.D.
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Abstract

A demonstration of technologies for determining the presence of dioxin and dioxin-like compounds in soil and sediment will be conducted under the U.S. Environmental Protection Agency's Superfund Innovative Technology Evaluation Program in Saginaw, Michigan, at Green Point Environmental Learning Center from April 26 to May 5, 2004. The primary purpose of the demonstration is to evaluate innovative monitoring and measurement technologies. The technologies listed below will be demonstrated.

- AhRC PCR™ Kit, Hybrizyme Corporation
- Ah-IMMUNOASSAY® Kit, Paracelsian, Inc.
- Coplanar PCB Immunoassay Kit, Abraxis LLC
- DF-1 Dioxin/Furan Immunoassay Kit, CAPE Technologies L.L.C.
- CALUX® by Xenobiotic Detection Systems, Inc.
- Dioxin ELISA Kit, Wako Pure Chemical Industries, Ltd.

This demonstration plan describes the procedures that will be used to verify the performance and cost of these technologies. The plan incorporates the quality assurance and quality control elements needed to generate data of sufficient quality to document each technology's performance and cost. A separate innovative technology verification report (ITVR) will be prepared for each technology. The ITVRs will present the demonstration findings associated with the demonstration objectives.

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Abbreviations, Acronyms, and Symbols

AhR	aryl hydrocarbon receptor
ARNT	aryl hydrocarbon receptor nuclear translocator
ASE	accelerated solvent extraction
ATSDR	Agency for Toxic Substances and Disease Registry
CALUX®	Chemical-Activated Luciferase Expression®
CAL/VER	Calibration verification test run
COC	chain of custody
DEQ	dioxin equivalent
DER	data evaluation report
D/F	dioxin/furan
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNR	Department of Natural Resources
DRE	dioxin responsive elements
EIA	enzyme immunoassay
ELC	Environmental Learning Center
ELISA	enzyme-linked immunosorbent assay
EMPC	estimated maximum possible concentration
EPA	Environmental Protection Agency
fp	false positive
fn	false negative
g	gram
GC	gas chromatography

HDPE	high-density polyethylene
HPLC/GPC	high-performance liquid chromatography/gel permeation chromatography
HRGC	high-resolution capillary gas chromatography
HRMS	high-resolution mass spectrometry
HRP	horseradish peroxidase
H&S	health and safety
IPR	initial precision and recovery
I-TEF	International Toxicity Equivalency Factor
ITVR	innovative technology verification report
LDPE	low-density polyethylene
LRMS	low-resolution mass spectrometry
µL	microliter
mL	milliliter
MDEQ	Michigan Department of Environmental Quality
mm	millimeter
MDL	method detection limit
MMT	Monitoring and Measurement Technology
MS	mass spectrometry
ND	non-detect
NERL	National Exposure Research Laboratory
nm	nanometer
NOAA	National Oceanic and Atmospheric Administration
OD	optical density
OPR	ongoing precision and recovery
ORD	Office of Research and Development
PAH	polycyclic aromatic hydrocarbons
PAR	Precision and Recovery Standard
PC	positive control
PCB	polychlorinated biphenyl

PCDD/F	polychlorinated dibenzo-p-dioxin/dibenzofuran
PCDH	polychlorinated diaromatic hydrocarbon
PCP	pentachlorophenol
PCR	poplymerase chain reaction
PE	performance evaluation
pg	picogram
POD-conjugate	peroxides conjugated with a dioxin analog
ppm	parts per million; microgram/g; µg/g
ppb	parts per billion; nanogram/g; ng/g
ppt	parts per trillion; picogram/g; pg/g
QA/QC	quality assurance/quality control
RPD	relative percent difference
rpm	revolution per minute
RSD	relative standard deviation
SDL	sample-specific detection limit
SIM	selected ion monitoring
SITE	Superfund Innovative Technology Evaluation
S:N	signal to noise ratio
SOP	standard operating procedure
TCDD	tetrachlorodibenzo-p-dioxin
TEF	toxicity equivalency factor
TEG	tetraethylene glycol
TEQ	toxicity equivalent
TOC	total organic carbon
total $TEQ_{D/F}$	total toxicity equivalents of dioxins/furans
total TEQ_{PCB}	total toxicity equivalents of World Health Organization polychlorinated biphenyls
total TEQ	total toxicity equivalents including the sum of the dioxin/furan and World Health Organization polychlorinated biphenyls
TSA	technical systems audit

WHO	World Health Organization
XDS	Xenobiotic Detection Systems

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Battelle acknowledges the advice and support of the following individuals in preparing this document: Stephen Billets, George Brilis, and Brian Schumacher of the U.S. Environmental Protection Agency's National Exposure Research Laboratory; Randy Allen of Hybrizyme Corporation; Noriyoshi Inoue of Paracelsian, Inc.; Fernando Rubio, Abraxis, LLC; Gary Hinshaw of Environmental Assurance Monitoring; Bob Harrison of CAPE Technologies L.L.C.; Andrew Chu, Jeff Sturkey, George Clark, and John Gordon of Xenobiotic Detection Systems, Inc.; and Hiroyuki Hayashi, Masako Hayakawa, and Emmy Leung of Wako Pure Chemical Industries, Ltd. Battelle acknowledges the support of the Dioxin SITE Demonstration Panel for their technical input to the plan and their review of this document. In particular, we recognize Michael Jury, Sue Kaelber-Matlock, and Al Taylor from the Michigan Department of Environmental Quality; Becky Goche and Doug Spencer of the U.S. Fish and Wildlife Service; and Andy Beliveau, Nardina Turner, Greg Rudloff, Allen Debus, Craig Smith, David Williams, Dwain Winters, Jon Josephs, Bob Mouringhan, Terry Smith, and Joe Ferrario of the U.S. Environmental Protection Agency.

Executive Summary

Performance verification of innovative environmental technologies is an integral part of the regulatory and research mission of the U.S. Environmental Protection Agency (EPA). The Superfund Innovative Technology Evaluation (SITE) Program was established by the EPA Office of Solid Waste and Emergency Response and the Office of Research and Development under the Superfund Amendments and Reauthorization Act of 1986. 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 innovative technologies and gather reliable performance and cost information to support site characterization and cleanup activities, and (3) develop procedures and policies that encourage use of innovative technologies at Superfund sites as well as other waste sites or commercial facilities. The intent of a SITE demonstration is to obtain representative, high-quality performance and cost data on innovative technologies so that potential users can assess a given technology's suitability for a specific application.

This plan summarizes the demonstration activities that will be conducted during the SITE Demonstration of monitoring and measurement technologies for dioxin and dioxin-like compounds. The demonstration will be conducted in Saginaw, Michigan, from April 26 to May 5, 2004. The demonstration is being conducted under the Monitoring and Measurement Technology Program, which is administered by the Environmental Sciences Division of the EPA's National Exposure Research Laboratory in Las Vegas, Nevada. The following six technologies will be demonstrated:

- AhRC PCR™ Kit, Hybrizyme Corporation
- Ah-IMMUNOASSAY® Kit, Paracelsian, Inc.
- Coplanar PCB Immunoassay Kit, Abraxis LLC
- DF-1 Dioxin/Furan Immunoassay Kit, CAPE Technologies L.L.C.
- CALUX® by Xenobiotic Detection Systems, Inc.
- Dioxin ELISA Kit, Wako Pure Chemical Industries, Ltd.

The performance and cost of each technology will be compared to those of conventional, off-site laboratory analytical methods. The performance and cost characteristics of one technology will not be compared to those of another technology. A separate innovative technology verification report (ITVR) will be prepared for each technology.

The demonstration has both primary and secondary objectives. The primary objectives are critical to the technology evaluation and require use of quantitative results to draw conclusions regarding technology performance. The secondary objectives pertain to information that is useful but does not necessarily require use of quantitative results to draw conclusions regarding technology performance.

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

P1. Determine the accuracy.
P2. Determine the precision.

P3. Determine the comparability of the technology to the reference laboratory methods.
P4. Determine the method detection limit (MDL).
P5. Determine the frequency of false positive and false negative results.
P6. Evaluate the impact of matrix effects.
P7. Estimate costs associated with the technology.

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

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

To address the demonstration objectives, both environmental and performance evaluation (PE) samples will be analyzed during the demonstration. The environmental samples will be collected from multiple sampling locations around the country so that a diverse population of environmental samples, with varying sources and contaminant concentrations, are represented. The PE samples will include certified, spiked, and blank samples that will be obtained from commercial providers. Upon completion of the demonstration, the technology and reference method results will be compared to evaluate the performance and associated cost of each technology. The ITVRs for the six technologies will be submitted to EPA for publication in December 2004.

Chapter 1
Introduction

The U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), National Exposure Research Laboratory (NERL) has contracted with the Battelle Memorial Institute (Battelle, Columbus, Ohio) to conduct a demonstration of monitoring and measurement technologies for dioxin and dioxin-like compounds in soil and sediment. The demonstration is being conducted as part of the EPA Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) Program from April 26 to May 5, 2004, in Saginaw, Michigan. The purpose of this demonstration is to obtain reliable performance and cost data on the technologies in order 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 demonstration plan describes the procedures that will be used to verify the performance of each measurement technology. The plan also incorporates a site health and safety plan and the quality assurance and quality control (QA/QC) elements needed to ensure that data of sufficient quality is generated to document each technology's performance. This plan has been prepared using the NERL's "A Guidance Manual for the Preparation of Site Characterization and Monitoring Technology Demonstration Plans"[(1)] and in accordance with the EPA National Risk Management Research Laboratory's "Quality Assurance Project Plan Requirements for Applied Research Projects."[(2)]

This demonstration plan describes the SITE Program, the scope of the demonstration, and the definition of dioxin and dioxin-like compounds (Chapter 1); the demonstration organization and responsibilities of the participants (Chapter 2); the six technologies that will be demonstrated (Chapter 3); sample collection, sample homogenization, and sample handling procedures (Chapter 4); the demonstration site and the sampling locations (Chapter 5); the demonstration approach, including the objectives, experimental design, data analysis procedures, and the demonstration schedule (Chapter 6); the confirmatory process, including the reference methods and the reference laboratory that will be used during the demonstration (Chapter 7); the data management procedures (Chapter 8); the QA/QC procedures (Chapter 9); the health and safety plan (Chapter 10); and references (Chapter 11).

1.1 Description of the SITE 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 SITE 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 innovative technologies and gather reliable performance and cost information to support site characterization and cleanup 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 intent of a SITE demonstration is to obtain representative, high-quality performance and cost data on one or more innovative technologies so that potential users can assess a given technology's suitability for a specific application. 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 other conventional 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. The Technology Transfer Program also supports the technologies by offering technical assistance, training, and workshops.

The demonstration of monitoring and measurement technologies for dioxin and dioxin-like compounds is being conducted as part of the MMT Program, which provides developers of innovative sampling, monitoring, and measurement technologies with an opportunity to demonstrate their technology's performance under actual field conditions. These technologies may be used to sample, detect, monitor, or measure hazardous and toxic substances in water, soil, soil gas, and sediment. The technologies include chemical sensors for *in situ* (in place) measurements, groundwater, soil, and sediment samplers, field-portable analytical equipment, and other systems that support field sampling and analysis.

The MMT Program promotes acceptance of technologies that can be used to (1) accurately assess the degree of contamination at a site, (2) provide data to evaluate potential effects on human health and the environment, (3) apply data to assist in selecting the most appropriate cleanup action, and (4) monitor the effectiveness of a remediation process. The program places a high priority on innovative technologies that provide more cost-effective, faster, or safer methods for producing real-time or near-real-time data than conventional, laboratory-based technologies. These innovative technologies are demonstrated under field conditions, and the results are compiled, evaluated, published, and disseminated by the ORD. The primary objectives of the MMT Program are as follows:

- Test and verify the performance of field sampling and analytical technologies that enhance sampling, monitoring, and site characterization capabilities

- Identify performance attributes of innovative technologies to address field sampling, monitoring, and characterization problems in a more cost-effective and efficient manner

- Prepare fact sheets, brochures, bulletins, newsletters, and other technical publications that enhance acceptance of these technologies for routine use.

The MMT Program is administered by the Environmental Sciences Division of the NERL in Las Vegas, Nevada. The NERL is the EPA's center for investigation of technical and management approaches for identifying and quantifying risks to human health and the environment. The NERL's mission components include (1) developing and evaluating methods and technologies for sampling, monitoring, and characterizing water, air, soil, and sediment; (2) supporting regulatory and policy decisions; and (3) providing the technical support needed to ensure effective implementation of environmental regulations and strategies. By demonstrating selected innovative field measurement technologies for dioxin, the MMT Program is supporting the development and evaluation of methods and technologies for field measurement of dioxin and dioxin-like compounds in a variety of soil and sediment matrices.

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, exploring leads from technology developers and industry experts, and reviewing responses to Commerce Business Daily announcements. 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, their 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 requirements for conducting the demonstration; and (4) select and provide the reference laboratory involved 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 QA/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 the performance, cost, advantages, limitations, and field applicability of each technology.

As part of the third step of the technology verification process, EPA publishes a verification statement and a detailed evaluation of each technology in an innovative technology verification report (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 is not published as an EPA document, but an unpublished 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 distributes fact sheets, newsletters, brochures, bulletins and ITVRs through direct mailings, at conferences, and on the Internet. Information on the SITE Program are available on the EPA ORD web site (http://www.epa.gov/ORD/SITE). Additionally, a Visitor's Day is held in conjunction with the demonstration so that potential users can have a first-hand look at the technologies in operation.

1.2 Scope of Demonstration

Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans, commonly referred to collectively as "dioxins," are of significant concern in site cleanup projects and human health assessments because they are highly toxic. Conventional analytical methods for determining dioxin concentrations 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 dioxin contamination at a site and could be used to direct or monitor cleanup activities. More rapidly acquired 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. More affordable and

quicker analytical techniques will not replace HRMS, but will complement an enhanced sampling design. However, before adopting an innovative alternative to traditional laboratory-based methods, an assessment of how commercially available technologies compare to conventional laboratory-based analytical methods using certified, spiked, and environmental samples is warranted.

The purpose of the demonstration is to evaluate measurement technologies for dioxin and dioxin-like compounds in soil and sediment in order to provide (1) potential users with a better understanding of each technology's performance and cost under well-defined field conditions and (2) developers with documented results that will assist them in promoting acceptance and use of their technologies. To meet these demonstration objectives, samples will be collected from a variety of dioxin-contaminated soil and sediment sampling locations around the country. Samples will be identified and supplied through several EPA Regional offices. The samples will be homogenized and characterized prior to use in the demonstration so that a variety of environmentally derived dioxin-contaminated samples with concentrations over a large dynamic range (< 50 to > 10,000 picogram/gram [pg/g]) can be analyzed. Six measurement technologies for dioxin and dioxin-like compounds will participate in a demonstration at a field site in Saginaw, Michigan, in April 2004. The technologies will be operated by the developers in mobile laboratories or construction trailers equipped with fume hoods at the site. Draft ITVRs are planned to be available for developer review in September 2004, for peer review in October 2004, and final ITVRs will be submitted to the EPA for publication in December 2004.

1.3 Definition of Dioxin and Dioxin-Like Compounds and Toxicity Equivalents

Dioxins and furans are halogenated polynuclear aromatic hydrocarbons that are considered toxic. Dioxins and furans are similar in structure as shown in Figure 1-1 and 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. 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 harmful. A total of seven dioxin and 10 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 the most toxic and serves as the marker compound for this class.

For risk assessment purposes, estimates of the toxicity of samples that contain a mixture of dioxin and furan congeners are often expressed as toxicity equivalents (TEQ). TEQ is 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 1-1) provides an equivalency factor for each congener's toxicity relative to the toxicity of 2,3,7,8-TCDD. In the 1990s, the most widely

accepted system of TEQs, known as the International Toxicity Equivalency Factor (I-TEF) system, was proposed by the North Atlantic Treaty Organization Committee on Challenges to Modern Society.[3] In 1997, a World Health Organization (WHO) Expert Group reassessed TEFs using a tiered approach, in which the results of animal toxicity studies, especially those involving chronic or sub-chronic exposure, were given more weight than the results of *in vitro* or biochemical studies.[4] In addition to re-evaluating the TEFs for humans, the group also determined separate TEFs for birds and fish. The WHO TEFs presented in Table 1-1 were determined for mammalian species that are applicable for human risk assessment purposes. The total TEQ from dioxin and furans (Total $TEQ_{D/F}$) in a sample is calculated by adding up all of the TEQ values from the individual dioxin and furan congeners.

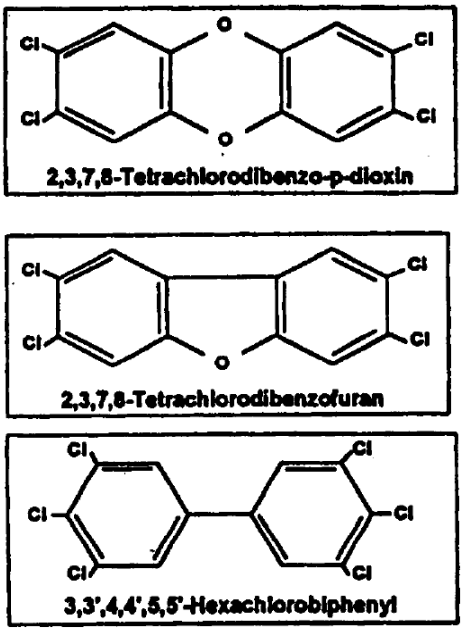

Figure 1-1. Structure of representative dioxin, furan, and PCB.

The close toxicological similarity of certain coplanar and mono-ortho substituted polychlorinated biphenyls (PCBs, see Figure 1-1) to dioxins has led to the extension of the TEF system to these "dioxin-like" PCBs. The dioxin-like PCBs, commonly referred to as, "WHO PCBs", that exhibit toxicity are coplanar or mono-ortho substituted and have structural and conformational similarities to dioxin compounds. Currently only 12 of the total 209 PCB congeners are thought to have dioxin-like toxicity; these 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 12 PCBs have been assigned TEF values and are routinely included in the calculation of TEQs in toxicity assessments. The total TEQ contribution from PCBs (referred to as total TEQ_{PCB}) is calculated by summing up the individual PCB TEQ values. The total TEQ in a sample is the sum of the total $TEQ_{D/F}$ and total TEQ_{PCB} values. TEQ concentrations for soils and sediments are typically reported in picogram per gram (pg/g) which is equivalent to parts per trillion.

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 Group noted that the TEF indicates an order of magnitude estimate of the toxicity of a compound relative to 2,3,7,8-TCDD.[4] The WHO Group reported that 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 non-additive effects when the congeners are present in complex mixtures. The WHO Group 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 aryl hydrocarbon (Ah) receptor binding compounds, such as polycyclic aromatic hydrocarbons (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 contribute to the total TEQ. This potentially can result in an underestimation of TEQs in environmental samples using the TEF approach. [4]

This demonstration was designed with the limitation of the TEQ concept in mind. The samples chosen contain a variety of combinations of dioxins, furans, and PCBs and at a wide range of concentration levels. Some samples are high in analytes with better understood TEFs, some are high in analytes with

TEFs that have more uncertainty. Some are high in other Ah receptor binding compounds such as polycyclic aromatic hydrocarbons (PAHs), while others are free of these possible TEQ contributing compounds. (Complete descriptions of the samples analyzed in this demonstration are in Chapter 5.) The purpose is to evaluate each of the technologies directly to the TEQ_{DF} and TEQ_{PCB} and assess how other factors which may contribute to a sample TEQ affect the technology's determination of TEQ_{DF} and TEQ_{PCB}. Each technology will be evaluated independently, and the statistical tests used will be described in that technology's ITVR.

Table 1-1. Toxicity Equivalency Factor Values

Compound[a]	TEF Value		Compound	TEF Value	
	I-TEF	WHO-TEQ [b]		I-TEF	WHO-TEQ
PCDDs			**PCDFs**		
2,3,7,8-TCDD	1	1	2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDD	0.5	1	1,2,3,7,8-PeCDF	0.05	0.05
			2,3,4,7,8-PeCDF	0.5	0.5
1,2,3,4,7,8-HxCDD	0.1	0.1	1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1	1,2,3,7,8,9-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1	1,2,3,6,7,8-HxCDF	0.1	0.1
			2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01	1,2,3,4,6,7,8-HpCDF	0.01	0.01
			1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDD	0.001	0.0001	OCDF	0.001	0.0001
Dioxin-like PCBs					
Coplanar			**mono-*ortho***		
3,3',4,4'-TCB (PCB 77)	0.0005	0.0001	2,3,3',4,4'-PeCB (PCB 105)	0.0001	0.0001
3,4,4',5-TCB (PCB 81)	—	0.0001	2,3,4,4',5-PeCB (PCB 114)	0.0005	0.0005
3,3',4,4',5-PeCB (PCB 126)	0.1	0.1	2,3',4,4',5-PeCB (PCB 118)	0.0001	0.0001
3,3',4,4',5,5'-HxCB (PCB 169)	0.01	0.01	2,3,4,4',5-PeCB (PCB 123)	0.0001	0.0001
			2,3,3',4,4',5-HxCB (PCB 156)	0.0005	0.0005
			2,3,3',4,4',5-HxCB (PCB 157)	0.0005	0.0005
			2,3',4,4',5,5'-HxCB (PCB 167)	0.00001	0.00001
			2,3,3',4,4'5,5'-HpCB (PCB 189)	0.0001	0.0001

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

[b] TEFs for human and mammals

1.4 Sources of Dioxins and Furans

Dioxins and furans enter the environment primarily by releases in the air by incineration that are subject to long-range transport and atmospheric deposition, by releases directly to land, by releases to water, and by occurrence in commercial products. The U.S. EPA has compiled a database of sources of dioxin-like compounds in the United States.[5] In this database, releases in air are by far the greatest contributors of dioxins and furans to the environment. Air releases come from municipal solid waste incinerators, backyard trash burning, medical waste incineration, secondary copper smelting, and cement kilns among others. Releases to land come from land application of sewage sludge and certain pesticides contaminated with dioxins and furans. Releases in water come largely from bleached pulp and paper mill processes. In addition, certain chemical products such as pentachlorophenol and various pesticides are contaminated with dioxin-like compounds as by-products or impurities.

1.5 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 from approximately $500 to $1,100 per sample per method, depending on the method selected, the level of QA/QC incorporated into the analyses, and reporting requirements.

1.5.1 High-Resolution Mass Spectrometry

EPA Method 1613, Revision B[6] and SW846 Method 8290 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 high-resolution capillary gas chromatography (HRGC)/HRMS analysis. The main differences between the two methods are that EPA Method 1613 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-gram (g) sample and 20-microliter (µL) final sample volume are presented in Table 1-2.

Table 1-2. Calibration Range of HRMS Dioxin/Furan Method

Compound	EPA Method 1613, Revision B	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

1.5.2 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/low-resolution mass

spectrometry (LRMS) analysis. The calibration ranges in Table 1-3 are based on a typical 10-g sample size and 100-µL final volume.

Table 1-3. 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

1.5.3 PCB Methods

There are more options for analysis of dioxin-like compounds such as PCBs. EPA Method 1668, Revision A[(7)] 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 co-eluting congeners. The calibration range for PCBs based on a typical 10-g sample and 50-µL final sample volume is 1 to 10,000 pg/g. PCBs also can be determined as specific congeners by GC/LRMS or as Aroclors[1] by GC/electron capture detection.

1.5.4 Summary of Analytical Methods for Dioxins and Dioxin-like Compounds

The analytical methods described in this section were all considered for use in this demonstration. Based on the data needs of this demonstration, EPA Method 1613B was selected as the reference method for dioxins and furans, and EPA Method 1668A was selected as the reference method for the WHO PCBs. Total $TEQ_{D/F}$ concentrations will be generated by Method 1613B and total TEQ_{PCB} concentrations will be generated by Method 1668A. These data will be summed to derive a total TEQ value for each sample. Prior to the start of the demonstration, characterization analysis by Method 1613B and LRMS modified Method 1668A will be used to determine which samples will be included in the study design. Additional discussion of the analytical methods will be presented in subsequent sections of this demonstration plan.

[1] Monsanto Corporation marketed products that were mixtures of 20 to 60 PCB congeners under the trade name Aroclor. Aroclor mixtures are identified by a number (e.g., Aroclor 1260) that represents the mixture's chlorine composition as a percentage (e.g., 60%).

Chapter 2
Demonstration Organization and Responsibilities

This chapter identifies key project personnel and summarizes their responsibilities in planning and executing the demonstration. Figure 2-1 is an organization chart that shows key project personnel and the lines of communication among them. Table 2-1 presents the key demonstration participants. During the demonstration, the participants will be asked to follow the health and safety procedures outlined in Chapter 10. However, each organization is directly and fully responsible for the health and safety of its own employees.

2.1 EPA Project Personnel

The EPA program manager, Stephen Billets, has overall responsibility for the project. Dr. Billets will review and concur with the project deliverables, including the demonstration plan, ITVRs, and DER. The EPA QA officer at the EPA NERL, George Brilis, is responsible for reviewing and concurring with the demonstration and quality assurance project plan. The roles for EPA in this demonstration include:

- Review and approve the demonstration plan.
- Review and approve the DER and ITVRs.
- Be present at the demonstration.
- Participate in Visitor's Day.
- Coordinate activities with the Battelle project manager.

2.2 Battelle Project Personnel

The Battelle project manager, Amy Dindal, is responsible for conducting day-to-day management of Battelle project personnel, maintaining direct communication with the EPA and the developers, and ensuring that all Battelle personnel involved in the demonstration understand and comply with the demonstration plan. Ms. Dindal is also responsible for distributing the draft and final demonstration plans to all key project personnel and for reviewing measurement and analytical data obtained during the demonstration. Battelle project personnel will assist Ms. Dindal in preparing project deliverables and in performing day-to-day project activities. In consultation with the EPA, Battelle project personnel are responsible for the following elements of the demonstration:

- Developing and implementing all elements of this demonstration plan.

- Scheduling and coordinating the activities of all demonstration participants.

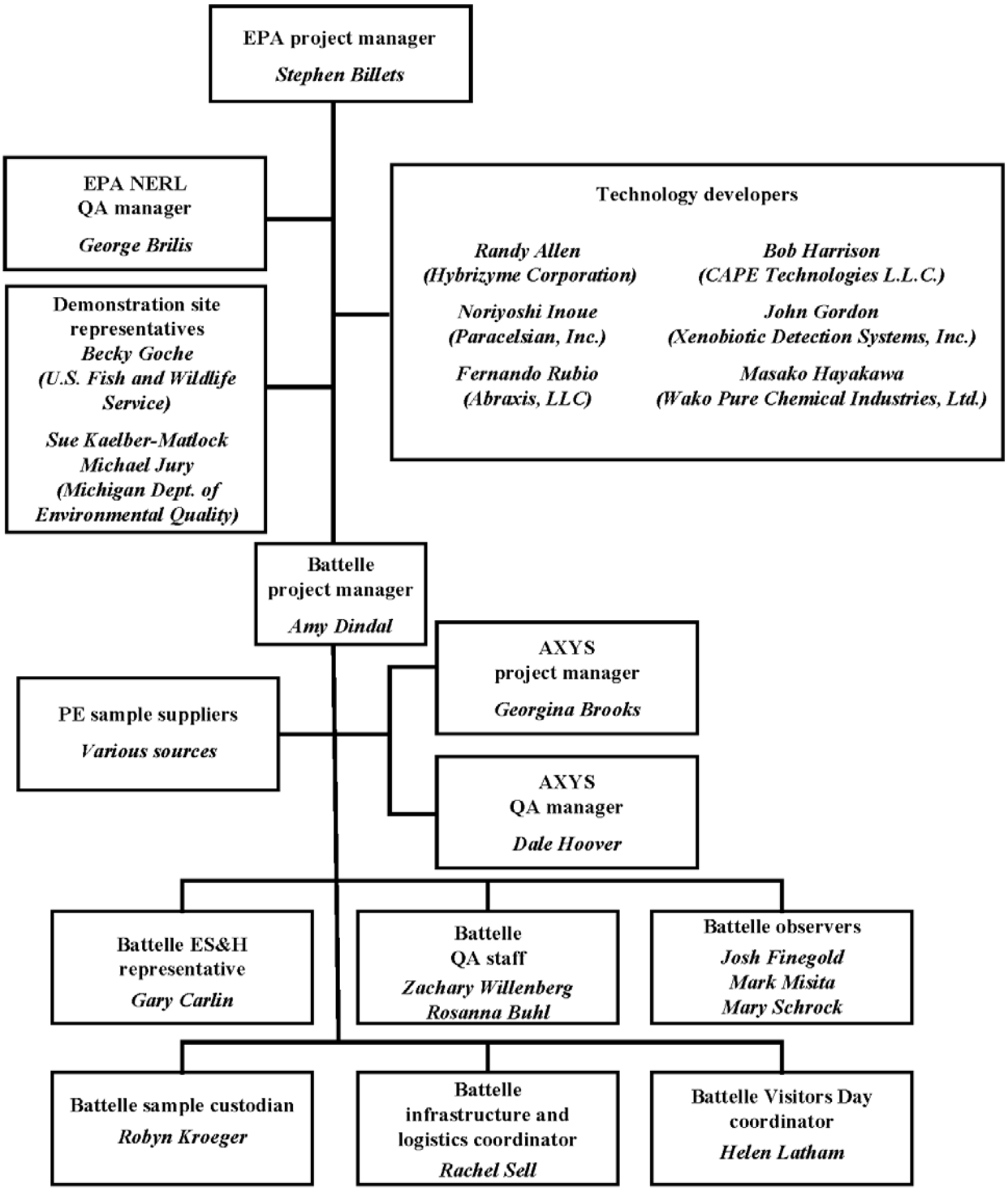

Figure 2-1. Organization chart for the dioxin demonstration.

Table 2-1. Demonstration Participants

Organization	Point of Contact	Contact Information
U.S. Environmental Protection Agency	Stephen Billets George Brilis	National Exposure Research Laboratory 944 East Harmon Avenue Las Vegas, Nevada 89119 Telephone: (702) 798-2232 Fax: (702) 798-2261 E-mail: billets.stephen@epa.gov
Battelle	Amy Dindal	505 King Avenue Columbus, Ohio 43201-2693 Telephone: (561) 422-0113 Fax: (561) 258-0777 E-mail: DindalA@battelle.org
Michigan Department of Environmental Quality	Sue Kaelber-Matlock Michael Jury	Remediation and Redevelopment Division 503 N. Euclid Avenue Bay City, Michigan 48706 Telephone: 989-686-8025, X 8303 Fax: 989-684-9799 E-mail: matlocks@michigan.gov
U.S. Fish and Wildlife Service	Becky Goche	Green Point Environmental Learning Center 3010 Maple Street Saginaw, Michigan 48602 Telephone: (989) 759-1669 E-mail: becky_goche@fws.gov
Abraxis LLC	Fernando Rubio	54 Steamwhistle Drive Warminster, Pennsylvania 18974 Telephone: (215) 357-3911 E-mail: frubio@abraxiskits.com
CAPE Technologies L.L.C.	Bob Harrison	3 Adams Street South Portland, Maine 04106-1604 Telephone: (207) 741-2995 E-mail: cape-tech@ceemaine.org
Hybrizyme Corporation	Randy Allen	Suite G-70 2801 Blue Ridge Road Raleigh, North Carolina 27607 Telephone: (919) 783-9595 E-mail: rallen@hybrizyme.com
Xenobiotic Detection Systems, Inc.	John Gordon	1601 E. Geer Street, Suite S Durham, North Carolina 27704 Telephone: (919) 688-4804 E-mail: johngordon@dioxins.com
Wako Pure Chemical Industries, Ltd.	Masako Hayakawa	1600 Bellwood Road Richmond, Virginia 23237-1326 Telephone: (877) 714-1920 E-mail: hayakawa.masako@wako-chem.co.jp
Paracelsian, Inc.	Noriyoshi Inoue	72 Hampton Road Scarsdale, New York 10583 Telephone: (914) 472-5152 E-mail: inomak@earthlink.net
AXYS Analytical Services	Georgina Brooks	2045 Mills Road Sidney, British Columbia, Canada V8L358 Phone: 250-655-5800 E-mail: gbrooks@axys.com

- Coordinating the collection of samples; performing sample homogenization; performing characterization analyses for dioxin/furans, PCBs, and PAHs; and sample aliquoting.

- Coordinating activities with the PE sample suppliers.

- Developing and maintaining sample control process and distributing samples during the demonstration.

- Auditing the reference laboratory (AXYS Analytical Services) to determine whether the operations are properly performed.

- Overseeing the operation of the developer technologies and documenting the operation of each technology during the demonstration.

- Summarizing, evaluating, interpreting, and documenting demonstration data for inclusion in the ITVRs and DER.

- Evaluating and reporting on the performance and cost of each technology.

- Preparing draft and final versions of six ITVRs (one for each technology).

- Preparing draft and final versions of the DER, consistent with the format and content of historical documents.

- Coordinating meetings among the EPA, the developers, and the demonstration panel (see Appendix A).

- Providing required planning, scheduling, cost control, documentation, and data management for field activities.

- Managing demobilization activities, including proper waste disposal.

- Immediately communicating any deviation from the demonstration plan during field activities to the EPA program manager and discussing appropriate resolutions of the deviation.

- Interfacing with the demonstration site representatives and making logistical preparations for the demonstration.

Tasks for specific Battelle staff will include:

- Battelle's ES&H representative, Gary Carlin, or his designee, will review the site-specific health and safety procedures and will audit field procedures during the demonstration to ensure compliance with the health and safety procedures presented in Chapter 10 of the demonstration plan. Mr. Carlin will also train the participants to the site safety and health plan on the first day of the demonstration.

- Battelle's QA manager, Zachary Willenberg, is responsible for overall project QA. Mr. Willenberg will be available to resolve any project-specific QA issues and will conduct an in-field technical systems audit (TSA) to assess whether Battelle is performing the demonstration activities in accordance with this demonstration plan. Mr. Willenberg, or his designee, will also review the

reference laboratory data packages. Battelle QA Auditor Rosanna Buhl will lead the on-site TSA at the reference laboratory during sample analysis.

- Rachel Sell will be Battelle's infrastructure and logistics coordinator for the demonstration. Ms. Sell will be responsible for coordinating details with the site representatives and for securing all of the equipment (such as rental of the mobile laboratories) and supplies (such as rental of gas cylinders) for the demonstration.

- Three Battelle observers will each be assigned to two technologies. The observers will be responsible for observing the technologies in operation during the demonstration and understanding the technical and operational features of the technology which is assigned to them. Josh Finegold will be responsible for observing Abraxis and CAPE Technologies. Mary Schrock will be responsible for observing Hybrizyme and Xenobiotic Detection Systems. Mark Misita will be responsible for observing Wako and Paracelsian.

- Robyn Kroeger will be Battelle's Sample Custodian. Ms. Kroeger will be responsible for filling and labeling all of the soil, sediment, and extract samples prior to the demonstration and for distributing the samples during the demonstration. Ms. Kroeger will also be responsible for archiving the samples after the demonstration activities have concluded.

- Helen Latham will coordinate the Visitor's Day activities. Ms. Latham will be responsible for ensuring that all guests during the Visitor's Day have signed in and received a name badge. Ms. Latham will also be responsible for distribution of handouts, managing of media organizations who attend the Visitor's Day, and photo-documentation of Visitor's Day activities.

2.3 Developer Personnel

The developers of the six technologies are responsible for providing, mobilizing, operating, and demobilizing their respective technologies at the demonstration site. The developer responsibilities include the following:

- Provide Battelle with information on the technologies.

- Review and concur with the demonstration plan.

- Notify Battelle in writing of technology-specific requirements, such as the type of power supply and the amount of work space needed, so that proper arrangements can be made for field demonstration of the technologies.

- Provide the personnel and all supplies needed for demonstration of the technologies unless otherwise arranged in advance with Battelle.

- Analyze the samples specified in the demonstration plan.

- Analyze developer-specified QC samples (for example, blanks or standards) in accordance with the technology specifications.

- Provide technology-specific demonstration results to Battelle at the end of the demonstration.

- Review and comment on the technology-specific ITVRs.

- Conduct all activities in accordance with the schedule to ensure a timely completion of the final reports.

2.4 Demonstration Site Representatives

The representatives for the demonstration site are Becky Goche, U.S. Fish and Wildlife Service and Sue Kaelber-Matlock and Michael Jury from Michigan Department of Environmental Quality (MDEQ). All work performed at each demonstration site will be scheduled, coordinated, and conducted with the permission of the demonstration site representatives, who will be the primary contacts for Battelle. Ms. Goche is responsible for obtaining the site access and submitting the request for the special use permit which is necessary for performing the demonstration at the site (see Appendix B). Mr. Jury is responsible for establishing adequate electrical power at the demonstration site and coordinating other logistics details with Ms. Sell. Ms. Kaelber-Matlock is responsible for participating in Visitor's Day. The demonstration site representatives are also responsible for reviewing and concurring with the demonstration plan.

2.5 Reference Laboratory Personnel

The reference laboratory for the project, AXYS Analytical Services, Ltd. (Sidney, British Columbia, Canada) will perform laboratory analyses. The laboratory project manager, Georgina Brooks, is responsible for overall planning, scheduling, budgeting, and reporting of laboratory activities. Mr. Dale Hoover is the AXYS QA manager. All work will be conducted under the direct supervision of Ms. Brooks. AXYS is also responsible for reviewing and concurring with the demonstration plan, and Ms. Brooks will immediately discuss appropriate resolutions of any deviation from the reference laboratory activities specified in the plan with the Battelle project manager. The impact of any deviations will be discussed with the EPA program manager.

2.6 Suppliers of Performance Evaluation Samples

The performance evaluation (PE) samples will be supplied from various sources (see Section 6.2.1). This will include purchasing standard reference materials and preparation of spiked samples. All activities, including purchasing standard reference materials and spiked sample preparation, will be conducted under the direct supervision of the Battelle project manager.

Chapter 3
Developer Technology Descriptions

This chapter contains technology descriptions for each of the six technologies that are participating in the demonstration. This information was provided by the developers with only editorial changes made by Battelle to ensure consistency and the needs of this document.

3.1 CAPE Technologies DF1 Dioxin/Furan Immunoassay Kit

The DF1 Dioxin/Furan Immunoassay Kit from CAPE Technologies is an enzyme immunoassay (EIA) test kit containing a polyclonal antibody specific for PCDD/Fs. Both semiquantitative screening and quantitative analysis are possible with the DF1 Dioxin/Furan Immunoassay Kit. Samples can be prepared for analysis by EIA using a variety of methods. Extracts of soil, sediment, food, water, fly ash, stack gas, tissue, or other samples that have been prepared by conventional extraction methods can be exchanged to a water-miscible solvent system for analysis using the CAPE immunoassay kit. The technology description, operating procedure, and advantages and limitations presented below are based on information provided by CAPE Technologies L.L.C.

Figure 3-1. CAPE Technologies DF1 Dioxin/Furan Immunoassay Kit.

3.1.1 Technology Description

The DF1 Dioxin/Furan Immunoassay Kit (Figure 3-1) is designed to analyze samples according to their TEQ concentrations by responding to the toxic PCDD/F congeners in approximate correlation with their TEFs. The test is capable of multiple congener recognition and preferentially targets congeners with high TEF values, i.e., those with the highest toxicity relative to 2,3,7,8-TCDD. The specificity of the test is predominantly for PCDD/Fs that contain 3 to 6 chlorines, with a strong preference for the 2,3,7,8 chlorinated congeners. This specificity roughly parallels the TEF values of the individual PCDD/F congeners. PCDD/Fs are typically extracted with organic solvents that are incompatible with EIA; therefore, a solvent exchange is required. PCDD/Fs have very low volatility and are retained during this solvent exchange in a small volume of a keeper solution (Triton X-100 detergent in tetraethylene glycol [TEG]) after evaporation of the original solvent. Methanol is added to dilute this solution, and the methanol-TEG-Triton mixture is added directly to the EIA tubes. The solubility of PCDD/Fs in methanol is augmented significantly by adding TEG and Triton X-100.

During the first EIA incubation, PCDD/Fs are specifically bound by the anti-dioxin antibodies, which have been immobilized on the EIA tube surface. After washing away the unbound material, the bound PCDD/Fs remain, and a competitor-horseradish peroxidase (HRP) conjugate is added. Bound PCDD/Fs occupy the dioxin binding sites of the antibodies in proportion to the PCDD/F content of the sample and prevent binding of the competitor-HRP conjugate. After a short incubation, unbound conjugate is removed, and the test tubes are washed thoroughly. The amount of conjugate bound by the anti-dioxin antibody is inversely related to the amount of PCDD/Fs originally present in the sample. Finally, a solution of chromogenic HRP substrate and hydrogen peroxide is added to the test tubes. Color development is directly proportional to enzyme concentration and inversely related to the PCDD/F concentration in the original sample. The test tubes are analyzed using a tube reader or spectrophotometer to measure the optical density (OD). The OD values of unknown samples are compared to the OD values of standards to determine the level of PCDD/Fs in the samples.

The final measured EIA response is the sum of the individual congener responses, which correlates with TEQ because the immunoassay cross-reaction profile for PCDD/Fs correlates with TEF values. Accuracy among samples may vary solely because of the variability of congener composition. To maximize accuracy, the variability of congener composition in the target sample population should be known. The best performance is achieved when all samples are from a single group that shares as many properties as possible (common source of contamination, similar congener composition, similar sample matrix, etc.).

The limit of detection for the DF1 Dioxin/Furan Immunoassay Kit claimed by CAPE Technologies is 4 pg of dioxin, which is sufficient for analysis at 500 parts per trillion (pg/g) using extract equivalent to 20 milligrams of sample. Sensitivity can be increased by adding more sample extract to the cleanup procedure and subsequently to the EIA. For example, by loading the EIA tube with the equivalent of 1 g of prepared sample, analysis can be performed at approximately 10 pg/g. When using increased sample loads, the manufacturer's recommendations for extract cleanup must be followed closely. Results must be related to the original sample concentration by back calculation using the proper dilution and volume factors. Matrix detection limits will vary according to matrix, sample size, and dilution factor. Up to 40 samples a day can be analyzed using the procedure described below.

DF1 immunoassay kit starter packages include the following:

- DF1-ST-A, a small starter package containing two DF1-12 kits (40 antibody-coated tubes and matching liquid reagents), one Grip-Rack, and one set of dioxin standards, plus two check samples of dioxin in toluene made by Wellington Labs.

- DF1-ST-B, a large starter package containing one DF1-60 kit (100 antibody-coated tubes and matching liquid reagents), one Grip-Rack, and one set of dioxin standards, plus two check samples of dioxin in toluene made by Wellington Labs.

After the purchase of one starter package, subsequent purchases are either the DF1-12 or the DF1-60 (described below). These kits do not include dioxin standards and check samples, which must be ordered separately. The DF1-12 kit for screening analysis of 12 samples includes 20 antibody-coated tubes and matching liquid reagents. The DF1-60 kit for screening analysis of 60 samples includes 100 antibody-coated tubes and matching liquid reagents. Figure 3-2 is a matrix describing the use of the DF-1 immunoassay kit.

ANALYTICAL OPTIONS MATRIX

CAPE Technologies Analytical Options	Option 1- customer performs extraction, cleanup, and analysis	Option 2- customer performs extraction, CAPE Technologies performs cleanup and analysis	Option 3- CAPE Technologies performs extraction, cleanup and analysis
cost	lowest, in the $70-90 per sample range for immunoassay and sample prep kits; final number depends on overhead and amount of QA performed	higher than option 1, typically in the $125-250 per sample range, but quoted on an individual job basis	higher than option 2, typically in the $150-300 per sample range, but quoted on an individual job basis
turnaround time	fastest; on-site use is possible; same or next day results, totally dependent on customer	customer extraction plus shipping plus CAPE turnaround (standard = 10 business days, rush = 5 days, priority = 2 days from receipt of samples)	shipping plus CAPE turnaround (standard = 10 business days, rush = 5 days, priority = 2 days from receipt of samples)
method specific training	training by Powerpoint file or in person; kit starter package for training and proficiency demo	none, but need to follow extraction instructions	none required
experience and expertise	chemist preferred, some analytical experience essential, immunoassay experience helpful	little required	none required
shipping of kits to customer	immunoassay and sample prep kits ship by commercial courier in days	extraction kits ship by commercial courier in days	none required
shipping of samples to CAPE Technologies	none required	organic solvent extracts reduced to small volume solid keeper can travel by commercial courier with no restrictions	possible import restrictions

Figure 3-2. DF1 immunoassay kit analytical options. Various options exist for using the DF1 Immunoassay Kit, including having samples analyzed by the customer or by CAPE Technologies. It should be noted that the customer should have technical experience with immunoassay techniques and *must* perform this analysis in an analytical laboratory or some other location that can provide the necessary equipment and infrastructure (i.e., fume hood, vortex mixer, sample evaporation system, etc.).

3.1.2 Operating Procedure

To ensure accurate and reliable results, every effort should be made to perform the dioxin/furan immunoassay at temperatures between 20°C (68°F) and 25°C (77˚F). The following is one option for sample preparation and was designed for same-day analysis at 500 pg/g, using a one-step cleanup. Other sample preparation options are available for lower concentrations. All sample extraction and extract cleanup components are in a kit form and are disposable.

1. Sodium sulfate is added to a soil sample and mixed. Dimethylformamide (DMF) is added to the soil sample, and the soil is extracted by shaking for two hours. The supernatant DMF extract is removed. DMF extracts are stable from weeks to months at room temperature.

2. Interferences are removed by chemical oxidation. Hexane is added to an aliquot of the DMF extract, then treated with 15% SO_3 in concentrated H_2SO_4 (fuming sulfuric acid). The supernatant hexane is removed and exchanged to a water-miscible organic solvent solution. This hexane-based fuming sulfuric acid cleanup is sufficient for most samples; but, in certain circumstances, an additional cleanup step may be required. This is the case for samples that contain large amounts of non-volatile

aliphatic oils. When the DMF extracts of such soils are cleaned using fuming sulfuric acid, the oil is not oxidized; it remains after evaporation of the hexane, causing a biphasic system when introduced to the EIA first incubation. Such EIA samples appear opalescent or milky, and their results are invalid because the biphasic system prevents capture of analyte by the antibody. For these samples, a new aliquot of DMF extract is cleaned by carbon adsorption. In this case, the final solvent in the cleanup procedure is toluene rather than hexane.

3. The cleaned sample in hexane or toluene is exchanged to a water-miscible organic solvent solution for EIA analysis. PCDD/Fs have very low volatility and are retained during this solvent exchange in Triton X-100 detergent in TEG after evaporation of the original solvent. Methanol is added to dilute this solution, and the methanol-TEG-Triton mixture is added directly to the EIA tubes.

4. An accurately measured volume of negative control, standard, or prepared sample is mixed with an aqueous sample diluent in test tubes with anti-dioxin antibody immobilized on the surface. The mixture is then incubated.

5. After incubation, antibody tubes are washed, and 0.5 mL of HRP competitor conjugate is added to each tube using a repeater pipettor. Bound PCDD/Fs occupy the dioxin binding sites of the antibodies in proportion to the PCDD/F content of the sample and prevent binding of the competitor-HRP conjugate. After a short incubation, unbound conjugate is removed, and the test tubes are washed thoroughly.

6. A solution of chromogenic HRP substrate and hydrogen peroxide is added to the test tubes. Color development is directly proportional to enzyme concentration and inversely related to the PCDD/F concentration in the original sample. Stop solution is added to each tube using a repeater pipettor to fix the amount of color development.

7. The test tubes are analyzed using a tube reader or spectrophotometer to measure the OD at 450 nanometers. The test is interpreted by measuring the signal produced by a sample and determining the concentration from a dose-response curve constructed from standards tested at the same time.

3.1.3 Advantages and Limitations

Extraction and cleanup using the DF1 Dioxin/Furan Immunoassay Kit is faster and simpler than for GC/mass spectrometry (MS). Confirmation of selected positive samples and a portion of the negative samples by GC/MS analysis is strongly recommended. Quantitative interpretation of data is possible in certain situations.

The low pg sensitivity of the DF1 Dioxin/Furan Immunoassay Kit is competitive with HRMS. This sensitivity allows for greater flexibility in designing immunoassay protocols and gives excellent tolerance of matrix interferences.

The dioxin/furan congener recognition profile correlates to the TEFs of the individual PCDD/F congeners. Because of this specificity, the DF1 Dioxin/Furan Immunoassay Kit has a proven correlation to TEQ.

The distribution of PCDD/Fs in soil and sediment samples can be extremely heterogeneous. Adequate sample number, distribution, and homogeneity are the responsibility of the analyst.

Samples that appear heterogeneous during the first incubation are invalid due to phase separation. Adequate sample cleanup must be assured by the analyst.

3.2 Hybrizyme Corporation AhRC PCR™ Kit

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. The technology description, operating procedure, and advantages and limitations below are based on information provided by Hybrizyme Corporation. The information was edited by Battelle to ensure consistency and to meet the needs of the demonstration plan.

3.2.1 Technology Description

This procedure uses Hybrizyme's AhRC PCR™ kit (Figure 3-3) to detect molecules in a test sample that bind to the AhR. 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 certain co-planar 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.

Figure 3-3. Hybrizyme's AhRC-PCR Test Kit.

The Hybrizyme technology, with appropriate sample processing, provides a quantitative analysis of dioxins. The AhRC PCR™ kit has recently been validated and approved for use in Japan for that purpose. Correlation data from these studies with HRGC-HRMS can be found on the Hybrizyme Web site (www.hybrizyme.com). The method for quantitative determination of dioxins may not be optimal for field use, since it requires specialized training and sample processing equipment to perform.

In the SITE program, Hybrizyme will use the AhRC PCR™ kit as it would be used by a field, mobile, or fixed-based laboratory to rapidly analyze samples for relative concentrations of AhR-reactive compounds. This method is inexpensive, easy to learn and uses disposable labware and relatively small quantities of solvent.

The AhRC PCR™ kit permits the determination of compounds demonstrating measurable binding affinity with the AhR in a manner that is largely unaffected by the chemical mixture in which they reside. By virtue of the sensitivity of the assay, many of these compounds can be directly measured in simple crude extracts. The Hybrizyme method will provide a measure of the additive contributions in a single measurement of any, or all, AhR-reactive compounds present in a sample extract.

The ease of use, speed of analysis, and cost of the Hybrizyme method allows it to be effectively used as a site-mapping tool, establishing a concentration gradient for AhR-reactive compounds across a large surface area. When employed in this manner, its operation resembles an uncalibrated thermometer. It has all the characteristics of a more conventional calibrated thermometer in that it is capable of providing reproducible responses for a given temperature being measured at different times, determining

temperature gradients, and comparing one temperature to another on a relative basis. In this example the units of the thermometer are arbitrarily defined.

Once a concentration grid has been established, samples already collected from predetermined “hot spots” can be analyzed by conventional HRGC-HGMS, or more sophisticated sample processing can be employed to obtain quantitative results from the AhRC PCR assay. When the Hybrizyme method is used as a screening tool, the fundamental procedures for sample preparation are simple and use relatively unsophisticated instrumentation. The results are both reproducible and reliable within the constraints of the sample processing method used to generate the reported value.

The Hybrizyme method can be performed on as many as three sets of 20 samples each per day by a team of two technicians. Using this technology, the boundaries of a contaminated site can be more rapidly established. Once the initial measurements are completed, remediation efforts can begin without the need to await final data as generated by HRGC-HRMS, saving both time and money. By eliminating the need to use such sophisticated techniques on all of the collected samples, many of which typically contain low to non-detectable levels, the production of useful data is expedited in all portions of a given study.

The equipment necessary to implement the Hybrizyme method can be purchased for under $45,000 U.S. A single AhR PCR™ kit is capable of producing 96 measurements; however, a portion of these assays must be dedicated to calibration and QA/QC procedures. The cost of the kit is $2,400 U.S. When used optimally, the cost of consumables per sample including the Hybrizyme kit, sample processing materials, and reagents would be under $35 per sample.

The Hybrizyme method is useful in pre-screening samples in the typical laboratory performing HRGC-HRMS analyses on relatively large sample sets. Experienced laboratory personnel could eliminate all samples having low to negligible contamination using the AhRC PCR kit. Simultaneously, this data would permit organizing the remainder of the samples from the lowest to highest concentration of AhR-binding compounds. As such, the laboratory will not spend inordinate amounts of time examining “non-detects” nor will it accidentally cross-contaminate its laboratory equipment by unknowingly processing samples containing elevated levels of the expected analytes.

3.2.2 Operating Procedure

1. Reconstitute PCR wash solution to 1 X with distilled or deionized water for use with an automated plate washer. Prime the plate washer with PCR wash solution.

2. Prepare standards and unknowns in methanol.

3. Prepare the 1 X capture reagent by diluting 40 µL of stock reagent into 600 µL of assay buffer for each strip used. Place desired number of strips in the strip frame and reseal the remainder in the foil pouch. Wash the strips using the "3XWASH" program of the plate washer. Using the multichannel pipetter, dispense 50 µL to each well in the strip. Shake 60 to 90 minutes.

4. Thaw the activation solution and mix gently during the process. Do not allow the activation solution to remain at room temperature for more than 20 minutes prior to use. For best results, mix the activation solution vials together prior to dispensing when performing multiple strips.

5. Dispense 50 µL of the assay buffer into each glass vial by using a multichannel pipette. The assay buffer should be at room temperature prior to use. Add 5 µL of standard or sample. The use of a

pipetter (i.e., P-10 Pipetman) with a filter tip is preferred. After adding sample to the entire row, tap gently to mix. Repeat the process for each row of glass vials.

6. Add 50 µL of activation solution to each glass vial using a multichannel pipette. Shake for 1 hour at room temperature.

7. Wash the strips using the "3XWASH" program of the plate washer. Using a multichannel pipetter, transfer 30 µL of each reaction to each well. Shake 30 minutes.

8. Wash the strips using the "PCRWash" program of the plate washer. This series of soaks and washes takes about 15 minutes. Thaw the primer/probe solution at this time.

9. Add 100 µL of primer/probe solution, 400 µL of water, and 500 µL of 2 X Universal Master Mix to make each 1,000 µL of 1 X Master Mix. Dispense 40 µL into each well.

10. Seal the wells with adhesive tape and cover with two compression pads. Insert the strips into a thermocycler and run the “PCR” template.

11. Analyze the data.

3.2.3 Advantages and Limitations

The advantages of the AhRC PCR™ assay result, in part, from the high degree of specificity that is required for the activated receptor to bind the DNA-probe, and the unmatched sensitivity of PCR.

Figure 3-4a. ABI Prism 7000.

The AhRC PCR™ assay couples the AhR with Nobel Prize-winning PCR technology to produce an easy to use, sensitive method for dioxin analysis. The AhRC PCR™ Kit can be shipped worldwide, yielding results from sample extracts within hours. It is ideally suited for stationary or mobile laboratories. The AhRC PCR™ assay takes place in disposable microwells or specialized PCR tubes, and the final product is measured by a real-time thermocycler. The thermocycler never comes in contact with the sample or the assay reagents. The assay system is simple to use, requiring only limited training and technical experience.

The AhRC PCR™ assay was developed for real-time PCR systems such as the ABI PRISM 7000 (Figure 3-4a), which can generate up to 96 results per run, or the Cepheid Smart Cycler (Figure 3-4b), designed to be totally transportable.

Figure 3-4b. Cepheid Smart Cycler.

3.3 Paracelsian, Inc., Ah-IMMUNOASSAY®

The Ah-IMMUNOASSAY® (Figure 3-5) is an enzyme-linked immunosorbent assay (ELISA) invented at Paracelsian, Inc. (patents pending and allowed). It is designed to screen for dioxin-like toxicity in environmental and tissue samples. The technology description, operating procedure, and advantages and limitations below are based on information provided by Paracelsian, Inc. and only editorial changes were made by Battelle to ensure document consistency and meet the needs of the demonstration plan.

3.3.1 Technology Description

The Ah-IMMUNOASSAY® makes it possible to screen large numbers of samples to eliminate non-positives before subjecting the positive samples to further analysis. The Ah-IMMUNOASSAY® takes advantage of the correlation between the toxicity of dioxin-like compounds and their interaction with the Ah-receptor. The Ah-IMMUNOASSAY® measures the ability of the test mixture to interact with the Ah-receptor, thus providing data for the total toxicity of the mixture as 2,3,7,8-TCDD equivalents. Samples to be tested in the Ah-IMMUNOASSAY® are added to a reagent mixture containing Ah-receptor and other components in a special ELISA plate and allowed to incubate at room temperature for two hours. At that time, any Ah-receptor transformed by dioxin-like compounds is bound to the plate, and the remaining material is washed away. Antibodies are added to the ELISA to detect the transformed, bound Ah-receptor, which is in turn detected colorimetrically. Color development is directly proportional to the amount of transformed Ah-receptor.

The detection limit is one pg 2,3,7,8-TCDD equivalent per ELISA plate well. The linear range is 1 to 64 pg TCDD equivalents per ELISA plate well. About 2 µl of final sample extract is required per ELISA plate well. The assay does not separate and identify single dioxin congeners, but measures the ability of the test mixture to interact with the Ah-receptor, thus measuring the total toxic potential of the mixture as 2,3,7,8-TCDD equivalents. Duplicate aliquots of each reference standard and sample dilution are recommended. Replicate absorbance readings of OD should show a coefficient of variation of 20% or less.

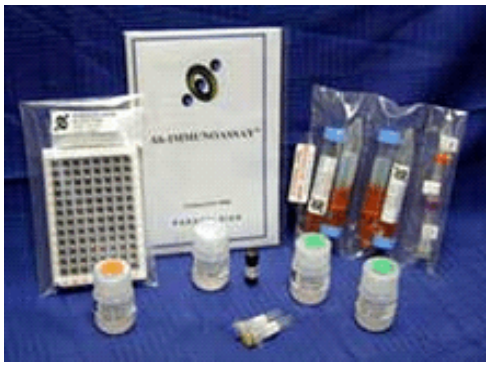

Figure 3-5 . Paracelsian, Inc., Ah-IMMUNOASSAY®.

The Ah-IMMUNOASSAY® Kit is supplied as a 96- or a 48-well version and contains

- Cytosol
- DRE Oligo
- Aryl hydrocarbon receptor nuclear translocator (ARNT) extract
- Activator
- Mixing tube (50 mL)
- Reagent reservoirs
- ELISA plate and cover
- 20 Wash buffer
- AB 1 and AB 2 (Antibodies 1 and 2)
- AB diluent
- Detection tablets
- Detection buffer
- Positive control (a-napthoflavone or 7,8-benzoflavone).

The Activated Cytosol components (Cytosol, DRE Oligo, ARNT Extract, and Activator) are shipped separately on dry ice. These components are stable for a minimum of one month when stored at -20°C and for three months when stored at -80°C. The remaining components are shipped at ambient temperature but should be refrigerated once received. They are stable for a minimum of one year when stored at 4°C.

The user is to supply calibration standards (normally 2,3,7,8-TCDD) and any materials needed to prepare samples for analysis in dimethyl sulfoxide (DMSO).

3.3.2 Operating Procedure

The following protocol is for an assay that uses all wells of the 96-well version of the kit. Steps 18 through 21, describing the procedure for the three replicate washes following each incubation, can be automated by a commercial plate washer. Likewise, any residual fluid remaining in the plate wells can be completely removed by a properly adjusted plate washer, thereby eliminating the need for the manual blotting in step 31.

1. Remove the refrigerated components of the kit from cold storage and warm to room temperature.

2. Remove the cytosol components (cytosol, DRE Oligo, ARNT extract, activator) from frozen storage. Thaw up to four 7.5-mL vials of cytosol in a beaker of tepid water. Steps 3 through 9 will generate the activated cytosol. Keep all intermediate mixtures in an ice bath as much as possible.

3. Pool the thawed tubes of cytosol into the 50-mL mixing tube.

4. Add the required volume of DRE Oligo to the pooled cytosol. Return any unused DRE Oligo to the freezer.

5. Mix by gently rocking the tube.

6. Add the required volume of ARNT to the cytosol, DRE Oligo mixture. Return any unused ARNT to the freezer.

7. Mix by gently rocking the tube.

8. Add the required volume of activator to the cytosol, DRE Oligo, ARNT mixture. Return any unused activator to the freezer.

9. Mix immediately (to avoid high local concentrations of salt) by gently rocking the tube. This mixture is the activated cytosol and must be kept on ice.

10. Add 10 µL each of one reference standard, one negative control, and up to 10 samples in DMSO (the negative control) to 1.0-mL aliquots of the activated cytosol and gently mix. Tubes for these preparations are supplied by the user. These mixtures compose the treated stock for each type of determination.

11. The 96-well ELISA plate layout will accommodate one negative control, seven dilutions of the TCDD reference standard (or a single dilution of the NAP Standard), and four dilutions of 10

samples, all in duplicate. If less than 96 wells are used, return the unused ELISA strips to 4°C storage in the foil bag with desiccant.

12. Remove the ELISA plate from the desiccated plastic bag and add 400 µL of each treated stock to the top row of the wells to give duplicate runs of each of the 10 samples and the single reference standard.

13. With a multichannel pipettor, add 200 µL of activated cytosol to all the remaining wells. One of the kit reservoirs will facilitate loading the pipettor.

14. Beginning with the first sets of duplicate wells, make serial dilutions (seven dilutions for the TCDD reference or the single dilution of the NAP, four dilutions for each sample) down the respective plate columns. Individual dilutions are mixed by a minimum of six repetitive aspiration and redispensing cycles of the well contents. The residual waste following the final dilution must be collected for proper disposal.

15. Attach the lid to the plate.

16. Incubate the plate for two hours at 30°C. Incubation at room temperature (i.e., 20°C) is satisfactory, but will result in a slightly lower response. Incubation at a higher temperature (i.e., 37°C) will also result in a lower response.

17. Prepare the 1X wash buffer by diluting the contents of the 20 X wash buffer bottle to 500 mL with reagent-grade water (250 mL for the 48-well kit). Store unused 1 X wash buffer at 4°C.

18. After the two-hour incubation, remove the well contents by aspiration into a waste receptacle for proper disposal.

19. Add 400 µL 1X wash buffer to all wells, loading the multichannel pipette from a kit reservoir.

20. Wait two minutes. Remove the well contents by aspiration into the waste receptacle for proper disposal.

21. Repeat steps 19 and 20 twice for a total of three separate washes.

22. Prepare the primary antibody stock by aliquoting the required amount of AB 1 into the appropriate volume of AB diluent. Mix gently. Store unused AB 1 and AB diluent at 4°C.

23. Deliver 200 µL AB 1 stock to all wells of the plate, loading the multichannel pipette from a kit reservoir.

24. Incubate the plate for one hour at 30°C.

25. After the one hour incubation, repeat steps 18 through 21.

26. Prepare the secondary antibody stock by aliquoting the required amount of AB 2 into the appropriate volume of AB diluent. Mix gently. Store unused AB 2 and AB diluent at 4°C.

27. Deliver 200 µL AB 2 stock to all wells of the plate, loading the multichannel pipette from a kit reservoir.

28. Incubate the plate for one hour at 30°C.

29. After the one-hour incubation, repeat Steps 18 through 21.

30. Prepare the detection reagent stock by dissolving the required number of detection tablets in the appropriate volume of detection buffer with gentle mixing while protected from light. Allow a minimum of 15 minutes for the tablets to completely dissolve. Unused detection buffer and tablets are kept at 4°C.

31. Following the incubation of the final wash, strike the inverted plate against paper toweling to remove residual fluid before proceeding to the next step.

32. Deliver 200 µL of the detection reagent stock from step 30 to all wells of the plate, loading the multichannel pipette from a kit reservoir.

33. Incubate the plate at 30°C while protected from light. Residual moisture from condensation and/or from contact with incubator water must be carefully blotted from the plate before reading. The plate is read at 405 nanometers (nm) after 15, 30, 45, and 60 minutes. Variations in incubation temperatures and plate reader characteristics may dictate the best incubation times for individual conditions.

A standard curve is used to determine the amount of TCDD equivalents present in a sample. The standard curve is generated by plotting the average absorbance (OD) measured at 405 nm for each of the TCDD reference standards on the vertical (Y) axis versus the corresponding quantity (pg) of TCDD on the horizontal (X) axis. Results are calculated manually using graph paper or a curve-fitting statistical software package. The TCDD equivalents for the samples are determined from the standard curve by interpolating from the absorbance value (Y axis) to the quantity (pg) of TCDD (X axis). The initial TCDD concentration in the sample is found by correcting for the sample dilution. The supplied NAP solution, at 2 µL per test well, may optionally be used as a single-point positive control that is equivalent to 32 pg TCDD.

3.3.3 Advantages and Limitations

Equipment required for the test is relatively inexpensive and readily available. The Ah-IMMUNOASSAY® is simple to perform and highly reproducible. It is very sensitive to the respective toxicity of the dioxins and related compounds of interest. Toxicity is measured as the ability of the sample to transform the AhR. The result, therefore, denotes total toxicity from all toxins that are present in the sample.

The assay does not identify individual dioxin congeners. Competing enzyme immunoassay methods are less sensitive, typically detecting only certain dioxin congeners without direct correlation to total toxicity. Some related toxic compounds are not detected at all by direct immunoassays. False negatives results with the Ah-IMMUNOASSAY® are rare. Positive results can be further characterized by selective pretreatment steps or by specific GC/MS testing.

3.4 Abraxis LLC Coplanar PCB ELISA Kit

The Abraxis coplanar PCB ELISA kit applies the principle of enzyme immunoassays for the qualitative or semi-quantitative analysis of coplanar PCBs in a variety of sample extracts. Extracts from soil, sediment, fish tissue, and others matrices can be exchanged to methanol for ELISA analysis. Water samples can be diluted 1:1 in methanol and analyzed directly in the assay. The technology description, operating procedure, and advantages and limitations below are based on information provided by Abraxis LLC. and only editorial changes were made by Battelle to ensure document consistency and meet the needs of the demonstration plan.

3.4.1 Technology Description

The Abraxis coplanar PCB ELISA kit (Figure 3-6) can screen samples according to their TEQ concentration. The specificity of the test is predominantly for those congeners with high TEF values; i.e. congeners 126 and 169. Samples extracted with organic solvents that are incompatible with ELISA can be evaporated and re-dissolved in methanol. For a quick screen of soil and sediment samples, the samples can be extracted in 20% acetone in hexane, diluted 1:10 in the provided diluent, and run directly in the assay.

Figure 3-6. Abraxis PCB ELISA Kit.

A solution containing a primary antibody (rabbit) that reacts with coplanar PCBs is added to a microplate containing a secondary antibody that captures the primary antibody. Calibrators (congener 126) and samples are added and allowed to incubate, followed by the addition of a coplanar PCB-HRP enzyme conjugate. Any coplanar PCBs that may be in the sample competes with the coplanar PCBs enzyme label conjugate for a finite number of antibody binding sites. At the end of the incubation period, the unbound conjugate is removed, and the plate is washed. A substrate/chromogen solution is then added and enzymatically converted from a colorless to a blue solution by the captured coplanar PCB-HRP conjugate on the plate. The reaction is then terminated by acidification. The coplanar PCBs concentration is determined by measuring the absorbance (at 450 nm) of the sample solution using a microplate reader and comparing it to the absorbance of the calibrators. The amount of color produced is inversely proportional to the amount of coplanar PCBs present in the sample.

The final value measured by ELISA is the sum of the various congeners responses, this value approximates TEQ_{PCB} because of the immunoassay kit cross-reaction profile for coplanar PCBs approximates TEF values. Accuracy among samples may vary solely because of the variability of congener composition. To help maximize accuracy, the variability of congener composition in the target sample should be known.

The primary use of the Abraxis coplanar PCB ELISA kit is to screen samples that have low coplanar PCB concentrations. The sensitivity of the test in water samples is claimed by Abraxis to be 4 parts per trillion (pg/mL). This value must be related to the original sample concentration by using the appropriate dilution and volume factors. Detection levels depend on how much sample is evaporated and the volume of solvent used to resuspend the sample. Matrix detection limits will vary according to the matrix being analyzed, sample size, and dilution factor. Up to 100 samples per day can be analyzed using the procedure described below.

The Abraxis coplanar PCB ELISA Kit consists of

1. Microtiter Plate coated with Goat-Anti Rabbit Antibody
   96-test kit: 8 X 12 strips

2. Coplanar PCB Antibody Solution
   Rabbit anti-coplanar PCB solution in a colored buffered saline solution with preservative and stabilizers.
   96-test kit: one 6-mL vial

3. Coplanar PCB Standards (Congener 126)
   Seven concentrations (0, 25, 50, 100, 250, 500, 1,000 ppt) in 50% methanol.
   96-test kit: one 1-mL vial

4. Coplanar PCB-HRP Enzyme Conjugate
   Coplanar PCB labeled with HRP diluted in colored buffered solution with preservative and stabilizers.
   96-test kit: one 6-mL vial

5. Diluent/Zero Standard
   50% methanol in distilled water (v/v) without any detectable PCB.
   96-test kit: one 30-mL vial

6. Color Solution
   A solution of hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine in an organic base.
   96-test kit: one 16-mL vial

7. Stopping Solution
   A solution of diluted acid.
   96-test kit: one 6-mL vial

8. Washing Buffer 5X Concentrate
   Buffer salts with detergent and preservatives.
   96-test kit: one 100-mL vial

3.4.2 Operating Procedure

To ensure accurate and reliable results, every effort should be made to perform the coplanar PCB ELISA at temperatures between 20ºC and 25ºC and to allow the reagents to be at the same temperature. The following sample preparation was designed for a quick screen at 625 pg/g. Other sample preparation options are available for lower concentration.

3.4.2.1 Preparation of Sample Extracts From Soil

1. Label soil collection bottles and extract collection vials.

2. Remove the screw cap from the soil collector bottle (containing dispersion device) and collect soil by weight using a digital balance. Place the bottle in an upright position on the balance and tare weight.

3. Weigh 2 g of soil in a 30-mL high-density polyethylene (HDPE) bottle.

4. Add 6 g of anhydrous sodium sulfate and mix until sample is free flowing.

5. Add 1 steel mixing ball.

6. Add 10 mL of 20% acetone in hexane. Rotate for one hour.

7. Remove the organic extract from the soil particulates by filtration, sedimentation, or centrifugation.

8. Transfer extract to a 40 mL screw cap extraction tube and oxidize using concentrated sulfuric acid (4 mL). Mix by agitation for a minute and allow phases to separate.

9. Remove the organic phase (top layer), transfer to a fresh extraction tube and add 4 mL of concentrated sulfuric acid. Mix by agitation for a minute and allow phases to separate.

10. Repeat step 9 until the acid phase is colorless.

11. Evaporate 1 mL of the organic phase using a nitrogen stream.

12. Redissolve in 0.25 mL of methanol.

13. Add 0.25 mL of water. If cloudy, centrifuge.

NOTE: This extraction procedure dilutes the sample by a factor of 2.5. Therefore, assay results need to be multiplied by 2.5 to obtain the final coplanar PCB concentration in the sample.

3.4.2.2 Dilution of Sample Extracts

Dilute sample (1:10) by adding 50 µL of extract to 450 µL of diluent/zero standard (provided).

3.4.2.3 Assay Procedure

1. Add 50 µL of anti-coplanar PCB antibody solution successively to each well.

2. Add 50 µL of the appropriate standard, control, or sample. Using duplicates or triplicates is recommended. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents. Incubate at room temperature for 30 minutes.

3. After the incubation, remove the covering and add 50 µl of enzyme conjugate solution to the individual wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents. Incubate at room temperature for 90 minutes.

4. After the incubation, remove the covering and vigorously shake the contents of the wells into a waste container. Wash the strips 3 times using the 1 X wash solution with a volume of at least 250 uL for each wash step. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels.

5. Add 150 uL of color solution successively to each well. Incubate for 20 to 30 minutes.

6. Add 50 uL of stopping solution to each well in the same sequence as for the other reagents.

7. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the stopping solution.

8. Construct a standard curve by plotting the $\%B/B_O$ for each standard on a vertical logit (y axis) versus the corresponding PCB 126 standard concentration on the horizontal logarithmic axis (x) on a graph paper. Alternatively, commercial ELISA programs can be used (4-parameter or logit-log).

9. To obtain the total coplanar PCB TEQ in a sample, multiply sample assay results by the cross-reactivity factor 0.01.

3.4.3 Advantages and Limitations

The Abraxis coplanar PCB ELISA kit can detect the total coplanar PCBs at various levels and eliminates the complex extraction and clean-up steps used with GC/MS.

The coplanar PCB kit can be used in the field or laboratory. The low part per trillion (ppt) sensitivity of the kit is competitive with HRMS.

The distribution of coplanar PCBs can be heterogeneous. Adequate number (statistically significant), distribution, and homogeneity are the responsibility of the analyst.

Samples that appear heterogeneous during assay incubations (cloudiness) are invalid. Adequate sample cleanup must be assured by the analyst.

The Abraxis coplanar PCB kit is cost-effective compared to other methods; however, it is a screening method only. The method does not provide quantitative results because of the variability of concentrations of individual congeners in real samples. The relationship between immunoassay response and TEQ may vary significantly for different samples. This ELISA kit provides an approximation.

Forty samples can be analyzed in less than 4 hours from sample extraction to results.

3.5 Wako Dioxin ELISA Kit

The Wako Dioxin ELISA Kit from Wako Pure Chemical Industries, Ltd. was developed to screen minute amounts of dioxin. With a microplate reader, samples can be assayed simultaneously for PCDD/Fs. The technology description, operating procedure, and advantages and limitations below are based on information provided by Wako Pure Chemical Industries, Ltd. and editorial changes were made by Battelle to ensure consistency and to meet the needs of the demonstration plan.

3.5.1 Technology Description

A monoclonal antibody specific to dioxin is mixed with a sample solution or the positive control (PC) provided with the Dioxin ELISA Kit. Peroxidase conjugated with a dioxin analog (POD-conjugate) is then added, reacting with a primary antibody to dioxin in the sample. The mixture is added to a

microplate coated with a secondary antibody that captures the antibody-POD-conjugate and incubated at 2° to 8°C for 18 to 20 hours. After washing the resultant microplate with a buffer, the antibody-POD-conjugate complex formed on the plate is reacted with substrate for peroxidase. The reaction is stopped by adding stop solution, and the microplate reader reads the signal.

The Dioxin ELISA Kit contains the secondary antibody microplate, the PC, buffers A and B, the primary antibody, peroxidase conjugate (lyophilized), sample solubilizer, wash solution concentrate, substrate, citrate buffer, stop solution, and a plate seal.

The monoclonal antibodies used for the Dioxin ELISA Kit indicate cross-reactivity nearly equal to the positive control (2,7,8-trichlorodibenzo [1,4] dioxin-1-yl) acrylic acid) and 2,3,7,8-TCDD. It is possible to find dioxin concentrations as the amount equivalent to 2,3,7,8-TCDD TEQ.

The Dioxin ELISA Kit sensitivity is claimed by Wako to be 1.6 to 100 pg per assay, and 96 samples can be assayed in two days. The procedure is summarized in Figure 3-7.

3.5.2 Operating Procedure

The Dioxin ELISA Kit should be used at room temperature (20° to 25°C) and stored at 2° to 10°C.

1. To prepare the PC solution (5,000 pg/mL), add 2.0 mL of methanol to the tube containing the positive control (PC). Then, gently stir the tube four or five times, and leave it at room temperature for 10 minutes. Gentle stirring is repeated once more before use. Do not vigorously shake or vortex the solution to avoid PC material adhering to the tube wall. The solution can be used within four weeks at 2° to 10°C after reconstitution.

2. Prepare the dilution solution by dispensing 2 mL of sample solubilizer in a tube and adding 2 mL of methanol.

3. Prepare 1/10-fold diluted PC solution (500 nanograms/mL) by adding 900 µL of dilution solution to a disposable culture tube rinsed with acetone and dried. Then add 100 µL of concentrated PC solution and gently stir the tube two or three times. Leave the tube at room temperature.

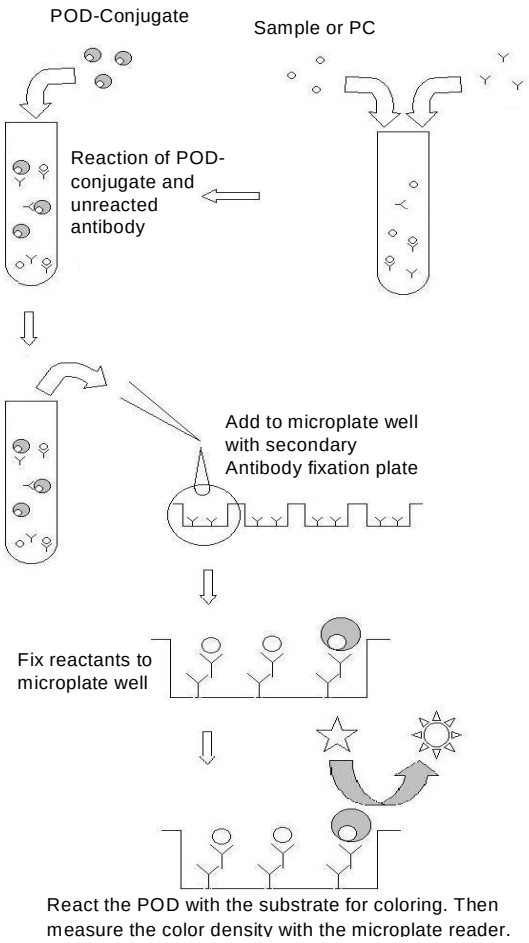

Figure 3-7. Wako Dioxin ELISA Kit procedure.

4. Prepare PC dilution solutions for the standard curve using the 1/10-fold diluted PC solution described above as follows:

Tube Number:	1	2	3	4	5	6	7
Concentration	0 pg/mL	40 pg/mL	100 pg/mL	250 pg/mL	500 pg/mL	1,000 pg/mL	2,500 pg/mL
Dilution solution	500 µL	460 µL	400 µL	250 µL	450 µL	400 µL	250 µL
Concentrated PC solution	—	—	—	—	50 µL	100 µL	250 µL
1/10-fold diluted PC solution	0 µL	40 µL	100 µL	250 µL	—	—	—
Total volume	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL

5. Dry the contents of each tube.

6. To prepare a dilution series of PC solutions, add 500 µL of Buffer B to each tube, lightly stir it with a vortex mixer, and centrifuge at 2,000 revolutions per minute (rpm) at room temperature to collect the liquid at the bottom.

7. To prepare the primary antibody stock solution, add 200 µL of purified water to the tube and gently stir, then leave the tube for 10 minutes at room temperature. The solution can be used within four weeks.

8. Rinse a beaker or a 5- to 10-mL vial with acetone and air dry on clean paper towels.

9. Prepare the primary antibody working solution using a 100-fold dilution of the primary antibody stock solution described above with Buffer A according to assay sample numbers and volumes. Stir the diluted solution gently several times, and leave it at room temperature for 10 minutes. Stir the solution several times just before use. This solution cannot be stored prior to use.

10. Prepare the POD-conjugate solution by adding 4 mL of Buffer A to the tube and gently stir several times. Leave the tube at room temperature for 10 minutes. Gently stir the tube just before use. The solution can be used within two weeks when stored at 2° to 10°C.

11. Fix the necessary number of microplate wells (coated with the secondary antibody) to a microplate holder with Scotch® tape. Place the holder in a closed box, and store it in a refrigerator for at least 30 minutes before use.

12. Add 100 µL of Buffer B to the tube containing the material obtained by sample pretreatment and gently stir the solution along the inner surface of the tube several times with vortex mixer. Briefly centrifuge the tube at 2,000 rpm for five minutes to collect the solution at the bottom.

13. Line up the tubes containing the dilution series of PC solutions and the samples dissolved in Buffer B in a tube rack. Dispense an equal volume of the primary antibody working solution to each tube.

14. Immediately agitate the solutions several times, then seal the tubes with Parafilm and leave them at room temperature for 30 minutes.

15. During the incubation, prepare chilled water containing ice in a water bath and immerse the tubes in the bath. After the 30 minute-incubation at room temperature, add 250 µL and 50 µL of POD-conjugate solution to the tubes containing the diluted PC solutions and those containing sample material, respectively.

16. Gently agitate the tubes several times and leave them for 10 minutes. Avoid vigorous shaking with the vortex because dioxins may adsorb to the tube wall.

17. Take the microplate holder from the box stored in a refrigerator. Dispense 100 μL of the reaction mixture to the wells. Briefly agitate the solutions in the wells. Seal the wells with Parafilm, return the holder to the box, and store the box in a refrigerator at 2° to 10°C for 18 to 20 hours.

18. On the second day, prepare the wash solution by diluting the wash solution concentrate (X6) with five volumes of purified water. The solution volume prepared can be adjusted depending upon the number of wells used. Usually, one well needs 1.2 mL of the wash solution. The solution can be used within two weeks after dilution when stored at 2° to 10°C.

19. Prepare the necessary volume of color-developing solution by mixing the substrate and the citrate buffer in a ratio of 1:50 just before use. (The tube used in this preparation should be cleaned with purified water.) Add the citrate buffer to the tube, then the substrate. Avoid vigorous agitation of the mixed solution because it may cause crystalization.

20. Take the well holder from the box stored in a refrigerator and remove the seal from the wells. Remove the reaction mixture from the wells, being careful not to spill the solution to the holder. Lightly tap the holder upside down on sheets of paper to remove the residual mixture in the wells. Dispense approximately 0.35 mL of wash solution to each well and then drain the solution, being careful not to spill the solution. Remove the solution from the well walls by tapping the holder upside down on the papers. Repeat washing twice. At the last washing, remove the solution by tapping as much as possible.

21. For the enzyme reaction, dispense 100 μL of the color-developing solution to all the wells. Seal with Parafilm, cover the holder with aluminum foil to block out light, and leave the holder at room temperature for 30 minutes. Stop the reaction by adding 100 μL of stop solution in the same well sequence as the color-developing solution. Tap the side of the holder to mix the solution. Read the signal with a microplate reader at 450 nm or at 450/650 nm within 15 minutes after stopping the reaction. Construct the standard curve obtained with the diluted PC solutions and estimate the dioxin concentrations of samples as 3,7,8-TCDD. The primary antibody used in this kit reacts with 2,3,7,8-TCDD and 3,7,8-TCDD with equal intensity.

22. In estimating the dioxin concentration, it is useful to plot the obtained values with a regression of polynomial quadratic equation or four-parameter logic after log-logit conversion.

3.5.3 Advantages and Limitations

Highly sensitive analysis is possible with the Dioxin ELISA Kit.

A non-toxic standard (2, 7, 8-trichlorodibenzo [1,4] dioxin-1-yl) acrylic acid) is used.

The 96-microplate format makes possible a simultaneous multisample assay. The assay process takes only two days.

3.6 Xenobiotic Detection Systems, Inc., CALUX®

CALUX® (chemical-activated luciferase expression) by Xenobiotic Detection Systems Inc. (XDS) technology is based on a reporter gene system using a genetically engineered cell line capable of detecting all of the WHO-recognized dioxins, furans and PCBs. Giving results for dioxins/furans and PCBs

separately or together, as well as being available as a screening and/or quantitative analysis. CALUX® by Xenobiotic Detection Systems is used to analyze soil, sediment, fly ash, stack gas emissions, food, feed, blood, and any other substance suspected of being contaminated with dioxins/furans and PCBs. The technology description, operating procedure, and advantages and limitations provided below for the CALUX® are based on information provided by Xenobiotic Detection Systems, Inc. and editorial changes were made by Battelle to ensure consistency and to meet the needs of the demonstration plan.

3.6.1 Technology Description

XDS has patented (patent # 5,854,010) a genetically engineered cell line that contains the firefly luciferase gene under trans-activational control of the AhR. This cell line can be used for the detection and quantification of the AhR agonists, the target receptor of dioxins, furans, and PCBs. The XDS term for the *in vitro* assay is the Chemical-Activated Luciferase Expression (CALUX®) by Xenobiotic Detection Systems assay. The most widely studied compounds that activate this system are the polychlorinated diaromatic hydrocarbons (PCDH), such as 2,3,7,8-TCDD. Many PCDH compounds are quantified relative to TCDD, since this is one of the most potent activators of AhR-mediated gene transcription. These relative quantifications are known as TEQs, and the results from the CALUX® by Xenobiotic Detection Systems assay provide a measure of TEQs in a sample. By using proprietary cleanup methods developed by XDS, it is possible to separate PCBs from dioxins/dibenzofurans and to determine what portion of the total TEQ in a sample is due to each of these classes of compounds. XDS has termed this procedure the Dioxin/Furan and PCB-Specific (DIPS) or DIPS-CALUX® by Xenobiotic Detection Systems bioassay.

Prices start at $200.00 for a dioxin screening (single) analysis and $250.00 for a dioxin and PCB analysis, with analysis provided as a fee for service at the XDS laboratories. Field analysis is available with 96 well plates being shipped to the site for analytical procedures to be performed by trained personnel. Costs per 96 well plates are approximately $2,400, with each plate capable of analyzing up to 40 samples along with standard curves and quality control standards. Rental of equipment and proprietary software to perform the CALUX® by Xenobiotic Detection Systems is also available.

3.6.2 Operating Procedure

Xenobiotic Detection Systems, Inc. has a patented genetically engineered cell line (mouse hepatoma H1L1) that contains the gene for firefly luciferase under transactivational control of the AhR. This cell line can be used for the detection and relative quantification of a sample's total dioxin I-TEQ. The XDS CALUX® bioassay for dioxin-like chemicals uses a patented sample processing procedure (U.S. patent # 6720431) that allows separation of coplanar PCBs and PCDDs/PCDFs so that estimates of I-TEQ can be made for each chemical class. This allows reporting of I-TEQ estimates for chlorinated dioxins/furans and for the PCBs.

The samples are extracted using a modification of the EPA 8290 extraction method. Briefly, the dried samples are ground and 1-g aliquots are placed in solvent-cleaned glass vials with polytetrafluoroethylene-lined caps. The sample is extracted with a 20% solution of methanol in toluene then twice with toluene. During each extraction step, the samples are incubated in an ultrasonic water bath. The three extracts from each sample are filtered, pooled, and concentrated by vacuum centrifugation. The sample extract is suspended in hexane and rapidly processed through a patented (U.S. patent # 6720431) two column chromatographic procedure to produce two extracts, one containing chlorinated dioxins/furans and one containing PCBs (see Figure 3-8). The extracts are exchanged into

DMSO and used to dose the genetically engineered cells in the CALUX® assay by XDS to provide I-TEQ estimates for PCBs and PCDD/PCDFs.

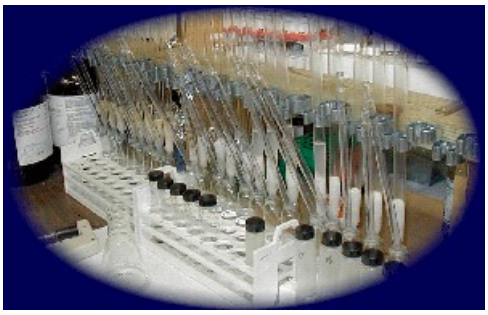

Figure 3-8. Xenobiotic Detection Systems patented sample processing procedure.

Prior to dosing the cells, the sample extracts in DMSO are suspended in cell culture medium. This medium is then used to expose monolayers of the H1L1 cell line grown in 96-well culture plates (see Figure 3-9). In addition to the samples, a standard curve of 2,3,7,8-TCDD is assayed (161, 80.5, 40.2, 20.1, 10.1, 5.0, 2.5, 1.2, and 0.6 ppt TCDD). The plates are incubated for a time to produce optimal expression of the luciferase activity in a humidified CO_2 incubator. Following incubation, the medium is removed and the cells are examined microscopically for viability. The induction of luciferase activity is quantified using the luciferase assay kit from Promega.

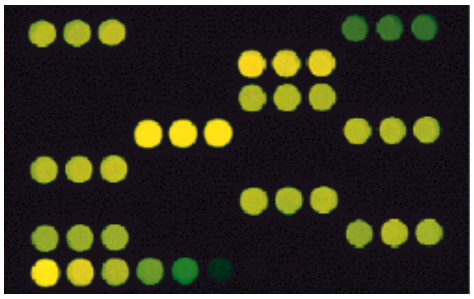

Figure 3-9. Luminescence produced when CALUX® cells are exposed to dioxin and dioxin-like chemicals.

3.6.3 Advantages and Limitations

CALUX® by Xenobiotic Detection Systems analyses are approximately 25% of the cost of HRGC/MS analyses, and sample sizes are small (10 g or less). Results can be made available within 28 hours in emergency situations. Expenses for laboratory setup are minimal, and the proprietary cleanup method eliminates false positives and false negatives.

CALUX® by Xenobiotic Detection Systems analyzes environmental, foodstuff, and biological samples. CALUX® by Xenobiotic Detection Systems also analyzes all 17 toxicologically active dioxin and 12 PCB congeners, and results are reproducible. Detection limits below 0.5 ppt TEQ scores are provided for both dioxin and PCB analyses. CALUX® by Xenobiotic Detection Systems does not, however, identify individual congeners.

CALUX® by Xenobiotic Detection Systems has been authenticated as a European Union-certified screening tool for dioxin in foodstuffs by BELTEST.

Chapter 4
Sample Collection, Sample Homogenization, and Sample Characterization

This chapter discusses the sample collection, sample homogenization, and sample characterization procedures used in the demonstration.

4.1 Sample Collection

This section describes the environmental sample collection activities performed at various sites across the country. Battelle performed an intensive search for sampling sites that had varying levels of dioxin contamination in either soil or sediments. This diversity will make the demonstration results more applicable to a larger user community, as well as to challenge the technologies using a variety of sample compositions. Samples were collected by EPA, an EPA contractor, or MDEQ and shipped to Battelle. When determining if a soil or sediment site had appropriate dioxin contamination, a guideline concentration range was provided to sample providers of < 50 pg/g to 5,000 pg/g. It was understood that this concentration range was to be used as a general guideline, and that those providing the sample descriptions were to provide concentration data based on best available information.

4.1.1 Procedure

This section describes the method that was used to collect the samples by each of the site personnel. Once necessary approvals and sampling locations had been secured, site personnel were shipped sample containers. Each site providing samples received one-gallon containers (Environmental Sampling Supply, Oakland, California, Part number 3785-1051, wide-mouth, 128-ounce HDPE round packer) for the collection of five or six samples. At least 2 gallons of material (soil or sediment) for each sample were needed; therefore, if a site was providing six samples, twelve 1-gallon containers were shipped to the site for the collection of six 2-gallon samples. With the 12 containers provided, it was anticipated that the six sample containers were filled in duplicate.

Shipments consisted of pre-cleaned, 1-gallon sample containers with instructions on how to fill the containers for each sample collected. Instructions for sample collection as well as how the containers were to be labeled and returned were included in a cover letter (see example in Appendix C) 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 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, pentachlorophenol [PCP]). Final instructions to sample providers indicated that collected samples were to be shipped back to Battelle 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 demonstration plan.

4.1.2 Sample Shipping to and Archival at Battelle

The samples were received at Battelle in the same coolers that were used to send the containers to the field. The environmental samples were stored at room temperature until homogenized.

4.2 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 inch. The pans were placed in an oven at 35 ºC and held there until the samples were visibly dry. This process took 24 to 72 hours, depending on the sample moisture. The trays were removed from the oven and allowed to come to room temperature by sitting in a fume hood for approximately 2 hours. Approximately 500 g of material was 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 and soil was blended and sieved. The blended and sieved sediment/soil in the tray were 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 actively used over a period of several days, at which time they were temporarily stored at room temperature.

It should be noted that none of the technologies participating in this demonstration require that samples be dried prior to analysis. As such, moisture effects on technology performance that will commonly be encountered with real-world soil and sediment samples, will not be evaluated during this demonstration.

4.2.1 Criteria for Determining Adequate Homogenization

Two criteria had to be met in order for the sample to be considered adequately homogenized. The first criteria 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. The second criteria was that no single RSD for an individual congener could be greater than 30%. If both of these criteria were met, the sample was considered homogeneous and considered for inclusion in the demonstration. If either of these criteria were not met, options for the

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

sample included: (a) discard it and not consider it for use in the demonstration, (b) reanalyze it to determine if the data outside the homogenization criteria were due to analytical issues, or (c) rehomogenize and reanalyze 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). All samples included in the demonstration met the criteria for acceptable homogenization. In a few cases, the composition of a particular sample was of interest for inclusion in the demonstration because of concentration or unique congener pattern, but the homogenization criteria were slightly exceed (i.e., no more than 30% RSD for TEQ and no more than 40% RSD for a particular congener). Since multiple replicates of every sample will be analyzed in the design, it was the recommendation of the demonstration panel (Appendix A) to still include that sample in the study because of the unique nature of the sample, but flag it as slightly exceeding the homogenization criteria.

4.3 Characterization of Environmental Samples

A total of 58 environmental samples were received from ten different sampling locations (see descriptions in Chapter 5). All of the environmental samples were homogenized, as described in Section 4.2, and analyzed by Battelle. The environmental samples were characterized for the 17 D/Fs by Method 1613B, the 12 WHO PCBs by LRMS Method 1668A, and 18 target PAHs by National Oceanic and Atmospheric Administration Status and Trends GC/MS method. All characterization analyses were performed by Battelle in their laboratories. The purpose of the characterization analyses was two-fold. First, the analyses determined the adequacy of the homogenization. Second, the approximate concentrations of the target analytes were determined. These two pieces of information served as the basis of sample selection for inclusion in the demonstration.

4.3.1 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 1613, Revision B. 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 was taken for analysis from each aliquot and spiked with $^{13}C_{12}$-labeled internal standards, and extracted with methylene chloride using accelerated solvent extraction (ASE) 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.

Each extract was analyzed by GC/HRMS in the selected ion monitoring (SIM) mode at a resolution of 10,000 or greater. A DB5 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 1613 with one additional calibration standard at concentrations equivalent to one-half the level of Method 1613's lowest calibration point. Using a DB5 column, 2,3,7,8-TCDF is not separated from other non-2,3,7,8-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/PCDF data were reported as both concentration (pg/g dry) and TEQs (pg TEQ/g dry).

4.3.2 PCBs

One aliquot of material from each sampling location was prepared and analyzed for the 12 WHO-designated dioxin-like PCBs by LRMS. Battelle's 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 was 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 gas chromatography on a DB5-XLB column and identified and quantified using electron ionization mass spectrometry. This method provides specific procedures for the identification and measurement of the selected PCBs in SIM mode.

4.3.3 PAHs

One aliquot of material from each sampling location was analyzed for PAHs. The PAHs were selected by the demonstration panel (see Appendix A). 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. Battelle's method for the identification and quantification of PAH in sediment and soil extracts by GC/MS is based on the National Oceanic and Atmospheric Administration (NOAA) status and trends method [(8)] and, therefore, certain criteria (i.e., initial calibrations and daily verifications) are different than those defined in traditional EPA methods 625 and 8270C. Up to 30 g of sample was 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 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 gas chromatography on a DB-5, 60-m column and were identified and quantified using electron impact mass spectrometry. Extracts were analyzed in the SIM mode to achieve the lowest possible detection limits.

4.4 Sample Handling, Sample Tracking, and Sample Management

In preparation for the demonstration, the bulk homogenized samples will be split into jars for distribution. Each 4-ounce, amber, wide mouth glass sample jar (Environmental Sampling Supply, Oakland, California, Part number 0125-0055) will contain approximately 50 g of sample. Seven sets of samples will be prepared for the six developers and one reference laboratory. A minimum of four replicate splits

of each sample will be 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) will be transferred from their original packaging to the jars to be used in the demonstration for the environmental samples, such that the environmental and PE samples will be visually indistinguishable.

The samples will be randomized in two fashions. First, the order in which the filled jars will be distributed will be randomized, such that the same developer will not always receive the first jar filled for a given sample set. Second, the order of analysis will be randomized so that each developer analyzes the same set of samples, but in a different order. PE materials will be randomized in the same manner, such that the PE samples are indistinguishable from other samples.

All jars will have two labels. The label on the top of the jar will be the analysis order (i.e., Developer Name 1) and contain sample numbers 1 through 209. A second label will be placed on the side of the jar will contain a coded identifier including a series of 10 numbers. The 10 numbers will be coded to include the site, replicate, developer, and matrix in the number series that can be readily decoded by Battelle but will be meaningless to the developers so the analyses will occur blindly.

Battelle will be responsible for sample distribution during the demonstration. All samples will be prepared for distribution at the start of the verification. When ready to perform analyses, each developer will go to the sample distribution point to pick up the samples. The samples will be distributed in batches of 10 and will be released at each developer's request. More than one batch of 10 samples can be relinquished at a time, if desired by the developer. Completion of a chain-of-custody (COC) form will document sample transfer and will be used to report results. An example COC is included in Appendix D. Samples that have been analyzed by the developer will be turned in to Battelle, along with COC/results form. Results will be entered onto the COC by the developer using black ink. Corrections will be made with a single line/initial/date procedure.

During the demonstration, all samples not in the possession of the developer will be stored in the Battelle Headquarters trailer. All trailers will be locked after hours and hired security guards will patrol the site from 7 pm to 7 am.

During the demonstration, the samples will be stored at room temperature. There is no concern about significant volatilization of the target contaminants because standard reference materials for dioxins are routinely stored at room temperature, and the samples used in this demonstration will be prepared in a manner similar to how SRMs are processed.

An archived set of samples will be at the demonstration site in case a sample is dropped, the integrity is comprised, or a jar is broken during transit to the site. After the demonstration, all unused demonstration samples will be returned to Battelle for archival in a freezer (approximately -20°C) at least until after the reports are final. At the conclusion of the study, the archive set of samples may be provided to EPA for additional use or as reference material.

Debris from the demonstration that can be discarded by routine waste removal services will be disposed of from the demonstration site. Sample by-products, including unused sample, aqueous and solvent-based effluents, and miscellaneous used supplies (e.g., glassware, pipette tips, shoe covers, and gloves) will be returned to Battelle for confirmation and quantification of by-products generated by each of the participating technologies. After categorizing and inventorying all of the materials, Battelle will be responsible for final waste disposal.

Chapter 5
Descriptions of Demonstration Site and Sampling Locations

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

5.1 Demonstration Site Description

This section describes the site selected for hosting the demonstration, along with the selection rationale and criteria. Several candidates 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, Battelle, 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.

After review of the information available, the site selected for the demonstration is the Green Point Environmental Learning Center (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 Refuge is one of over 540 National Wildlife Refuges managed by the U.S. Fish and Wildlife Service, a part of the United States government, in the Department of the Interior. The Green Point ELC offers a variety of environmental educational programs for students of Saginaw and surrounding communities. Furthermore, it offers over 2.5 miles of trails that wind through bottomland hardwood forest, fields, wetlands, and along the banks of the Tittabawassee River. These trails offer opportunities to study various habitats and the wildlife associated with them. Green Point ELC also has a bird watching area, ponds, restored grasslands, and learning center displays.

The Green Point ELC is situated within the Tittabawassee River flood plain. The Michigan Department of Environmental Quality (MDEQ) has 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. The MDEQ is taking more samples in the flood plain to determine the extent of contamination. Soil samples taken from areas outside the flood plain have been at normal background levels. The source of the contamination has not been clearly determined and is under investigation. MDEQ is monitoring the level of dioxin in soil and considering the regulatory levels of soil of 50 ppt as a screening level, indicating a need for further study to 1,000 ppt (i.e., 1 ppb) as an action level.

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 MDEQ, EPA Region 5, and local U.S. Fish and Wildlife expressed interest in supporting the demonstration by providing site access for the demonstration. In addition, MDEQ personnel will provide logistical support required for the demonstration and will support a Visitor's Day during the demonstration.

- Space Requirements and Feasibility—The demonstration will take place in the parking lot adjacent to the Green Point ELC, not directly on the area of soil and sediment contamination. The site has electrical power and adequate space to house the trailers and mobile labs that will be used for the demonstration. Furthermore, the site is close to an international airport and hospitals and has a sufficient number of lodgings and restaurants. Weather in Michigan can be unpredictable in the month of April; however, all participants will be provided heated containment (a mobile laboratory or hard-sided trailer) so that the weather should not impact the demonstration.

- Site Diversity—The area encompassing the Green Point site has different levels and types of dioxin contamination in both the soil and sediment needed to evaluate the monitoring and measurement technologies described in Chapter 3.

5.2 Description of Sampling Locations

This section provides an overview of the 12 sampling sites and methods of selection. Table 5-1 summarizes each of the locations, what type of sample (soil or sediment) was provided, and the number of samples from each location. Samples provided consisted of either soil or sediment and will be described below based on this distinction. It should be noted that it is not an objective of the demonstration to characterize the concentration of dioxins, furans, and PCBs in material from a specific sampling site. It is, however, necessary to ensure comparability between technology results and the reference laboratory results. This will be accomplished by homogenizing each matrix, such that all sub-samples of a given matrix have consistent contaminant concentrations. As a result, homogenized samples are not necessarily representative of original concentrations at the site.

5.2.1. 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, PCP, and PAHs). This information was provided by the site owners/sample providers (e.g., EPA, EPA contractors, and MDEQ), and only editorial changes were made by Battelle.

5.2.1.1. Warren County, North Carolina

Five different 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 from approximately 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. 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 new 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 EPA and the State of North Carolina. The site was deleted from the National Priorities List on March 7, 1986.

5.2.1.2. Tittabawassee River Flood Plain

MDEQ sampled the Tittabawassee River flood plain soils from three sites in the flood plain. 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 range. Two samples were collected from two locations at Freeland Festival Park in Freeland. The first sample was taken above the river bank, and the second sample was taken near a brushy forested area 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 farmed 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 have not been analyzed, but concentrations are expected to be less than 1 ppm. The DNR property is approximately a 10-minute walk from where the demonstration will be conducted at the Green Point ELC.

5.2.1.3 Midland, Michigan

Soil samples were collected by 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 flood plain. The first sample location was soil at the Chippewa Nature Center adjacent to their parking lot in the woods. The soil was a brown sandy loam with an estimated TEQ concentration of less than 10 ppt. The next sample location was in Midland, in the greenbelt between Lynn and Patrick Streets. The soil was dark brown clay loam with an estimated TEQ concentration of 200 ppt. The third location sampled in Midland was near the intersection of Swede and Patrick. Again, the estimated TEQ concentration is 200 ppt. The next soil sample was gathered from a traffic island at the intersection of Saginaw and Bay City Roads. The sample consisted of brown sandy topsoil with an estimated TEQ concentration of 600 ppt. The fifth and final Midland sample location was in the green space between Saginaw Road and sidewalk just south of the Mark Putnam Road intersection. The estimated TEQ concentration is up to 1,000 ppt.

5.2.1.4 Winona Post

The Winona Post site in Winona, Missouri was a Superfund cleanup of a woodtreater facility. Contaminants at the site included pentachlorophenol, dioxin, diesel, and PAHs. Over a period of at least 40 years, these contaminants were apparently deposited into an onsite 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 2 ½-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 mg/kg for pentachlorophenol and 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.

5.2.1.5 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 and continued to expand operations at the facility and accelerated its growth in the 1940s. A variety of raw materials have been used at the facility over the years including inorganic compounds, organic solvents, and other organic compounds. The source of the dioxin contamination in the site soils was the manufacture of 2,4,5-T. (Agent Orange is a mixture of chemicals containing equal amounts of two active ingredients: 2, 4-D and 2,4,5-T.) Manufacture of the chemical herbicide began at the site in 1948 and ceased in 1969. The site has a dioxin profile from ppt to low ppb range. No PCBs or PAHs have been identified in the soil.

5.2.2. Sediment Sampling Sites

This section provides specific 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., EPA, EPA contractors, and MDEQ), and only editorial changes were made by Battelle.

5.2.2.1 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 Corp 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.

5.2.2.1.1 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 non-point sources). This Bay is downstream from a dioxin Superfund site that contains some of the highest dioxin concentrations yet found in the United States, and also is downstream from a mercury Superfund site. The dioxin concentration in the area sampled for this demonstration is approximately 450 pg/g. Average PCB concentrations range from 300 to 740 ppb. Fine-grained sediments make up 50% to 90%. Average total organic carbon (TOC) is about 4%.

5.2.2.1.2 Raritan Bay

Surrounded by industry and residential discharges, Raritan Bay has dioxin contamination in the area, but 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 are similar to percentages in Newark Bay.

5.2.2.2 Tittabawassee River Sediments

MDEQ sampled six locations in the Tittabawassee River flood plain. The first location is 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 is 260 pg/g; however, concentrations as high as 2,100 pg/g TEQ are possible in this area. The second site is on the Tittabawassee River approximately 100 yards downstream from old Smith's Crossing Bridge in Midland, Michigan. The sediment is brown and sandy with organic material. The estimated TEQ concentration is 870 pg/g; but, again, concentrations as high as 2,100 pg/g TEQ are possible in the area. The third site is on Tittabawassee River at the Emerson Park Golfside Boat Launch. The sediment is gray black silty sand, with many leaves and high organic matter. The estimated TEQ concentration is < 5 pg/g. The fourth site is on the Tittabawassee River adjacent to Imerman Park in Saginaw County across from the fishing dock. The sediment is sand with some silt. The estimated TEQ concentration is between 100 and 2,000 pg/g TEQ. The fifth site is on the Tittabawassee River approximately 1 mile downstream of Center Road Boat Launch in Saginaw Township. The sediment consists of sand and gravel with some shells and not much organic matter. The estimated TEQ concentration is between 100 and 1,000 pg/g TEQ. The sixth site also is on the Tittabawassee River across from the Center Road Boat Launch. The sediment is fine sand with high organic matter. The estimated TEQ concentration is 1,000 pg/g TEQ.

5.2.2.3 Saginaw River Sediments

MDEQ sampled Saginaw River flood plain sediments with the assistance of staff from the U.S. EPA Great Lakes National Program Office. Samples were collected at six locations in the flood plain area. The first sampling location is 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 is granular with some organic material. The estimated TEQ concentration is 100 ppt. The next Saginaw River sample was taken upstream of Genesee Bridge on the right side of river. The sample is a brown fine sand from about 15 feet of water. Again, the estimated TEQ concentration is 100 ppt. The third location is in the Saginaw River downstream of the Saginaw wastewater treatment plant in about eight feet of water. The sample is gray silty clay with an unknown TEQ concentration. The fourth location is in the Saginaw River in about eight feet of water. The sample is a black sandy material. The estimated TEQ concentration for this location is again unknown. The fifth location is 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 consists of dark black silt with some sand. The estimated TEQ concentration is unknown, but PCB concentrations are expected to be high. The sixth and final sampling location is near the mouth of the Saginaw River in about five feet of water. The sediment is a mix of fine black silt and layers of sand and shells. The estimated TEQ concentration for this location is also unknown.

5.2.2.4 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, thought to be the same 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 were carried out at the facility: PCP, creosote, and chromium-copper-arsenic (or CCA). The site was listed on 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) dioxin/furan concentrations. Due to the processes that occurred on this site, the samples also contain varying levels of PAHs and PCP, but should not contain PCBs.

Table 5-1. Summary of Environmental Sampling Locations

Sample Type	Sampling Location	Number of Samples
Soil	Warren County, North Carolina	5
	Tittabawassee River Flood Plain, Michigan	6
	Midland, Michigan	6
	Winona Post, Missouri	6
	Nitro, West Virginia	6
Sediment	Newark Bay, New Jersey	6
	Raritan Bay, New Jersey	6
	Tittabawassee River Flood Pain, Michigan	6
	Saginaw River, Michigan	6
	Brunswick, Georgia	5

Chapter 6
Demonstration Approach

This chapter presents the objectives, design, data analysis procedures, and schedule for this technology demonstration.

6.1 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 judgments regarding comparability to conventional methods. The demonstration has both primary and secondary objectives. Primary objectives are critical to the technology evaluation and require the use of quantitative results to draw conclusions regarding a technology's performance. Secondary objectives pertain to information that is useful but will not necessarily require the use of quantitative results to draw conclusions regarding a technology's performance. Each report will summarizes the findings of these objectives and provide sufficient documentation for a user to choose an alternative to conventional technology.

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

P1. Determine the accuracy.
P2. Determine the precision.
P3. Determine the comparability of the technology to EPA standard methods.
P4. Determine the method detection limit (MDL).
P5. Determine the frequency of false positive (fp) and false negative (fn) 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 are as follows:

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

The objectives for the demonstration were developed based on input from the Dioxin SITE Demonstration Panel members (Appendix A), 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.

6.2 Overview of Demonstration Samples

The goal of the demonstration is to perform a detailed evaluation of the overall performance of the technology for use in contaminated site evaluation. The demonstration objectives will be centered around providing performance data that support action levels for 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.[9] If samples are determined to have dioxin TEQ levels between 50 and 1000 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 will be evaluated that bracket the ATSDR guidance levels. Table 6-1 lists the primary and secondary performance objectives for this demonstration and which sample types will be used in each evaluation. The PE samples will be used to determine the accuracy of the technology and will consist of purchased soil and sediment standard reference materials with certified concentrations of known contaminants and newly-prepared spiked samples. The PE samples will also be used to evaluate precision, comparability, MDL, and false positive/ negative results. Environmentally contaminated samples will be collected from dioxin-contaminated sites around the country (as described in Chapters 4 and 5) and will be used to evaluate the precision, comparability, MDL, false positive/negative results, and matrix effects. Extracts, prepared in toluene, will be used to evaluate precision, MDL, and matrix effects. All samples will be 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 6-2 is an outline of the number of each sample type that will be included in the experimental design, and Figure 6-1 illustrates a distribution of the environmental sample concentrations, according to total $TEQ_{D/F}$ as determined by the characterization analyses (see Section 4.3). The following sections describe each sample type in greater detail.

Table 6-1. Distribution of Samples for the Evaluation of Performance Objectives

Performance Objective	Type of sample that will be evaluated	Total $TEQ_{D/F}$ (pg/g) range of samples
P1: Accuracy	PE	< 5 to > 5,000
P2: Precision	PE, environmental, extracts	< 1 to > 15,000
P3: Comparability	PE, environmental, extracts	< 1 to > 15,000
P4: MDL	PE, environmental extracts	< 1 to > 50
P5: False positive/negative results	PE, environmental	< 5 to > 15,000
P6: Matrix effects	environmental, extracts	< 1 to > 15,000
P7: Cost	PE, environmental, extracts	n/a
S1: Skill level of operator	PE, environmental, extracts	n/a
S2: Health and safety	PE, environmental, extracts	n/a
S3: Portability	PE, environmental, extracts	n/a
S4: Sample throughput	PE, environmental, extracts	n/a

n/a - not applicable

Table 6-2. Proposed Number and Type of Samples to be Analyzed in the Demonstration

Sample Type	Estimated D/F Concentration Range	No. of Samples
Performance Evaluation	< 5 to 6,500 pg/g total TEQ	58
Environmental	1 to 15,000 pg/g total TEQ	128
Extracts	< 1 to 1,000 pg/mL	23
Total number of samples per technology		209

6.2.1 PE Samples

PE standard reference materials are available through the National Institute of Standards and Technology (Gaithersburg, Maryland), Cambridge Isotope Laboratories (Andover, Massachusetts), Wellington Laboratories (U.S. Distributor TerraChem, Shawnee Mission, Kansas), LGC Promochem (United Kingdom), and Environmental Resource Associates (Arvada, Colorado). One or more of these sources

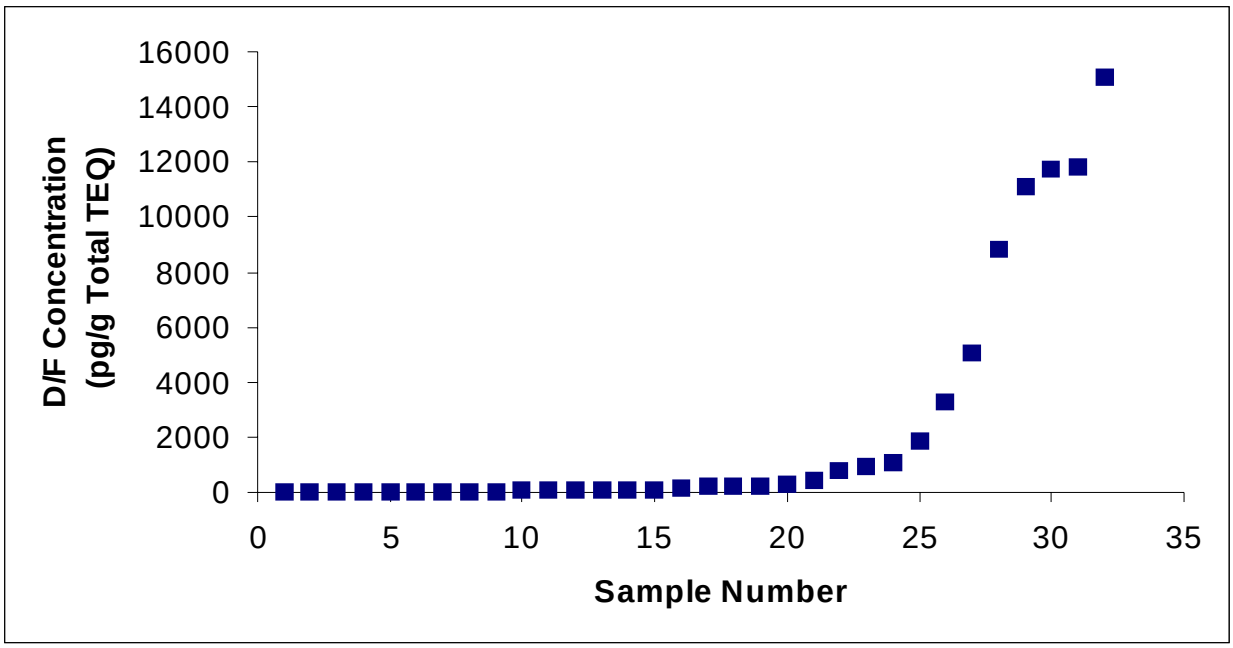

Figure 6-1. Approximate distribution of environmental sample concentrations.

will be utilized to obtain PE samples for use in this demonstration. PE samples will consist of three types of samples:

- **Standard reference materials or certified samples:** These will include soil and/or sediment samples with certified concentrations of dioxin, furan, and/or PCBs that are commercially available for purchase. These samples are supplied with a certificate of analysis, including a range of acceptable results based on analytical verification data.

- **Spiked samples:** These will include a certified dioxin, furan, PCB, and PAH-clean matrix spiked with known levels of dioxin and/or other contaminants. The spiked samples will be included primarily to allow for more samples to be analyzed across the analytical range of interest, since the

availability of a wide-concentration range of certified reference standards is limited. The concentrations of the spiked samples will be verified analytically by the reference laboratory for accuracy to within 10% of the spike concentration. The results for these samples will be compared to the spike concentration (rather than to one analytical verification result) if the measured concentration is within 10% of the spike concentration. These samples will also have a range of acceptable results provided by the supplier, but this range will be based on an arithmetic calculation and will not be based on analytical data.

- **Blank samples:** These samples will be certified free of dioxins (2,3,7,8-TCDD < 0.1 pg/g), furans (2,3,7,8-TCDF < 0.1 pg/g), WHO PCBs (< 10 pg/g), and PAHs, (< 350 ng/g).

PE samples will be included at levels bracketing the ATSDR action levels (i.e., < 50 to > 1,000 pg/g TEQ), and will cover a similar concentration range and congener patterns as the environmental samples.

6.2.2 Environmental Samples

Prior to the demonstration, samples were collected from 10 sampling locations from around the country and shipped to Battelle. The sampling locations, which are described in Chapter 5, were identified by EPA Regional staff and MDEQ. The EPA and MDEQ staff who identified specific sampling locations were part of the Dioxin SITE Demonstration Panel (Appendix A) and were responsible for arranging for sample collection and providing information about the sites (Chapter 5). Once received at Battelle, the environmental samples were homogenized and characterized for dioxin/furans (EPA Method 1613B), PCBs (LRMS modified EPA Method 1668A), and 18 target PAHs (NOAA method) to establish the basic composition of the samples. Environmental samples were selected for inclusion in the demonstration based on the preliminary characterization data. Because the soil and sediment samples were dried and homogenized, they will essentially be indistinguishable to the developers. As such, the soil and sediment samples will be jointly referred to as "environmental" samples, with no distinction made between soil or sediment other than in Chapter 5 where the sampling locations are described in detail.

6.2.3 Extracts

To evaluate the detection and measurement performance of each technology independent of the sample extraction method, soil and sediment samples will be extracted using toluene and soxhlet extraction. These extractions will be performed by AXYS Analytical Services (the reference laboratory, see Chapter 7), consistent with the procedures to extract the demonstration samples. The extracts will represent a 10 g soil/sediment sample extraction and will be reported in pg/mL. Total extract volume per 10 g aliquot will be 300 mL, but the sample extracts will be concentrated and provided to the developers as 10 mL extracts. The extracts will not be processed through any clean-up steps. The extracts will be derived from soil/sediment samples that are also included in the suite of environmental samples, so these samples will be analyzed by the developers starting from both the environmental matrix and the extract. All environmental sample extractions will be prepared in the same solvent (toluene), and the developers will be responsible for any solvent exchange steps. The extract samples will also include toluene-spiked solutions that are not extractions of actual environmental samples. Because adequate homogenization at trace quantities will be difficult to achieve, one set of extract samples will be spiked at low levels (< 1 pg/mL) and used as part of the MDL evaluation.

6.3 Pre-Demonstration Study

The best way to predict and prevent problems from occurring during the demonstration is to perform a "dry run" exercise. This was accomplished through a pre-demonstration study. The pre-demonstration samples were sent to all six developers for evaluation in their laboratories, as well as to the reference laboratory. The pre-demonstration study served as a final readiness check for the developer so that modifications could be made to their procedure if warranted by site-specific conditions. It was also a test of the demonstration plan to ensure a well-established process of sampling, compositing, homogenizing, splitting, extract preparation and aliquoting, and shipping of samples to the developers and the reference laboratory. The pre-demonstration study was comprised of 15 samples, including PE samples, environmental samples, and extracts. A distribution of the sample concentrations, as determined by the characterization analyses (see Section 4.3), is presented in Figure 6-2. The samples selected for the pre-demonstration study covered a wide range of concentrations and included a representative of each environmental site that will be analyzed during the demonstration.

The pre-demonstration study was conducted in two phases. In Phase 1, the developers were sent six soil/sediment samples and provided the D/F, PCB, and PAH characterization data with the samples, so that each developer could perform a self-evaluation of their technology's performance. In Phase 2, seven

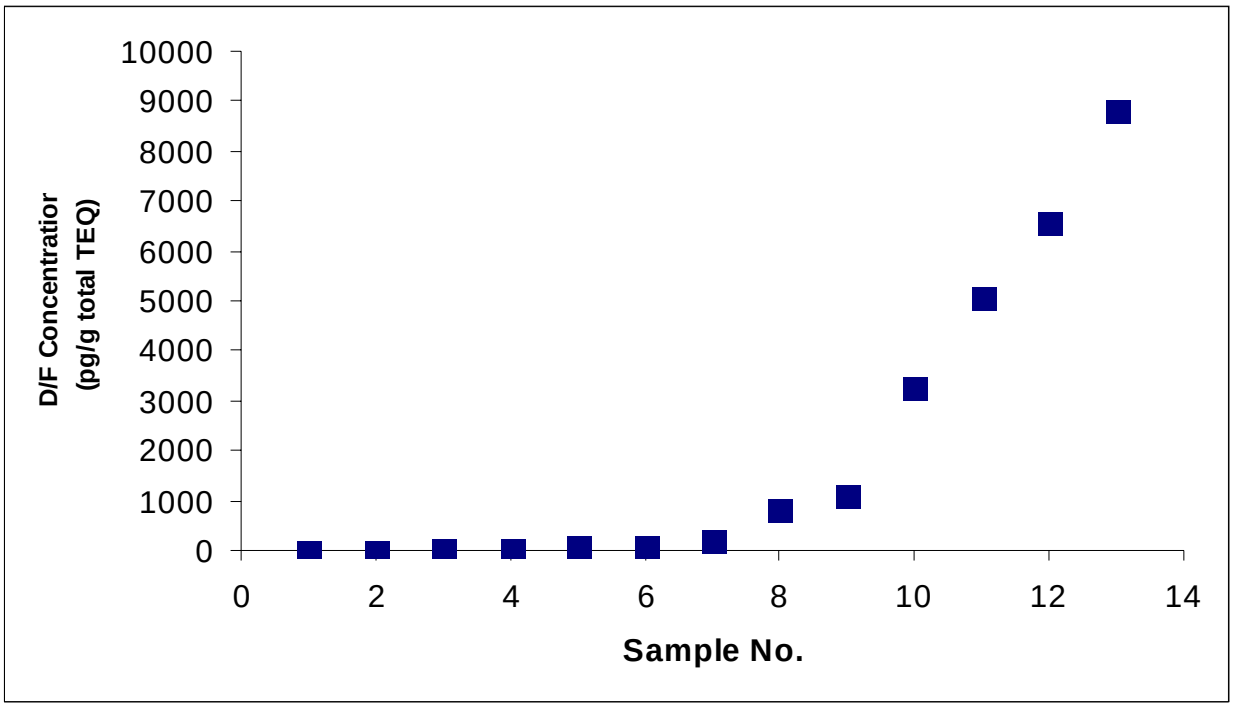

Figure 6-2. Approximate range of pre-demonstration soil/sediment sample concentrations.

additional soil/sediment samples and two extracts were sent to the developers for blind evaluation. The reference laboratory analyzed all 15 pre-demonstration samples blindly. Battelle collected the pre-demonstration results from the developers and the reference laboratory, and then returned the developer/reference laboratory correlated data back to each developer so that the developers could use the HRMS pre-demonstration sample data to refine the performance of their technologies prior to participating in the field demonstration.

6.4 Demonstration Schedule

The developers will analyze the demonstration samples in the mobile laboratories or trailers equipped with fume hoods at the demonstration site in Saginaw, Michigan. The schedule for the demonstration is as follows. Developers will begin shipping equipment and supplies to the demonstration site the week of April 19. On Thursday, April 22, the mobile laboratories and trailers will be installed. The developers can begin arriving as early as April 22 and will have access to the demonstration site as soon as the trailers and mobile laboratories are installed and ready for occupancy. The developers can work at the site on April 23, 24, and 25 setting up their oeprations and running their own quality control samples. The demonstration will officially commence on Monday, April 26, with a safety and site-specific training meeting at the auditorium in the Green Point ELC at 8 am. During this meeting, the health and safety plan will be reviewed so that all participants understand the safety requirements for the demonstration. Site health and safety (H&S) personnel, identified in Chapter 10, will be readily accessible throughout the course of the demonstration in case H&S questions arise. During the kick-off meeting, logistics will also be discussed, such as how samples will be distributed and results reported. It is anticipated that the developers will analyze the demonstration samples for 3 to 9 days, depending upon the sample throughput of each technology. Ideally, all 209 samples would be analyzed on-site, but the sample throughout of some of these technologies would require three weeks or more in the field to analyze 209 samples. Consequently, it was decided that the number of samples to be analyzed in the field by each developer would be determined at the developer's discretion. The developers are requested to analyze all 23 extract samples and at least half of the soil/sediment samples (93 samples). If a developer does not complete all 209 analyses in the field, the remaining samples will be shipped to the developer's laboratory at the conclusion of the demonstration activities and results will be due to Battelle at a pre-determined time after leaving the site. The time that the results generated off-site will be due to Battelle will be specified and adhered to by the developer. The estimated schedules for each developer for the field and laboratory portions of the demonstration are presented in Figures 6-3 and 6-4, respectively.[1] The schedule for the reference laboratory is also included.

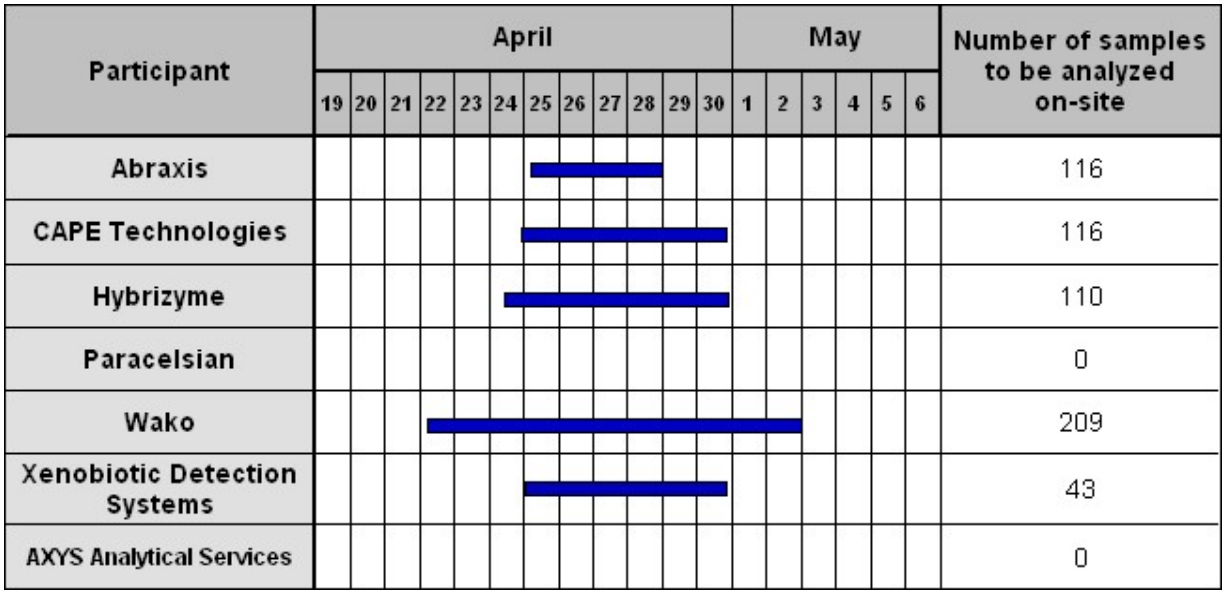

Figure 6-3. Estimated participant schedule for field component of demonstration.

[1]Please see Addendum for additional information regarding Paracelsian's participation.

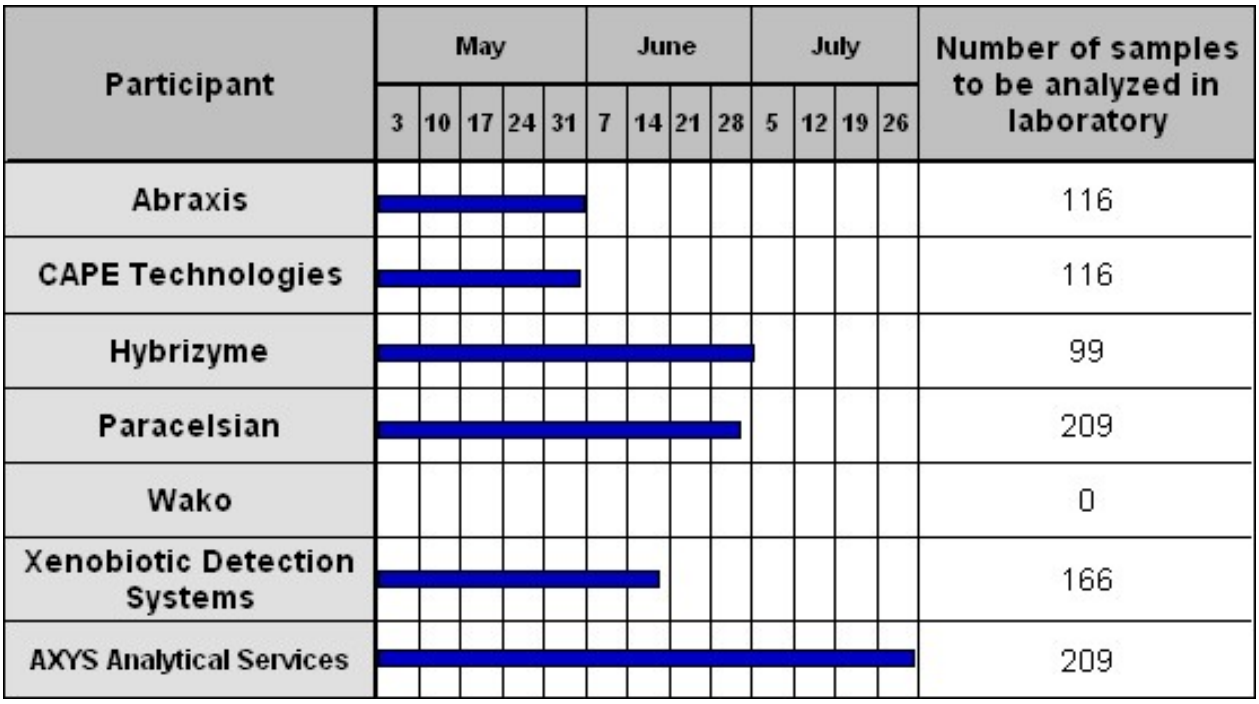

Figure 6-4. Estimated participant schedule for laboratory portion of demonstration.

6.5 Demonstration Design

Tables 6-3 through 6-6 include a generic summary of the samples to be included in the demonstration. The samples will be prepared, homogenized, and split into subsamples according to the processes outlined in Chapter 4. Samples will be labeled with a unique identifier and randomized blind analysis will be performed by the developers. Certified, spiked, uncontaminated (i.e., blank), and environmental samples will be randomly inserted into the testing scheme. Chain-of-custody procedures will be used to transfer samples from Battelle to the developers. Some sample information will be provided when the samples are distributed. All samples that are believed to have at least one D/F or PCB congener greater than 10,000 pg/g will be marked with an asterisk for safety purposes. The sites for the environmental samples will be identified to the reference laboratory for congener pattern recognition. Wako and CAPE Technologies requested that samples be identified as “soil” or “sediment”; none of the other developers wished to have that information supplied. This sample information will be supplied when the first batch of samples are released.

Table 6-3 Designations for Generic Data Qualifiers

Designation	Total $TEQ_{D/F}$ (pg/g)	WHO PCB [a] (pg/g)	PAHs [a] (ng/g)
L [b]	< 50	< 1,000	< 1,000
M	50 - 1,000	1,000 - 10,000	1,000 - 10,000
H	> 1,000	> 10,000	> 10,000
U [c]	not characterized	not characterized	not characterized

[a] Characterized for target list only; indicates approximate value of each target analyte
[b] Includes non-detects
[c] Applies to SRMs only where certified data not available for certain PAHs or PCBs

Table 6-4 Distribution of Performance Evaluation Samples

Sample Type	Generic Data Qualifier			No. of Replicates per Sample
	D/F	PCB	PAH	
PE# 1	L	L	U	7
PE# 2	H	U	U	4
PE# 3	M	L	U	7
PE# 4	M	H	U	4
PE# 5	M	H	L	4
PE# 6	L	L	L	4
PE# 7	L	L	L	4
PE# 8	L	L	M	4
PE# 9	L	L	L	4
PE# 10	L	H	L	4
PE# 11	L	M	L	4
PE# 12	certified blank	certified blank	certified blank	8
Total Number of PE samples				***58***

Table 6-5 Distribution of Environmental Samples

Sample Type	Generic Data Qualifier			No. of Replicates per Sample
	D/F	PCB	PAH	
Environmental Site #1 Warren Co, North Carolina	M	H	L	4
	H	H	H	4
	H	H	H	4
Environmental Site #2 Tittabawassee River (soil), Michigan	L	L	L	4
	M	L	L	4
	M	L	L	4
Environmental Site #3 Newark Bay, New Jersey	L	L	L	4
	M	L	L	4
	L	L	L	4
	L	L	L	4
Environmental Site #4 Raritan Bay , New Jersey	L	L	L	4
	L	L	L	4
	L	L	L	4
Environmental Site #5 Winona, Missouri	H	L	M	4
	H	M	M	4
	H	L	M	4
Environmental Site #6 Tittabawassee River (sediment), Michigan	L	L	L	4
	M	L	L	4
	L	L	L	4
Environmental Site #7 Brunswick, Georgia	L	L	M	4
	L	L	L	4
	H	L	H	4
Environmental Site #8 Saginaw River, Michigan	M	M	L	4
	H	M	L	4
	M	L	H	4
Environmental Site #9 Midland, Michigan	M	L	L	4
	M	L	L	4
	M	L	L	4
	L	L	L	4
Environmental Site #10 Nitro, West Virginia	L	H	L	4
	H	H	L	4
	H	H	L	4
Total number of environmental samples				***128***

Table 6-6 Distribution of Extract Samples[a]

Sample Type	Generic Data Qualifier			No. of Replicates per Sample
	D/F	PCB	PAH	
Extract# 1	L	L	L	4
Extract# 2	H	L	M	4
Extract# 3	L	L	L	7
Extract# 4	M	M	L	4
Extract# 5	L	H	L	4
Total number of extracts				***23***

[a] All developers will analyze the 23 extract samples on-site during the demonstration. Extract samples will be prepared by the reference laboratory in their typical extraction solvent (toluene).

Independent technical observers, Battelle staff who are familiar with use of these technologies, will watch the developer analyses as they are being performed to meet the second objectives of the demonstration to understand the operational aspects of the technologies. It is likely that all observers will intermittently observe each technology in operation over the course of the demonstration, but one observer will be assigned primary responsible for each technology. The checklists that will be used to document the observers notes and facilitate the observations are presented in Appendix E. These observations will be summarized and reported in each developer's ITVR. Each developer will have the opportunity to review the observer notes for their technology prior to inclusion in the report. Photo-documentation will supplement written observations recorded by Battelle staff. However, Battelle will not photograph any portion of the developer's technology without approval.

The amount and type of hazardous and non-hazardous waste that is generated during the demonstration will be recorded and confirmed by Battelle. All sample by-products produced during testing will be returned to Battelle for quantification and characterization prior to disposal.

The results of the demonstration will likely be impacted by fatigue if the developers work more than 12 hours per day, so the developers will be advised to work no more than a 12-hour day for each day of testing (e.g., 7 am – 7 pm). The exact start and end time for each demonstration day will be decided on-site amongst all the participants. It is requested that the developers stay on approximately the same start time, as requests to start and end significantly later than the other participants (for example, working noon to midnight when all of the other participants are working approximately 7 am to 7 pm) will not be granted.

The developers will be responsible for operating their own technologies and for providing all equipment and supplies needed for its operation during the demonstration, unless otherwise arranged with Battelle prior to the demonstration. The developers will also be responsible for supplying their own personal protective equipment such as gloves and lab coats.

6.6 Assessment of Primary and Secondary Objectives

The term, “dioxin and dioxin-like compounds” groups several hundreds of compounds in a category because these compounds are similar in chemical structure and biological activity. The reference laboratory will measure individual congeners that are the most significant sources of toxicity. The seventeen 2,3,7,8-substituted PCDD/F will be determined by Method 1613B; 12 WHO PCBs will be reported using Method 1668A. The developer technologies will report TEQ, total TEQ or DEQ (dioxin equivalents), but none will report data for individual congeners. As shown in Table 6-7, some technologies will report (1) total $TEQ_{D/F}$ (2) total TEQ_{PCB}, or (3) total TEQ, including contributions from D/F and PCBs. For the reference laboratory data, congener concentrations will be converted to TEQ and subsequently summed to determine total TEQ, using the TEFs established by WHO in 1998[4] (see Table 1-1).

For the reference laboratory data, non-detects will be assigned a TEQ value of zero and ½ of the detection limit. Detection limits will be reported as SDLs (Sample-Specific Detection Limits). SDLs will be determined from 2.5 times the noise in the chromatogram, converted to an area and then to a concentration using the same calculation procedure as for detected peaks. Any value that meets all quantification criteria (>SDL and ratio) will be reported as a concentration. A"J" flag will be applied to any reported value that is between the SDL and the lowest level calibration. The concentration of any detected congener that does not meet all quantification criteria (such as ratio or peak shape) will be reported but given a "K" flag to indicate estimated maximum possible concentration (EMPC). TEQs will be reported in 2 ways to cover the range of possible TEQ values:

1. All ND and EMPC values will be assigned a zero concentration in the TEQ calculation.

2. NDs will be assigned a concentration of one half the SDL. EMPCs will be assigned a value equal to the EMPC.

In both cases, any TEQ which has = 10% contribution from J flagged or K flagged data will also be flagged as J or K or both as appropriate.

Table 6-7. Comparison Between Developer and Reference Laboratory Data

Developer	Data will be reported as ...	Comparison to Reference Laboratory Data	Detection Limit Claimed by Developer (pg TEQ/g)	Reference Data From Method ...
CAPE Technologies	Total $TEQ_{D/F}$	Total $TEQ_{D/F}$	1	1613B
Hybrizyme	AhR units	Total $TEQ_{D/F}$ Total TEQ[a]	10	1613B 1613B+1668A
Paracelsian	Total DEQ[b]	Total $TEQ_{D/F}$ Total TEQ	1	1613B 1613B+1668A
Abraxis	Total TEQ_{PCB}	Total TEQ_{PCB} PCB 126 TEQ	6.25	1668A
Wako	2,3,7,8 TCDD EQ[c] pg/g	2,3,7,8-TCDD TEQ Total $TEQ_{D/F}$	20	1613B
Xenobiotic Detection Systems	TEQ D/F TEQ PCBs	total $TEQ_{D/F}$ total TEQ_{PCB}	0.3	1613B 1668A

[a] Total TEQ is the sum of the total $TEQ_{D/F}$ and total TEQ_{PCB}
[b] DEQ = equivalents to 2,3,7,8-TCDD
[c] EQ = equivalents

Because each technology will report data slightly differently, as described in Table 6-7, and each technology functions and is calibrated by different procedures, specific comparisons with the reference laboratory data will be technology-dependent and fully described in each ITVR. However, the similar statistical principles will be applied to the evaluation of each technology, as described below.

6.6.1 Primary Objective P1: Accuracy

The determination of accuracy for each technology's measurements will be based on their agreement with the certified or spiked levels of PE samples. For each technology, PE samples containing concentrations from across the analytical range of interest will be analyzed. The technology measurements from the 58 PE samples will be evaluated to determine whether there is a statistically significant difference between the technology measurements and the certified value or spiked level. Percent recovery values relative to the certified or spiked concentrations will also be calculated. Battelle will evaluate whether a statistically significant difference exists between a given technology's results and the reference values by performing a two-tailed, paired, Student's t-test. The null hypothesis will be that the mean difference between the technology results and the certified or spiked value is zero. The PE samples will also be analyzed by the laboratory reference method for confirmation of certified and spiked values.

To evaluate accuracy, the average of replicate results from the field technology measurement will be compared to the certified or spiked value of the PE samples to calculate percent recovery. The equation to be used will be:

$$R = \overline{C} \; / \; C_R \times 100$$

where $\overline{C}$ is the average concentration value calculated from the technology replicate measurements and C_R is the certified value. For the spiked samples, if the reference laboratory's average measured value is within 10% of the spiked concentration value, the spiked concentration value will be used as the certified value. If the average measured value by the reference laboratory is > 10% different, the reference laboratory's average measured value will be the certified value.

6.6.2 Primary Objective P2: Precision

A technology's precision refers to its reproducibility. Higher precision leads to less uncertainty in the results. To evaluate each technology's precision, all samples (including PE, environmental and extract samples) will be analyzed in at least quadruplicate. Seven replicates of three different samples will also be analyzed to evaluate method detection limits. Replication is necessary because precision will be evaluated at both low and high concentration levels, and across different matrices. The statistic used to evaluate precision is RSD. The equation used to calculate standard deviation (*SD*) between replicate measurements will be:

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

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

The equation used to calculate RSD between replicate measurements will be:

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

Low RSD values indicate high precision. For a given set of replicate samples, the RSD of a given technology's results will be 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.

6.6.3 Primary Objective P3: Comparability

A third primary performance objective is comparability, i.e., the degree of agreement between each technology and reference laboratory results. Battelle maximized data comparability by using the homogenization procedures and applying criteria for acceptable results prior to a sample being included in the demonstration, as described in Section 4.2.

For comparability, Battelle will evaluate whether a statistically significant difference exists between the measurements provided by a given technology and the laboratory reference method by performing a two-tailed, paired, Student's t-test. If the data are found to be non-normally distributed, a nonparametric Wilcoxon signed-rank test will be performed to determine if the two sets of results are statistically the same or different.

Technology results will also be compared to the corresponding reference laboratory by calculating a relative percent difference (RPD) for the average of each paired and replicate measurement. The equation for RPD is as follows:

$$RPD = \frac{(M_R - M_D)}{average(M_r, M_D)}$$

where M_R is the reference laboratory measurement and M_D is the developer measurement. RPD values less than 25% will indicate good agreement between the two measurements. Because the absolute value will not taken, negative RPD values can be obtained (which would indicate that the technology measurements were less than the reference laboratory measurements). As such, the median RPD value will be calculated (rather than the average RPD where the negative and positive values would be neutralized) to provide a summary calculation of comparability between each technology's results and reference laboratory measurements.

As described in Section 1.3, the reference laboratory concentration data will be converted to TEQ using the WHO TEF values. TEQs will be calculated by the developer technologies in unique ways that may or may not be directly comparable to the HRMS total $TEQ_{D/F}$, total TEQ_{PCB}, or total TEQ results, depending upon the developer calibration techniques (i.e., calibration to a single congener) and/or the functionality of the developer's technology and its relative responsiveness to the WHO-established TEFs. The correlation of the developer results with the HRMS results will be evaluated and it will be noted if the developer technology does or does not track the HRMS results on a relative scale. Assessments will be made as to how well the developer results tracked the HRMS results in light of possible confounding factors such as the sample containing other Ah Receptor binding compounds, cross-reactive compounds, or extremely high concentrations of particular analytes.

6.6.4 Primary Objective P4: Method Detection Limit

A fourth primary performance objective is to determine the MDL for each technology. To determine the MDLs, the developer will analyze seven aliquots of two low-level PE soil samples and seven aliquots of a low-level spiked extract. Battelle will use these data to calculate an MDL for each technology. The concentration of the samples will be dependent on the detection capability of each technology, but will ideally be three to five times the reporting limit for each technology.

The MDL calculation procedure to be followed is described in 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, which will be true for the demonstration because of the homogenization and sample preparation procedures that will be followed.
Battelle will use the Student's t-value and standard deviation to calculate the MDL for each technology in soil and sediment as shown in the following equation:

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

where $t_{(n-1,1-\infty=0.99)}$ = Student's t-value appropriate for a 99 percent confidence level and a standard deviation estimate with n-1 degrees of freedom.

6.6.5 Primary Objective P5: False Positive/False Negative Results

Battelle will investigate the tendency for each technology to return fp results, that is, results reported above the reporting limits for the field technology but below the reporting limits of the reference laboratory. The frequency of fp results will be reported as a fraction of results available for fp analysis. Similarly, the frequency of fn results will also be examined. For this purpose, Battelle will evaluate

results for samples reported as having concentrations above the reporting limits of the reference laboratory method to identify cases of the reference laboratory reporting detections and a technology reporting non-detects. As with the analysis of false positives, the statistic analyzed for each technology will be the percentage of fn out of all samples reported as detections by the laboratory method.

A point of clarification regarding the "reporting limits" of a technology and the "method detection limits" as described in Section 6.6.4. The reporting limits to be used in this evaluation will be the value designated by each technology developer and the reference laboratory, as the lowest level to be reported for each sample. This will vary from developer to developer. The MDL determination will include an evaluation of the stated reporting limits, with a sample 3 to 5 times the reporting limit of each technology analyzed 7 times, to assess the closeness of the technology's calculated MDL to the reporting limits used in the demonstration.

6.6.6 Primary Objective P6: Matrix Effects

The likelihood of matrix-dependent effects on performance will be investigated by evaluating the data sets in multiple ways. This will include evaluation of results in the following ways: samples from the ten different environmental sampling locations individually and as a group to determine if performance was different for environmental samples versus PE samples; grouping the data by matrix (i.e., soil, sediment, extract); evaluating samples with similar congener patterns (e.g., samples with high hepta- and octa-dioxins); assessing the performance with samples containing high levels of contaminants other than dioxins (e.g., PCBs or PAHs); and evaluation of in-field versus laboratory conducted measurements (where appropriate). These evaluations will also include looking at the performance of the technology for the samples which were analyzed both as extracts and as environmental soil/sediment samples. Differences in results provided for the soil/sediment samples and their associated extract samples may indicate a matrix or homogeneity issue if the comparison of the developer and HRMS results for the extract samples are in better agreement than for the analysis of the associated soil/sediment sample. If the soil/sediment sample HRMS and developer results are highly correlated, but the comparative results for the extracts are not, it may indicate that the toluene extract was not compatible with the developer's procedure if toluene is not their extraction solvent.

6.6.7 Primary Objective P7: Technology Costs

Since conventional laboratory-based analytical methods for measuring dioxins are relatively costly, the cost of each field technology is an important evaluation factor. With input from each technology developer, Battelle will document the full cost of each technology and compare those costs to typical and actual costs for D/F and PCB analytical methods. At a minimum, cost inputs will include equipment, consumable materials, mobilization and demobilization, and labor. Battelle will document what equipment was provided by the developer and what was provided by Battelle.

6.6.8 Secondary Objective S1: Skills and Training Required to Properly Operate the Technology

As described in Section 6.5, Battelle observers will be assigned to each of the technologies. These notes and observations will determine the number and skill-level of the operators. The observers will also determine the type of background and training required to properly operate the technology. The evaluation of this secondary objective will also include how user-friendly the technologies are. The developers will have the opportunity to review and comment on the observers notes before the observations are incorporated into the report to ensure accuracy.

6.6.9 Secondary Objective S2: Document Health and Safety Aspects Associated with the Technology

Because dioxins are a class of toxic compounds, it is important to understand the health and safety aspects associated with each technology. This will include health and safety issues when operating the technology as well as the amount and type of hazardous and non-hazardous waste generated by the technology. The outcomes from this evaluation will be based on the observer notes.

6.6.10 Secondary Objective S3: Document the Portability of the Technology

This evaluation will document if the technology can be readily transported to the field and how easy the technology was to operate in the field. The Battelle observers will be responsible for the collection and reporting of this information.

6.6.11 Secondary Objective S4: Evaluate Sample Throughput

Because HRMS methods are time consuming, it will be important to note the sample throughput (i.e., number of samples that can be processed in a typical work day). This secondary objective will be evaluated based on the observer notes, which will make particular note of the time limiting steps of the procedures, as well as the documentation of sample custodianship that will be recorded on the COC/results forms (Appendix D). The number of operators involved in the sample analyses will also be discussed.

6.7 Schedule of Events

Table 6-8 is a summary of events since the award of the contract to perform this demonstration. The reports from the data generated in this demonstration will be submitted to EPA for publication in December 2004.

Table 6-8. Schedule of Demonstration Events

Event	Schedule for Completion	Actual Completion Date
Contract award	n/a	June 18, 2003
Prepare and distribute developer survey	July 18, 2003	July 18, 2003
First Conference Call	**July 28, 2003**	**July 29, 2003**
Distribute summary notes from the conference call	August 5, 2003	July 31, 2003
Develop preliminary strategy for sample homogenization	August 12, 2003	August 12, 2003
Prepare one-page demonstration flyer for Dioxin 2003 Conference	August 22, 2003	August 22, 2003
Obtain dioxin-contaminated soil from one site and test homogenization procedure	September 30, 2003	October 7, 2003
Draft homogenization procedure	October 3, 2003	October 1, 2003
Second Conference Call	**October 8, 2003**	**October 8, 2003**
Identify, obtain, and homogenize samples from additional sites	November 28, 2003	November 13, 2003
Third Conference Call	**December 4, 2003**	**December 4, 2003**
First draft demonstration plan to EPA, developers, peer reviewers, and 1 or 2 technical advisors	December 12, 2003	December 12, 2003
Final receipt of environmental samples	December 19, 2003	December 24, 2003
PE samples sent to dioxin laboratories and audits scheduled	January 9, 2004	January 12, 2004
Comments due to Battelle on first draft demonstration plan	January 15, 2004	January 15, 2004
Fourth Conference Call	**February 5, 2004**	**February 5, 2004**
Reference laboratory selected	February 3, 2004	February 20, 2004
Pre-demonstration samples distributed	February 10, 2004	Phase 1: February 12, 2004 Phase 2: March 16, 2004
Developer and reference laboratory pre-demonstration results due to Battelle	March 31, 2004	April 16, 2004
Distribute second draft demonstration plan to EPA, developers, and entire Dioxin SITE Demonstration Panel (includes peer reviewers, technical advisors, and observers) for final review	March 31, 2004	April 2, 2004
Pre-demonstration results distributed to developers	April 9, 2004	April 16, 2004
Fifth Conference Call	**April 8, 2004**	**April 8, 2004**
Comments due to Battelle on third draft demonstration plan	April 12, 2004	April 12, 2004
Demonstration plan finalized	April 16, 2004	April 20, 2004
Field demonstration (Saginaw, Michigan)	April 26 through May 5, 2004 Visitor's Day on April 28	
Audit of reference laboratory	May 24, 2004	
First draft report to EPA	August 2, 2004	
Remaining five draft reports to EPA	September 6, 2004	
Reports to developers for review	October 1, 2004	
Reports to peer review	November 1, 2004	
Final reports submitted to EPA	December 17, 2004	
Contract ends	December 30, 2004	

Chapter 7
Confirmatory Process

This chapter describes the process performed by Battelle for the selection of the reference method and laboratory. The reference laboratory has provided the method performance information that is presented in this chapter.

7.1 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 both high resolution mass spectrometric methods. Method 1613B includes more labeled internal standards which, therefore, 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 dioxin/furan reference method.

The Dioxin SITE Demonstration Panel (Appendix A) proposed that an equal amount of reference data be generated to determine the PCB contribution to the TEQ, since risk assessment is often times based on TEQ values that are not class specific. As such, the complimentary HRMS method for PCB TEQ determinations, Method 1668A, was selected as the reference method for PCBs.

7.2 Reference Laboratory Selection

Battelle generated a list of ten prospective laboratories, based on conversations with EPA Regional staff who routinely use dioxin laboratories, personal experiences, and recommendations from the Dioxin SITE Demonstration Panel. The ten laboratories were contacted by Battelle and were sent a questionnaire to complete (see Appendix F). The 14 questions were geared towards understanding the capabilities of the laboratories, their experience with analyzing dioxin samples for EPA, and their ability to meet the needs of this demonstration. The responses from the questionnaires were studied and two laboratories were selected for the next phase of the selection process. The two laboratories were sent three blind audit samples and were audited by two auditors from Battelle. The day-long audit at each laboratory 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 follow-up questions to each laboratory to address gaps in the observations. This was done to ensure consistency in the process.

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, so AXYS was selected as the reference laboratory for this demonstration. The statement of work that was provided to AXYS to perform the reference methods is provided in Appendix G.

7.3 Reference Laboratory Sample Preparation and Analytical Methods

The purpose of this section is to describe the reference methods that will be used in the demonstration sample analyses. Both Method 1613B[6] and Method 1668A[7] allow for method modifications, as long as the modifications are documented and the requirements for method performance (i.e., performance specifications for quality control samples) can be met. The following sections briefly describe the methods and the modifications to the methods that AXYS performs. For brevity, the entire methods are not reproduced in this document, but the applicable sections where AXYS will perform modifications have been cited for convenient reference.

7.3.1 Dioxin/Furan Analysis

All procedures are carried out according to protocols as described in AXYS Summary Method Doc MSU-018 Rev 2 18-Mar-2004 (AXYS detailed SOP MLA-017 Rev 9 May-2004), which is based on EPA Method 1613B, with the modifications summarized below. Briefly, samples are spiked with a suite of isotopically labeled surrogate standards prior to analysis, solvent extracted, and cleaned up through a series of chromatographic columns that may include gel permeation, silica, Florisil, carbon/Celite, and alumina columns. The extract is concentrated and spiked with an isotopically labeled (internal) standard. Analysis is performed using a high-resolution mass spectrometer coupled to a high-resolution gas chromatograph equipped with a DB-5 capillary chromatography column (60 m, 0.25 mm i.d., 0.1 µm film thickness). A second column, DB-225 (30 m, 0.25 mm i.d., 0.15 µm film thickness), is used for confirmation of 2,3,7,8-TCDF identification.

The following is a summary of AXYS's 1613B method modifications. The italicized text is the portion of the method that is being modified, followed by the AXYS modification.

Section 2.1.2 The labeled compounds are spiked into a sample containing 10 g (dry weight) of solids. Samples containing multiple phases are pressure filtered and any aqueous liquid is discarded. Coarse solids are ground or homogenized. Any non-aqueous liquid from multi-phase samples is combined with the solids and extracted in an SDS extractor. The extract is concentrated for cleanup.
Modification: Non-aqueous liquid from the multiphase sample is combined with the solid phase and extracted by Dean Stark soxhlet.

Section 7.2.1: Sodium sulfate, reagent grade, granular, anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 400 ℃ for one hour minimum, cooled in a dessicator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon in the crystal matrix), that batch of reagent is not suitable for use and should be discarded. Extraction with methylene chloride (as opposed to simple rinsing) and baking at a lower temperature may produce sodium sulfate that is suitable for use.

Modification: Anhydrous sodium sulphate (Na_2SO_4) is purchased in powder form (not granular) and is baked overnight prior to use. There is no solvent rinse with dichloromethane.

Section 7.10: From stock solutions, or from purchased mixtures, prepare this solution to contain the labeled compounds in nonane at the concentrations shown in Table 3.
Note: See Appendix H for Table 3. All tables in Appendix H are reproduced verbatim from the method.
Modification: The concentration of the labeled compound spiking solution is 100 ng/mL (except for OCDD which is 200 ng/mL), the concentration of the labeled clean-up standard solution is 10 ng/mL and the concentration of the labeled injection internal standard solution is 100 ng/mL. The spiking volume for all these labeled standard solutions is 20 µL. The resulting concentrations in the final extracts are as specified in the method.

Section 7.11: Prepare Cl -2,3,7,8-TCDD in nonane at the concentration shown in Table 3. The cleanup standard is added to all extracts prior to cleanup to measure the efficiency of the cleanup process.
Modification: The concentration of the clean-up standard spiking solution is 10 ng/mL and the sample spiking volume is 20 µL. The resulting concentrations in the final extracts are as specified in the method.

Sections 7.13 Combine the solutions in Sections 7.9 through 7.12 to produce the five calibration solutions shown in Table 4 in nonane. 14.0 HRGC/HRMS Analysis 15.0 System and Laboratory Performance.
Note: See Appendix H for Table 4.
Modification: An additional lower level calibration solution, 0.2 times the concentration of CS1, is prepared and included in the initial calibration series. Initial calibration is based on a six-point series.

Section 7.14 Precision and Recovery (PAR) Standard-Used for determination of initial (Section 9.2) and ongoing (Section 15.5) precision and recovery. Dilute 10 µL of the precision and recovery standard (Section 7.9.1 or 7.9.2) to 2.0 mL with acetone for each sample matrix for each sample batch. One mL each are required for the blank and OPR with each matrix in each batch.
Modification: The concentrations of the PAR spiking solutions are 0.2/1.0/2.0 ng/mL for tetra/penta, hexa, and hepta/octa, respectively, and the spiking volume is 1 mL. The resulting final concentration in the extracts are as specified in the method.

Section 11.5 Preparation of Samples Containing Greater Than 1% Solids.
Modification: Aqueous samples containing > 1% visible solids are prepared and extracted using the same procedure as samples containing ≤1% visible solids. This involves extracting the solids by soxhlet and the filtrate by separatory funnel extraction and combining the extract from the two phases.

Section 12 Extraction procedures include separatory funnel (Section 12.1) and solid phase (Section 12.2) for aqueous liquids; Soxhlet/Dean-Stark (Section 12.3) for solids, filters, and SPE disks; and Soxhlet extraction (Section 12.4.1) and HCl digestion (Section 12.4.2) for tissues. Acid/base back-extraction (Section 12.5) is used for initial cleanup of extracts. Macro-concentration procedures include rotary evaporation (Section 12.6.1), heating mantle (Section 12.6.2), and Kuderna-Danish (K-D) evaporation (Section 12.6.3). Microconcentration uses nitrogen blowdown (Section 12.7).
Modification: Samples with sufficiently low moisture content may be mixed with Na_2SO_4 and extracted using the regular soxhlet apparatus in 80:20 toluene:acetone.

Section 12.4 Add 30-40 g of powdered anhydrous sodium sulfate to each of the beakers (Section 11.8.4) and mix thoroughly. Cover the beakers with aluminum foil and allow to equilibrate for 12-24 hours.

Modification: The equilibration time for the sodium sulphate drying step is that required to produce a dry, free flowing powder (minimum thirty minutes). This may be less than the 12-hour minimum specified.

Section 12.5.1 Spike 1.0 mL of the cleanup standard (Section 7.11) into the separatory funnels containing the sample and QC extracts from Section 12.1.4.1, 12.3.9.1.3, or 12.3.9.2.
Modification: Samples are spiked with cleanup standard right after extraction and before reduction; not spiked into the separatory funnels containing the extracts prior to the acid/base wash.

Section 12.6.1.1 Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45 ℃. On a daily basis, preclean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for a contamination check if necessary. Between samples, three 2-3 mL aliquots of solvent should be rinsed down the feed tube into a waste beaker.
Modification: Rotary evaporator baths are maintained at 35°C. Mimic proofs are collected instead of collecting proofs each day and archiving.

Section 13.0 Extract Cleanup.
Modification: Extracts may be cleaned up on silica, alumina, and carbon chromatographic columns using a Fluid Management System automated cleanup system.

Sections 14.0 HRGC/HRMS Analysis 15.0 System and Laboratory Performance. , 16.0 Qualitative Determination, Table 8, Table 9:
Note: See Appendix H for Tables 8 and 9.
Modification: M/Z channels 354/356 and 366/368 are used to confirm and quantify the native and surrogate penta-substituted dioxins, respectively; this change from the method's specification is made in the instrument method in order to avoid a persistent interference in the 356/358 and 368/370 M/Z channels. The theoretical ratio for the P5CDD M/M+2 ions is 0.61; therefore, the acceptance range is 0.52 - 0.70.

Section 17.0 Quantitative Determination
Modification: $Conc_i$ - the concentrations of target analytes, and the labeled compound concentrations and recoveries, are calculated using the equations below. These procedures are equivalent to those described in the method, but are more direct.

$$Conc_i = \frac{A_i}{A_{si}} \times \frac{M_{si}}{RRF_{i,si}} \times \frac{1}{M_x}$$

where A_i = summed areas of the primary and secondary m/z channels for the analyte peak of interest (compound *i*)

A_{si} = summed areas of the primary and secondary m/z channels for the labeled surrogate peak used to quantify *i*)

M_x = mass of sample taken for analysis

M_{si} = mass of labeled surrogate (compound *si*) added to sample as calculated by the concentration of standard spiked (pg/mL) multiplied by the volume spiked (mL)

$RRF_{i,si}$ = mean relative response factor of *i* to *si* from the six-point calibration range and defined individually as:

$$RRF_{i,si} = \frac{A_{si}}{A_{rs}} \times \frac{M_{rs}}{M_{si}}$$

Concentrations of surrogate standards are calculated using the following equation:and, the percent

$$Conc_{si} = \frac{A_{si}}{A_{rs}} \times \frac{M_{rs}}{RRF_{si,rs}}$$

recoveries of the surrogate standards are calculated using the following equation:

$$\%\text{Recovery} = \frac{A_{si}}{A_{rs}} \times \frac{M_{rs}}{RRF_{si,rs}} \times \frac{1}{M_{si}} \times 100$$

where A_{rs} and A_{si} are the summed peak areas (from the primary and secondary m/z channels) of recovery standard and labeled surrogate added to the sample

M_{rs} and M_{si} are the masses of recovery standard and labeled surrogate added to the sample

$RRF_{si,rs}$ is the mean relative response factor of the labeled surrogate to the recovery standard as determined by the six-point calibration range and defined individually as:

$$RRF_{si,rs} = \frac{A_i}{A_{si}} \times \frac{M_{si}}{M_i}$$

Section 17.5 If the selected ion current profile area at either quantitation m/z for any compound exceeds the calibration range of the system, a smaller sample aliquot is extracted.
Modification: Extracts may be diluted with solvent and reanalyzed by GC/MS isotope-dilution to bring the instrumental response to within the linear range of the instrument. For very high-level samples where a smaller sample aliquot may not be representative, extracts may be diluted and respiked with labeled quantification standards and reanalyzed by GC/MS to bring the instrumental response analytes within range. Final results may be recovery corrected using the mean recovery of labeled quantification standards.

7.3.1.1 Effects of these Modifications on Method Performance

It should be noted that the performance of this method including these modifications will be verified during the in-process audit of the reference laboratory and by the laboratory's performance on QC samples. These checks will confirm that the above-listed modifications will not negatively affect the accuracy or precision of the reference laboratory results.

7.3.2 PCB Analysis

The method is 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, incorporating the AXYS modifications described below. Details of all procedures are documented in AXYS method MLA-010, *Analytical Method for*

Determination of 209 PCB Congeners by EPA Method 1668A. Briefly, samples are spiked with isotopically labeled surrogate standards, solvent extracted and cleaned up on a series of chromatographic columns which may include silica, Florisil, alumina, carbon/Celite and gel permeation columns. The final extract is spiked with isotopically labeled recovery (internal) standards prior to instrumental analysis. Analysis of the extract is performed on an HRMS coupled to a HRGC equipped with a SPB-Octyl chromatography column (30 m, 0.25 mm i.d., 0.25 µm film thickness). Resolution of the PCB 156/157 coelution may be achieved by high resolution GC/MS using a DB-1 chromatography column (30 m, 0.25 mm id, 0.25 µm film thickness). Where required to eliminate interferences, extracts may be cleaned up by carbon columning to isolate the toxic PCBs of interest; analysis may then be conducted using the DB-1 column as the primary GC column rather than the SPB Octyl column.

The following is a summary of AXYS's 1668A method modifications. The italicized text is the portion of the method that is being modified, followed by the AXYS modification.

Section 4.2.1 Glassware should be rinsed with solvent and washed with a detergent solution as soon after use as is practical. Sonication of glassware containing a detergent solution for approximately 30 seconds may aid in cleaning. Glassware with removable parts, particularly separatory funnels with fluoropolymer stopcocks, must be disassembled prior to detergent washing. 4.2.2 After detergent washing, glassware should be rinsed immediately; first with methanol, then with hot tap water. The tap water rinse is followed by another methanol rinse, then acetone, and then methylene chloride.
Modification: The protocol for washing reusable glassware includes a detergent wash, water rinse and baking at 325°C for 8 hours. Immediately prior to use, glassware is solvent rinsed with toluene and hexane.

Section 4.7 Cleanup of tissue-The natural lipid content of tissue can interfere in the analysis of tissue samples for the CBs. The lipid contents of different species and portions of tissue can vary widely. Lipids are soluble to varying degrees in various organic solvents and may be present in sufficient quantity to overwhelm the column chromatographic cleanup procedures used for cleanup of sample extracts. Lipids must be removed by the anthropogenic isolation column procedure in Section 13.6, followed by the gel permeation chromatography procedure in Section 13.2. Florisil (Section 13.7) is recommended as an additional cleanup step.
Modification: The first cleanup column for tissue extracts is a gravity gel permeation column (SX-3 Biobeads). An anthropogenic isolation column 7.5.3 is not used.

Section 6.5.1 Pyrex glass wool-Solvent-extracted using a Soxhlet or Soxhlet/Dean-Stark extractor for 3 hours minimum
Modification: Glass wool is cleaned by rinsing twice with toluene and twice with hexane.

Section 7.12 Labeled Toxics/LOC/window-defining standard spiking solution-This solution is spiked into each sample (Section 9.3) and into the IPR (Section 9.2.1), OPR (Section 15.5), and blank (Section 9.5) to measure recovery. Dilute the Labeled Toxics/LOC/window-defining stock solution (Section 7.9.1) with acetone to produce a concentration of the labeled compounds at 2 ng/mL, as shown in Table 3. When 1 mL of this solution is spiked into an IPR, OPR, blank, or sample and concentrated to a final extract volume of 20 FL, the concentration in the final extract volume will be 100 ng/mL (100 pg/FL). Prepare only the amount necessary for each reference matrix with each sample batch. 7.13 Labeled cleanup standard spiking solution-This solution is spiked into each extract prior to cleanup to measure the efficiency of the cleanup process. Dilute the Labeled cleanup standard stock solution (Section 7.9.2) in methylene chloride to produce a concentration of the cleanup standards at 2 ng/mL, as shown in Table 3. When 1 mL of this solution is spiked into a sample extract and concentrated to a final

volume of 20 μL,the concentration in the final volume will be 100 ng/mL (100 pg/μL). 9.0 Quality assurance/quality control 11.0 Sample preparation
Note: See Appendix H for Table 3.All tables in Appendix H are reproduced verbatim from the method.
Modification: The concentration of the labeled toxics/LOC and the cleanup standard spiking solutions is 100 ng/mL and the sample spiking volume is 20 μL. The resulting final concentrations in the extracts are as specified in the method.

Section 7.14 Labeled injection internal standard spiking solution-This solution is added to each concentrated extract prior to injection into the HRGC/HRMS. Dilute the Labeled injection internal standard stock solution (Section 7.9.3) in nonane to produce a concentration of the injection internal standards at 1000 ng/mL, as shown in Table 3. When 2 μL of this solution is spiked into a 20 μL extract, the concentration of each injection internal standard will be nominally 100 ng/mL (100 pg/μL). Note: The addition of 2 μL of the Labeled injection internal standard spiking solution to a 20 μL final extract has the effect of diluting the concentration of the components in the extract by 10%. Provided all calibration solutions and all extracts undergo this dilution as a result of adding the Labeled injection internal standard spiking solution, the effect of the 10% solution is compensated, and correction for this dilution should not be made.
Modification: Concentration of the labeled injection internal standard spiking solution (recovery standard) is modified so that a volume of 5 μL is added. The resulting amount of standard added to the final extract is the same as specified in the method. The solution is spiked into a 15-μL extract volume for a final extract volume of 20 μL.

Section 7.2.1 Solution drying-Sodium sulfate, reagent grade, granular, anhydrous (Baker 3375, or equivalent), rinsed with methylene chloride (20 mL/g), baked at 400 °C for 1 hour minimum, cooled in a desiccator, and stored in a pre-cleaned glass bottle with screw-cap that prevents moisture from entering. If, after heating, the sodium sulfate develops a noticeable grayish cast (due to the presence of carbon).
Modification: Powdered, not granular, sodium sulphate is baked at 325°C for 8 hours rather than at 600°C for 24 hrs.

Section 7.5.1 Activated silica gel-100-200 mesh, Supelco 1-3651 (or equivalent), rinsed with methylene chloride, baked at 180 °C for a minimum of 1 hour, cooled in a desiccator, and stored in a precleaned glass bottle with screw-cap that prevents moisture from entering.
Modification: Silica is activated by baking at 450°C in a muffle oven for at least 8 hours.

Section 7.5.4.1.1 Fill a clean 1- to 2-L bottle 1/2 to 2/3 full with Florisil and place in an oven at 130-150 °C for a minimum of three days to activate the Florisil.
Modification: Florisil is baked at 450°C in a muffle oven for at least 8 hours, then deactivated with water to 2.1% deactivation.

Section 11.5.6 Decant excess water. If necessary to remove water, filter the sample through a glass-fiber filter and discard the aqueous liquid.
Modification: Unless requested by the client, the aqueous portion after filtration of aqueous samples with > 1% solids is not discarded but is extracted.

Section 11.5 Preparation of samples containing greater than one percent solids 11.5.2 Spike 1.0 mL of the Labeled Toxics/LOC/window-defining standard spiking solution (Section 7.12) into the sample. 12.3 Soxhlet/Dean-Stark extraction of samples containing particles.
Modification: Solid samples are dried by mixing with anhydrous sodium sulphate. The dried solid is extracted using a soxhlet extraction apparatus. The surrogate spike is incorporated after the drying step. The extracting solvent for solids is dichloromethane.

Section 11.8 Fish and other tissues-Prior to processing tissue samples, the laboratory must determine the exact tissue to be analyzed. Common requests for analysis of fish tissue include whole fish-skin on, whole fish-skin removed, edible fish fillets (filleted in the field or by the laboratory), specific organs, and other portions. Once the appropriate tissue has been determined, the sample must be homogenized. 12.4 Soxhlet extraction of tissue
Modification: The surrogate spike is incorporated into the sample after the drying step to eliminate the possibility of disproportional loss of volatile labeled and target compounds.

Section 12.4.2 Assemble and pre-extract the Soxhlet apparatus per Sections 12.3.1-12.3.4, except use the methylene chloride:hexane (1:1) mixture for the pre-extraction and rinsing and omit the quartz sand.
Modification: The pre-cleaning of the soxhlet apparatus is carried out using toluene instead of dichloromethane.

Section 12.4.9 Percent lipid determination-The lipid content is determined by extraction of tissue with the same solvent system (methylene chloride:hexane) that was used in EPA's National Dioxin Study (Reference 16) so that lipid contents are consistent with that study.
Modification: Lipid analysis is carried out by sub-sampling two 2-g portions of the extract from a total 30-g extract weight. The cleanup standard is spiked into the extract after soxhlet extraction and before any lipid analysis or rotary evaporation is done.

Section 12.6.1.1: Assemble the rotary evaporator according to manufacturer's instructions, and warm the water bath to 45 °C. On a daily basis, pre-clean the rotary evaporator by concentrating 100 mL of clean extraction solvent through the system. Archive both the concentrated solvent and the solvent in the catch flask for a contamination check if necessary. Between samples, three 2- to 3-mL aliquots of solvent should be rinsed down the feed tube into a waste beaker.
Modification: Rotary evaporation is done at 30 deg. C. Daily cleaning of the rotary evaporators include dismantling the vapor tube, sonicating the vapor tube for 20 minutes in toluene, and soaking the vapor tube sheath and locking caps in soapy water overnight. The vapor tube is rinsed with hexane and the vapor tube sheath and locking cap are rinsed with water, methanol, and hexane prior to air drying and assembly. Mimic proofs are run periodically but are not archived daily.

Section 12.7.4 When the volume of the liquid is approximately 100 µL, add 2 to 3 mL of the desired solvent (methylene chloride for GPC and HPLC, or hexane for the other cleanups) and continue concentration to approximately 100 FL. Repeat the addition of solvent and concentrate once more.
Modification*:* Before Florisil or alumina cleanup procedures, a solvent exchange is done by reducing under nitrogen to 300 uL and bulking up to 1 mL in hexane. If toluene is present the extract is reduced to 50 uL under nitrogen and bulked up to 1 mL.

Section 12.7.7 If the extract is to be concentrated for injection into the GC/MS (Section 14), qantitatively transfer the extract to a 0.3-mL conical vial for final concentration, rinsing the larger vial with hexane and adding the rinse to the conical vial. Reduce the volume to approximately 100 µL. Add 20 µL of nonane to the vial, and evaporate the solvent to the level of the nonane. Seal the vial and label with the sample number. Store in the dark at room temperature until ready for GC/MS analysis. If GC/MS analysis will not be performed on the same day, store the vial at < -10 °C.
Modification: Toluene (1 mL) is added to the eluate from the final column prior to rotary evaporation and nitrogen blow down concentration steps.

Section 13.1.1 Gel permeation chromatography (Section 13.2) removes high molecular weight interferences that cause GC column performance to degrade. It should be used for all soil and sediment

extracts. It may be used for water extracts that are expected to contain high molecular weight organic compounds (e.g., polymeric materials, humic acids). It should also be used for tissue extracts after initial cleanup on the anthropogenic isolation column (Section 13.6).
Modification: GPC chromatography, by a gravity column, is routinely used only for tissue extracts. The GPC cleanup is optional for all other matrices.

Section 13.3.1 Place a glass-wool plug in a 15-mm ID chromatography column (Section 6.7.4.2). Pack the column bottom to top with: 1 g silica gel (Section 7.5.1.1), 4 g basic silica gel (Section 7.5.1.3), 1 g silica gel, 8 g acid silica gel (Section 7.5.1.2), 2 g silica gel, and 4 g granular anhydrous sodium sulfate (Section 7.2.1). Tap the column to settle the adsorbents.
Modification: Routine layered silica column is as follows: 0.5 g neutral silica, 2 g 28% basic silica, 0.5 g neutral silica, 4 g 44% acidic silica, 4 g 22% acidic silica, 1 g neutral silica.

Section 13.3.4 Rinse the receiver twice with 1-mL portions of hexane, and apply separately to the column. Elute the CBs with 25 mL of hexane and collect the eluate.
Modification: The sample is loaded onto the column followed by 2-3 rinses of a least 1 mL, and eluted with 100 mL of hexane.

Section 14.2 Add 2 μL of the Labeled injection internal standard spiking solution (Section 7.14) to the 20 μL sample extract immediately prior to injection to minimize the possibility of loss by evaporation, adsorption, or reaction. If an extract is to be reanalyzed and evaporation has occurred, do not add more Labeled injection internal standard spiking solution. Rather, bring the extract back to its previous volume (e.g., 19 μL) with pure nonane (18 μL if 2 μL injections are used).
Modification: The volume of labeled injection internal standard (recovery standard) added to the extract is 5 μL, for a final extract volume of 20 μL. Hexane rather than nonane is used as the solvent to bring extract back to volume for reanalysis or to dilute extracts.

Section 17.5 If the selected ion current profile area at either quantitation m/z for any congener exceeds the calibration range of the system, dilute the sample extract by the factor necessary to bring the concentration within the calibration range, adjust the concentration of the Labeled injection internal standard to 100 pg/μL in the extract, and analyze an aliquot of this diluted extract. If the CBs cannot be measured reliably by isotope dilution, dilute and analyze an aqueous sample or analyze a smaller portion of a soil, tissue, or mixed-phase sample. Adjust the CB congener concentrations, detection limits, and minimum levels to account for the dilution.
Modification: Extracts are diluted with hexane. The concentration of the labeled injection internal (recovery) standard is not readjusted to 100 pg/uL when dilutions are performed.

Table 6, page 92:
Note: See Appendix H for Table 6.
Modification: The acceptance ranges for the labeled compounds dichlorobiphenyls (PCBs 4L and 15L) in OPRs, IPRs and samples have been lowered to 15% in recognition of the higher volatility of these compounds. This is a project-specific change to Sections 4.2 and 10.0 of SOP MLA-010.

Section 17.0 Quantitative Determination
Modification: $Conc_i$ - the concentrations of target analytes, and the labeled compound concentrations and recoveries, are calculated using the equations below. These procedures are equivalent to those described in the method but are more direct.

$$Conc_{si} = \frac{A_{si}}{A_{rs}} \times \frac{M_{rs}}{RRF_{si,rs}}$$

where A_i = summed areas of the primary and secondary m/z's for the analyte peak of interest (compound i)

A_{si} = summed areas of the primary and secondary m/z's for the labeled surrogate peak used to quantify i)

M_x = mass of sample taken for analysis

M_{si} = mass of labeled surrogate (compound si) added to sample as calculated by the concentration of standard spiked (pg/mL) multiplied by the volume spiked (mL)

$RRF_{i,si}$ = mean relative response factor of i to si from the five-point calibration range and defined individually as:

$$RRF_{i,si} = \frac{A_i}{A_{si}} \times \frac{M_{si}}{M_i}$$

Concentrations of surrogate standards are calculated using the following equation:

$$Conc_i = \frac{A_i}{A_{si}} \times \frac{M_{si}}{RRF_{i,si}} \times \frac{1}{M_x}$$

and, the percent recoveries of the surrogate standards are calculated using the following equation:

$$\%\text{Recovery} = \frac{A_{si}}{A_{rs}} \times \frac{M_{rs}}{RRF_{si,rs}} \times \frac{1}{M_{si}} \times 100$$

where A_{rs} and A_{si} are the summed peak areas (from the primary and secondary m/z channels) of recovery standard and labeled surrogate added to the sample;

M_{rs} and M_{si} are the masses of recovery standard and labeled surrogate added to the sample, and;

$RRF_{si,rs}$ is the mean relative response factor of the labeled surrogate to the recovery standard as determined by the five-point calibration range and defined individually as:

$$RRF_{si,rs} = \frac{A_{si}}{A_{rs}} \times \frac{M_{rs}}{M_{si}}$$

7.3.2.1 Effects of these Modifications on Method Performance

It should be noted that the performance of this method including these modifications will be verified during the in-process audit of the reference laboratory and by the laboratory's performance on QC

samples. These checks will confirm that the above-listed modifications will not negatively affect the accuracy or precision of the reference laboratory results.

7.3.3 Options for High-Level Samples

For extremely high level samples (> 1,000,000 pg/g), AXYS may dilute with solvent to the limit for reliable isotope dilution quantification (labeled response $\geq$ low calibration native response and S:N > 10:1 for both ions). For those samples that do not meet this criterion, a diluted portion of the extract will be respiked with an additional aliquot of surrogate standard, reinjected, and final concentrations for dilution/respike factors and surrogate recoveries using the mean surrogate recovery (excluding any surrogate responses compromised by high-level native presence) will be quantified.

Chapter 8
Data Management

To ensure that the demonstration data are scientifically valid, defensible, and comparable, appropriate procedures will be used to perform data management. This chapter describes (1) data reduction, (2) data review, (3) data reporting, and (4) data storage procedures for the demonstration.

8.1 Data Reduction

Each analytical method selected for the demonstration and each developer technology's instruction manual contain detailed instructions and equations for generation of results. Each developer will be responsible for reducing their own data and providing final results to Battelle in the form described in Table 6-7. The reference laboratory will generate concentration data for the dioxin/furan congeners using EPA Method 1613B and for the WHO PCBs using modified Method 1668A. The reference laboratory concentration data will be converted to TEQ using the WHO 1998 guidance as described in Chapters 1 and 6. The reference laboratory will generate final concentration and TEQ data, and Battelle will review those results through data validation (see Section 8.2.3). Comparisons between the developer and reference laboratory data will be dependent on how the developer is reporting their data (see Sections 1.3 and 6.6.3 for further discussion on this topic).

8.2 Data Review

A review of technology and laboratory analytical data will be conducted by each developer and the reference laboratory, respectively. Battelle will also conduct a review of all field and laboratory data. The review processes that will be used for developer and laboratory analytical data are described below.

8.2.1 Data Review by Developers

Each developer will review all results generated by its measurement technology. The developer will review all demonstration sample data as well as QC results (such as positive controls/spikes and method blanks) for their technology. The developer will report results to Battelle on the COC/results form (Appendix D) and in the units described in Table 6-7.

Battelle will generate a Microsoft™ Excel spreadsheet of each developer's results, including only sample number and developer result, and send the spreadsheet to each developer for review. This will allow the developer to review the results that have been entered by Battelle in electronic form to minimize transcription or interpretation errors. Battelle will not perform any data analysis with the developer data until the spreadsheet has been reviewed by the developer.

8.2.2 Data Review by Reference Laboratory

The procedures for data review by the reference laboratory are provided in Appendix I.

8.2.3 Data Review by Battelle

In addition to the review process that will be used by the reference laboratory, the Battelle project manager or designee (e.g., such as a Battelle dioxin expert) will review all laboratory and developer results, based on demonstration objectives. The Battelle project manager or designee (such as the QA manager) will also conduct a complete data validation for 100 percent of the data as an independent check of the reference laboratory results. If this validation reveals no oversights or problems, Battelle will consider all data to be acceptable. If oversights or problems are identified, the reference laboratory project manager will be consulted. The reference laboratory data will be compared to the data generated by Battelle during the characterization analyses (see Section 4.3). This will be a key comparison which will confirm the overall quality of the data set. Battelle's assessment of the data and QC results will be summarized for discussion with the EPA program manager and incorporated into the DER. The checklists for performing the data validations are presented in Appendix J.

During its data review, Battelle will identify project outlier data using statistical testing and will report these data to the EPA program manager. Project outlier data are defined as sample data outside specified acceptance limits established about the central tendency estimator (the arithmetic mean) of the data set for a given area or for all areas taken together. For data known or assumed to be normally distributed, the specified acceptance limits will be the 95 percent confidence limits defined by the Student's two-tailed t-test. Consistent procedures will be used to identify outliers for both reference laboratory and developer data. No data will be rejected simply because they are statistical outliers, but data may be reported with and without the statistical outliers as appropriate. Battelle will conduct a thorough check to identify the reasons for the outliers and will provide the EPA program manager with an explanation of why some data appear to be outliers.

8.3 Data Reporting

Each developer and the reference laboratory will prepare and submit data packages reporting the results of developer and laboratory results, respectively. The reference laboratory will also prepare and submit electronic data deliverables (EDD). Battelle will use these data to prepare the ITVR for each developer technology and the DER for the entire demonstration. Described below are the data reporting requirements for (1) developer data packages, (2) reference laboratory data packages, (3) ITVRs, and (4) the DER.

8.3.1 Developer Data Packages

The developers will compile their results on standard forms provided by Battelle (see Appendix D). The forms will contain sample identification numbers and spaces for a developer to enter their results as appropriate (i.e., each form will be unique to each developer). Electronic reporting of results will not be required. The developers will only be required to report their sample results. Developer-supplied QC sample results will be requested for the DER. Raw data, copies of logbook pages, standards preparation logs, etc. that are included in a typical laboratory data package will not be required from the developers.

8.3.2 Reference Laboratory Data Packages

The procedure for data package preparation for the reference laboratory is presented in Appendix K.

8.3.3 Innovative Technology Verification Reports

In accordance with the demonstration plan, Battelle will evaluate the performance and cost data collected for each dioxin measurement technology demonstrated and will prepare an ITVR for the technology. Each ITVR will be a focused report of about 100 pages and will include the following:

- An introduction
- A description of the measurement technology
- Site descriptions and the demonstration design
- Deviations from the demonstration plan
- A description of the reference method and its performance
- A description of the technology's performance
- An economic analysis
- A summary of demonstration results
- A verification statement.

Battelle will prepare individual ITVRs in accordance with the format specified in the "Handbook for Preparing Office of Research and Development Reports"[10] and project-specific guidance from the EPA program manager. The reports will be written in such a way that a reader with a basic science background can understand their contents and make an informed decision regarding the performance of the technologies. The ITVRs will undergo a rigorous review process that will include reviews by the EPA program manager, the developers, and external peer reviewers provided in accordance with the detail and content of previous reports.

8.4 Data Evaluation Report

Battelle will prepare a DER containing tabular summaries of investigative and QA/QC data from the demonstration as well as results of technical system and performance audits. The DER will primarily discuss the following:

- Pre-demonstration activities
- Demonstration activities
- Post-demonstration activities
- All developer and reference laboratory demonstration sample data
- QA/QC data, including completed audit, observer, and data validation checklists
- Audit reports.

8.5 Data Storage

The reference laboratory analysts responsible for performing measurements will enter raw data into logbooks or on datasheets. In accordance with standard document control procedures, the laboratory will maintain on file the original logbooks or data sheets, which will be signed and dated by the laboratory analysts responsible for them. Similar procedures will be used for all data entered directly into the laboratory information management system. Separate instrument logs will also be maintained by the laboratory to allow reconstruction of the run sequences for individual instruments. The reference laboratory will maintain all raw data, including raw instrument output on tape or diskette, on file for 5 years after the submission of the data packages to Battelle. Data documents will be kept in secure

archive file cabinets accessible only to designated laboratory personnel. The data will be disposed of upon receipt of EPA instructions to do so or after 5 years, whichever is sooner. A central project file for the demonstration will be established in the Records Management Office at Battelle's Columbus headquarters. This file will be a repository for all relevant field and laboratory project documentation. Battelle will offer the central file to the EPA at the end of the demonstration project but will maintain in the central file until the end of the SITE contract if requested to do so.

Chapter 9
QA/QC Procedures

This chapter describes the QA/QC procedures that will be implemented in this demonstration to ensure that the data generated are of high quality.

9.1 QA/QC Objectives

The overall QA objective for the demonstration is to produce well-documented data of known quality. Data quality will be measured in terms of the data's precision, accuracy, representativeness, completeness, and comparability. Table 9-1 contains the objectives for the data quality indicators which applies to both the developer and reference laboratory data. If analytical data from the reference laboratory fail to meet the QA objectives described in this section (except for comparability, which does not apply), the source of the errors will be investigated and corrective actions will be taken if necessary and possible. (Corrective actions associated with the reference method are discussed in detail in Section 9.2.) If analytical data from the field technologies did not meet the QA objectives, the discrepancies will be described in the ITVRs.

Table 9-1. Data Quality Indicator Objectives for Reference Laboratory and Developer Data

Data Quality Indicator	Calculation	Objective
Precision	RSD of replicate samples	Average of all RSDs < 20 percent
Accuracy	Percent recovery of certified or spiked PE values	75 percent to 125 percent
Representativeness	Valid samples from each soil and sediment type	At least one valid sample result generated from each soil and sediment sampling location
Comparability to reference method[a]	average absolute median RPD	< ± 25 percent
Completeness	Percent of total samples analyzed and valid results provided	98 percent

[a] Applies only to developer data

9.2 Internal QC Checks

9.2.1 Reference Method QC Checks

Tables 9-2, 9-3, 9-4, and 9-5 summarize the QC checks that will be performed by the reference laboratory as described in AXYS Summary Method Doc MSU-018 Rev. 2 18-Mar-2004 (AXYS detailed SOP MLA-017 Rev 9 May-2004) and AXYS Summary Method Doc MSU-020 Rev 03 24-Mar-2004 (AXYS detailed SOP MLA-010 Rev 5 Sep-2003).

Table 9-2. QC Acceptance Criteria for EPA Method 1613B [a]

Native Compound	Test Conc ng/mL	IPR[b] RSD (%)	IPR[b] X(%)	OPR[c] (%)	I-CAL %	CAL/VER[d] (%)	Labeled Cmpd %Rec. in Sample
2,3,7,8-TCDD	10	28	83-129	70-130	20	78-125	-
2,3,7,8-TCDF	10	20	87-137	75-130	20	84-120	-
1,2,3,7,8-PeCDD	50	15	76-132	70-130	20	78-130	-
1,2,3,7,8-PeCDF	50	15	86-124	80-130	20	82-120	-
2,3,4,7,8-PeCDF	50	17	72-150	70-130	20	82-122	-
1,2,3,4,7,8-HxCDD	50	19	78-152	70-130	20	78-125	-
1,2,3,6,7,8-HxCDD	50	15	84-124	76-130	20	78-125	-
1,2,3,7,8,9-HXCDD	50	22	74-142	70-130	35	82-122	-
1,2,3,4,7,8-HxCDF	50	17	82-108	72-130	20	90-112	-
1,2,3,6,7,8-HxCDF	50	13	92-120	84-130	20	88-114	-
1,2,3,7,8,9-HxCDF	50	13	84-122	78-130	20	90-112	-
2,3,4,6,7,8-HxCDF	50	15	74-158	70-130	20	88-114	-
1,2,3,4,6,7,8-HpCDD	50	15	76-130	70-130	20	86-116	-
1,2,3,4,6,7,8-HpCDF	50	13	90-112	82-122	20	90-110	-
1,2,3,4,7,8,9-HpCDF	50	16	86-126	78-130	20	86-116	-
OCDD	100	19	86-126	78-130	20	79-125	-
OCDF	100	27	74-146	70-130	35	75-125	-
Surrogate Standards							
$^{13}C_{12}$-2,3,7,8-TCDD	100	37	28-134	40-120	35	82-121	40-120
$^{13}C_{12}$-2,3,7,8-TCDF	100	35	31-113	40-120	35	71-130	40-120
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	39	27-184	40-120	35	70-130	40-120
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	34	27-156	40-120	35	76-130	40-120
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	38	16-279	40-120	35	77-130	40-120
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	41	29-147	40-120	35	85-117	40-120
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	38	34-122	40-120	35	85-118	40-120
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	43	27-152	40-120	35	76-130	40-120
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	35	30-122	40-120	35	70-130	40-120
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	40	24-157	40-120	35	74-130	40-120
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	37	29-136	40-120	35	73-130	40-120
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	35	34-129	40-120	35	72-130	40-120
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	41	32-110	40-120	35	78-129	40-120
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	40	28-141	40-120	35	77-129	40-120
$^{13}C_{12}$-OCDD	200	48	20-138	25-120	35	70-130	25-120
Cleanup Standard							
$^{37}Cl_4$-2,3,7,8-TCDD	10	36	39-154	40-120	35	79-127	40-120

[a] QC acceptance criteria for IPR, OPR, and samples based on a 20-µL extract final volume.
[b] IPR: Initial precision and recovery demonstration.
[c] OPR: Ongoing precision and recovery test run with every batch of samples.
[d] CAL/VER: Calibration verification test run at least every 12 hours.

Table 9-3. Method 1613B Specifications for QC Samples, Instrumental Analysis, and Analyte Quantification

QC Parameter	Specification
Analysis Duplicate	Must agree to within ±20% of the mean (applicable to concentrations 10 times the DL)[a]
Procedural Blank	TCDD/F < 0.5 pg/sample, PeCDD/F, HxCDD/F, HpCDD/F < 1.0 pg/sample, OCDD/F < 5 pg/sample
Detection Limit	SDL requirements 1 pg/sample
Analyte/Surrogate Ratios	Response must be within the calibrated range of the instrument. Coders may use data from more than one chromatogram to get the responses in the calibrated range.
Ion Ratios	Must be within ±15% of theoretical
Sensitivity	S:N>10:1 for all compounds for 0.1 pg/µL (CS-0.2), plus For bloods: S:N>3:1 for 0.025 pg/µL 2,3,7,8-T4CDD

[a] Duplicate criterion is a guideline; final assessment depends upon sample characteristics, overall batch QC and on-going lab performance.

Table 9-4. Method 1668A QC Acceptance Criteria for Chlorinated Biphenyls in CAL/VER, IPR, OPR, and Samples [a]

Congener	Congener number[b]	Test conc (ng/mL)	CAL/VER[c] (%)	IPR[d] RSD (%)	IPR[d] X (%)	OPR[e] (%)	Labeled compound recovery in samples (%) % (no carbon column)	Labeled compound recovery in samples (%) % (carbon column)
2-MoCB	1	50	75-125	40	60-140	70-130		
4-MoCB	3	50	75-125	40	60-140	70-130		
2,2'-DiCB	4	50	75-125	40	60-140	70-130		
4,4'-DiCB	15	50	75-125	40	60-140	70-130		
2,2'6-TrCB	19	50	75-125	40	60-140	70-130		
3,4,4'-TrCB	37	50	75-125	40	60-140	70-130		
2,2'6,6'TeCB	54	50	75-125	40	60-140	70-130		
3,3',4,4'-TeCB	77	50	75-125	40	60-140	70-130		
3,4,4',5-TeCB	81	50	75-125	40	60-140	70-130		
2,2',4,6,6'-PeCB	104	50	75-125	40	60-140	70-130		
2,3,3',4,4'-PeCB	105	50	75-125	40	60-140	70-130		
2,3,4,4',5-PeCB	114	50	75-125	40	60-140	70-130		
2,3',4,4',5-PeCB	118	50	75-125	40	60-140	70-130		
2',3,4,4',5-PeCB	123	50	75-125	40	60-140	70-130		
3,3',4,4',5-PeCB	126	50	75-125	40	60-140	70-130		
2,2',4,4',6,6'-HxCB	155	50	75-125	40	60-140	70-130		
2,3,3',4,4',5-HxCB	156	50	75-125	40	60-140	70-130		
2,3,3',4,4',5'-HxCB[f]	157	50	75-125	40	60-140	70-130		
2,3',4,4',5,5'-HxCB	167	50	75-125	40	60-140	70-130		
3,3',4,4',5,5'-HxCB	169	50	75-125	40	60-140	70-130		
2,2',3,4',5,6,6'-HpCB	188	50	75-125	40	60-140	70-130		
2,3,3',4,4',5,5'-HpCB	189	50	75-125	40	60-140	70-130		
2,2',3,3',5,5',6,6'-OcCB	202	50	75-125	40	60-140	70-130		
2,3,3',4,4',5,5',6-OcCB	205	50	75-125	40	60-140	70-130		
2,2',3,3',4,4',5,5',6-NoCB	206	50	75-125	40	60-140	70-130		

Congener	Congener number[b]	Test conc (ng/mL)	CAL/VER[c] (%)	IPR[d] RSD (%)	IPR[d] X (%)	OPR[e] (%)	Labeled compound recovery in samples (%) % (no carbon column)	Labeled compound recovery in samples (%) % (carbon column)
2,2',3,3,'4,5,5',6,6'-NoCB	208	50	75-125	40	60-140	70-130		
DeCB	209	50	75-125	40	60-140	70-130		
Labeled Compounds								
$^{13}C_{12}$-2-MoCB	1L	100	65-135	50	20-135	15-140	15-130	-
$^{13}C_{12}$-4-MoCB	3L	100	65-135	50	20-135	15-140	15-130	-
$^{13}C_{12}$-2,2'-DiCB	4L	100	65-135	50	20-135	20-140	20-130	-
$^{13}C_{12}$-4,4'-DiCB	15L	100	65-135	50	20-135	20-140	20-130	-
$^{13}C_{12}$-2,2',6-TrCB	19L	100	65-135	50	35-135	30-140	30-130	-
$^{13}C_{12}$-3,4,4'-TrCB	37L	100	65-135	50	35-135	30-140	30-130	-
$^{13}C_{12}$-2,2',6,6'-TeCB	54L	100	65-135	50	35-135	30-140	30-130	-
$^{13}C_{12}$-3,3',4,4'-TCB	77L	100	65-135	50	35-135	30-140	30-130	25-130
$^{13}C_{12}$-3,4,4',5-TeCB	81L	100	65-135	50	35-135	30-140	30-130	25-130
$^{13}C_{12}$-2,2',4,6,6'-PeCB	104L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2,3,3',4,4'-PeCB	105L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2,3,4,4',5-PeCB	114L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2,3',4,4',5-PeCB	118L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2',3,4,4',5-PeCB	123L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-3,3',4,4',5-PeCB	126L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB	155L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2,3,3',4,4',5-HxCB[f]	156L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2,3,3',4,4',5'-HxCB[f]	157L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB	167L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-3,3',4,4',5,5'-HxCB	169L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB	188L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB	189L	100	65-135	50	35-135	30-140	40-130	25-130
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB	202L	100	65-135	50	35-135	30-140	40-130	-
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB	205L	100	65-135	50	35-135	30-140	40-130	-
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB	206L	100	65-135	50	35-135	30-140	40-130	-
$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB	208L	100	65-135	50	35-135	30-140	40-130	-
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6,6'-DeCB	209L	100	65-135	50	35-135	30-140	40-130	-
Cleanup Standard								
$^{13}C_{12}$-2,4,4'-TriCB	28L	100	60-130	45	45-120	40-125	40-130	-
$^{13}C_{12}$-2,3,3',5,5'-PeCB	111L	100	60-130	45	45-120	40-125	40-130	-
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB	178L	100	60-130	45	45-120	40-125	40-130	-

[a] QC acceptance criteria for IPR, OPR, and samples based on a 20-µL extract final volume.
[b] Suffix "L" indicates labeled compound.
[c] CAL/VER: Calibration verification test run at least every 12 hours.
[d] IPR: Initial precision and recovery demonstration.
[e] OPR: Ongoing precision and recovery test run with every batch of samples.
[f] PCBs 156 and 157 are tested as the sum of two concentrations.

Table 9-5. Method 1668A Specifications for QC Samples, Instrumental Analysis, and Analyte Quantification

QC Parameter	Specification
Analysis Duplicate	Must agree to within ±20% of the mean (applicable to concentrations >10 times the DL)[a]
Procedural Blank	Analyte concentrations in blank samples for PCB congeners 77, 81, 114, 123, 126 and 169 must be less than 2 pg/congener, and concentrations of PCB congeners 156, 157, 167 and 189 must be less than 10 pg/congener. Concentrations of all other individual PCB congeners or coelutions must be less than 50 pg/congener in blank samples. The sum of all 209 congeners must be less than 200 pg.
Detection Limit	Typical detection limits for individual congeners range from 0.5 to 2.0 pg.
Initial Calibration	For 5-point calibration, a relative standard deviation of the RRF's ≤20% for all compounds. Ion ratios for all congeners must be within ±15% of theoretical for CS-0.2. Minimum S:N ratio 10:1 for CS-0.2
Continuing CAL/VER	Concentrations for all labeled surrogate PCB congeners must fall within ±35% of expected concentrations. Concentrations for all native PCB congeners must fall within ±25% of expected concentrations. Concentrations for labeled cleanup PCB Congeners must fall within 60% - 130% of expected concentrations.
Analyte/Surrogate Ratios	Response must be within the calibrated range of the instrument. Coders may use data from more than one chromatogram to get the responses in the
Ion Ratios	Ion ratios must fall within ±15% of the theoretical values for positive identification of all targets in the calibration standards and samples.
Sensitivity	Minimum S:N ratio 10:1 for CS-0.2

[a] Duplicate criterion is a guideline; final assessment depends upon sample characteristics, overall batch QC, and on-going lab performance.

9.2.2 Developer Technology QC Checks

Quality control checks to be performed by the developers will be at each developer's discretion, although it is highly recommended that quality controls such as blanks, spikes, and duplicates, be systematically analyzed throughout the demonstration. Developer QC data will be reported to Battelle for inclusion in the DER. The developers process for QC sample analysis will be evaluated and recorded by the Battelle observers.

9.3 Audits, Corrective Actions, and QA Reports

The assessment stage involves procedures to verify that project efforts are in compliance with the quality system as the project is being implemented, and that upon conclusion of the data gathering stage of the project, the collected data meet the performance and acceptance criteria (e.g., data quality objectives) specified in the planning stage of the project. The QA manager or designee conducts audits at planned, scheduled intervals; implements provisions for timely responses and implementation of corrective actions if needed; and completes the evaluation process with written reports to technical and management staff. The Battelle project manager will ensure that this individual has sufficient authority, access to project staff, access to documents and records, and organizational freedom to conduct the assessment.

9.3.1 Technical Systems Audits

Battelle will conduct a TSA during the time when the reference laboratory is analyzing the demonstration samples. This in-process audit will include a technical systems audit and a quality systems audit and will last one full-day. The work plan for this audit is included in Appendix L. This audit is very similar in focus and scope as the audit that was performed to select AXYS as the reference laboratory. The audit will consist of a short opening conference, a full day of observation of laboratory procedures, records, and interviews with laboratory staff, and a brief closing meeting. Auditors will inspect whether the observations/questions/issues

identified during the first TSA (conducted prior to selection of the reference laboratory) are adequately addressed/implemented by the reference laboratory. The TSA will focus on topics specifically related to this project such as: sample and standards tracking, sample and standard preparation, instrument calibration, sample analysis, sample integration and data reduction, and acceptability of quality control data. Records to be reviewed are:

- Sample receipt records and holding location/conditions
- Standard (spiking and calibration solution) receipt records and certificates of analysis
- Standard preparation records
- Support equipment records (balances, thermometers)
- Sample preparation and spiking records
- Preparation of instrument standards
- Preparation of spiking solutions
- Instrument calibration and maintenance records
- Instrument sample run logs
- Instrument set-up conditions, temperature programs, calibration and acquisition methods
- Sample chromatograms
- Instrument reports
- Quantification techniques
- QC sample results
- Pre-demonstration sample results
- Data management procedures
- Quality assurance procedures
- Data validation and verification procedures

Auditors will also review corrective actions from the pre-selection audit. Auditors may submit follow-up questions to the reference laboratory after the audit to address gaps in the observations.

A separate TSA will be performed by Battelle at the demonstration site to ensure that the demonstration plan is being implemented properly by Battelle. This will be a self evaluation. The Battelle QA manager will review notebooks, logsheets, chain-of-custody documentation, and observer checklists. The Battelle project manager will be debriefed by the Battelle QA manager at the conclusion of the on-site TSA.

The EPA Quality Manager has the authority to conduct an independent technical systems audit at any time during this demonstration.

9.3.2 Corrective Action Procedures

Corrective action procedures from TSA findings for the in-process reference laboratory audit or the field demonstration TSA will be documented by the responder and approved by the auditor. Acknowledgment of the response will be provided by the Battelle project manager. The Battelle auditor will then establish a time line for monitoring that the required parties will properly act upon the corrective action(s) within the required time frame. With the Battelle project manager, the Battelle auditor will set measurable milestones for determining that progress is being made on the corrective action and that the corrective action is effective.

9.3.3 QA Reports

The outcome of each assessment will be fully documented. The Battelle project manager will archive all audit documentation collected during the project and include it in the DER.

The Battelle QA manager or his designee will report the findings of each audit to the Battelle or AXYS Project Manager, as appropriate, who will then address the audit findings and provide an appropriate response. QA

reports require a written response by the person performing the inspected activity, and acknowledgment of the audit by the Battelle project manager.

Authority to report all TSA results is designated to the Battelle QA manager or his designee. These reports should

- Identify and document problems that affect quality and the achievement of objectives required by the demonstration and quality assurance project plan and any associated SOPs,
- Identify and cite noteworthy practices that may be shared with others to improve the quality of their operations and products,
- Propose recommendations (if requested) for resolving problems that affect quality,
- Independently confirm implementation and effectiveness of solutions, and
- Provide documented assurance (if requested) to line management that, when problems are identified, further work performed is monitored carefully until the problems are suitably resolved.

Responses to adverse findings are addressed immediately during a debriefing after the assessment is completed, and preferably at the site of the assessment.

Responses to each adverse finding will be documented in a letter or memo to the Battelle project manager. The letter or memo will indicate for each adverse finding the corrective action(s) taken or planned. The letter or memo will be signed by the appropriate developer or reference laboratory representative.

The Battelle QA manager or his designee will review the responses to each adverse finding and will follow up with the Battelle, developer, or reference laboratory representative on any findings that were not adequately addressed. Once all corrective actions associated with the QA report have been verified, the Battelle QA manager or designee will approve the QA report. The QA report and responses to adverse findings will be sent to the Battelle project manager for review and approval. The QA report and responses will be maintained in the QA project files and will be included in the DER.

Chapter 10
Health and Safety Plan

This chapter contains the site health and safety plan for field activities for the dioxin monitoring and measurement demonstration. This plan will be reviewed and signed by all demonstration participants before work begins at the site. The Battelle Project Manager will conduct daily safety briefings before the start of analysis each day to advise and remind the developers of health and safety issues.

SECTION 1: GENERAL INFORMATION AND DISCLAIMER	
CLIENT NAME: U.S. Environmental Protection Agency Superfund Innovative Technology Evaluation (SITE) Program	PROJECT NAME: SITE DEMONSTRATION OF DIOXIN MONITORING AND MEASUREMENT TECHNOLOGIES FOR SOIL AND SEDIMENT
EPA PROJECT MANAGER: Steve Billets	
BATTELLE Project Manager: Amy Dindal	
BATTELLE Site Safety Officer: Amy Dindal	
BATTELLE ES&H Representative: Gary Carlin	DATE: April 26, 2004

The purpose of this demonstration is to gather technologies capable of measuring dioxin and dioxin-like compounds in soil and sediment in one field location. The demonstration will be conducted near (but not on) a dioxin-contaminated site. The demonstration site will be in the parking lot of the Green Point Environmental Learning Center in Saginaw, MI. Developers of measurement technologies will operate their own technologies inside a trailer or mobile laboratory designated for their use. Battelle will distribute the samples for analyses and observe the operation of the technologies. Battelle will have its own trailer to support overseeing the demonstration, and it will be known as the Battelle Headquarters.

This Site Specific Health and Safety Plan (HASP) has been prepared for use by participants in this SITE MMT Demonstration. The plan is written for the specific LEVEL D site conditions, purposes, tasks, dates and personnel specified. If these conditions change, this plan must be amended and reviewed by those named in Section 18. This HASP satisfies the requirements of 29 Code of Federal Regulations (CFR) 1910.

Although it does not specifically apply, all site activities shall be performed in accordance with 1910.120(b)(1)(iv) and (v). Battelle will inform the demonstration participants of the site emergency response procedures and any potential fire, explosion, health, safety or other hazards by making this HASP and site information obtained by others available on-site. All participants are responsible for: (1) attending the health and safety briefing given by the Battelle Site Safety Officer (SSO) covering the requirements of this HASP; (2) providing their own personal protective equipment (PPE); (3) providing documentation that their employees have been health and safety trained in accordance with applicable federal, state and local laws and regulations; (4) providing evidence of medical surveillance and medical approvals for their employees (as applicable); and (5) complying with the direction and guidance provided by the SSO.

Prior to any demonstration samples being analyzed on site, a pre-work health and safety briefing shall be conducted by the SSO. This briefing shall discuss the chemical and physical hazards associated with this demonstration.

Visitors will be briefed on the site-specific health and safety procedures by the SSO. Visitors will be escorted by Battelle staff or by the developers trained on this HASP.

Under this plan, the SSO and the Battelle project manager, or their designees, are authorized and obligated to stop any site work activity that place or may place any staff member, subcontractor, visitor, or participant at risk of being injured or that violate specific requirements set forth within the HASP.

The requirements described within this HASP apply to all site activities and all Battelle staff, EPA, participants, or visitors working on the site. Violators of this HASP will be addressed on a case-by-case basis. Actions taken will range, at a minimum, from a verbal warning up to removal from the site depending on severity of the infraction. Initial enforcement shall be implemented by the SSO, the Battelle project manager, or their designee. All enforcement activities must be documented and maintained by the SSO. If the SSO is not on-site, an alternate will be designated.

SECTION 2: PROJECT INFORMATION

(1) SITE INFORMATION

Site Name:	Green Point Environmental Learning Center
Address	3010 Maple St.
	Saginaw, MI 48602

Site Contact:	Becky Goche
Phone Number:	989-759-1669
Site Safety Officer (SSO) Contact:	Amy Dindal
Site Phone Number	614-893-2260 (Battelle headquarters cellular)

(2) SITE CLASSIFICATION (check all that apply)	(3) ENTRY OBJECTIVES (check all that apply)
✓ **Hazardous (RCRA/CERCLA/State)**	❑ Site Inspection (General)
❑ Construction	❑ Well Drilling Observation
❑ Landfill (Non-Hazardous)	❑ Sampling, Air
❑ UST/LUST	❑ Sampling, Water
❑ Manufacturing	❑ Sampling, Soil
✓ **Active**	✓ **Other:**
❑ Inactive	**Demonstration of dioxin monitoring and measurement technologies. All work to be conducted within temporary containment facilities (i.e., trailers and mobile laboratories).**
✓ **Other:**	
All Battelle related activities will be conducted outside of HAZWOPER site.	DATE(S) OF FIELD VISIT(S): April 26 - May 6 2004

(4) BATTELLE TASKS

B1. Coordinate site activities associated with the dioxin monitoring and measurement technologies project.

B2. Assist developers, visitors, client as necessary.

TASKS PERFORMED BY OTHERS

1 Developers will conduct analysis of soil, sediment, and extraction samples utilizing their respective equipment.

(5) PROJECT ORGANIZATION AND COORDINATION – The following personnel are designated to carry out the stated project job functions on site. (Note: One person may carry out more than one job function.)

PROJECT MANAGER	Amy Dindal
SITE SAFETY OFFICER (SSO)	Amy Dindal
ALTERNATIVE SITE SAFETY OFFICER(S)	Gary Carlin; Rachel Sell
PUBLIC INFORMATION OFFICER	Steve Billets, U.S. EPA
SITE RECORD KEEPER	Amy Dindal
SAMPLE CUSTODIAN	Robyn Kroeger
ALTERNATE SAMPLE CUSTODIAN(S)	Amy Dindal; Rachel Sell
SITE PERSONNEL WITH CPR/FA*	Mark Misita

* If this person is not on-site, medical facilities that are ~ 3 miles away will be relied upon for medical care.

SECTION 2: PROJECT INFORMATION

(6) ON SITE CONTROL

- Entry to the demonstration site will be controlled. Authorized personnel (developers, Battelle staff, EPA, Michigan Department of Environmental Quality, U.S. Fish and Wildlife Service) will be allowed free access to demonstration site. Unauthorized personal, such as visitors, will only be allowed to enter the trailers and mobile labs on Visitor's Day or with approval from Battelle.
- An on-site Battelle Headquarters trailer will be established and clearly marked and will be the central communication location.
- Facility gates will be closed when Green Point is not open (typical hours of operation are 7:30 am to 4:00 pm). Security guards will patrol the grounds when participants are not there.
- Battelle staff will be present whenever sample analyses are on-going.
- All temporary containment facilities (e.g., trailers and mobile labs) will be locked when not occupied.
- On Visitor's Day, parking areas will be clearly marked and visitors will be given specific instructions concerning entry into the demonstration site.

SECTION 3: PHYSICAL HAZARDS

(1) IDENTIFY POTENTIAL PHYSICAL HAZARDS TO PARTICIPANTS (check all that apply)

❑	Confined Space	❑	Steep/Uneven Terrain	❑	Drums Handling
❑	Heavy Equipment	❑	Heat Stress	❑	Noise
❑	Moving Parts	√	**Extreme Cold** **Cold weather conditions may exist during the time of this project.**	❑	Non-Ionizing Radiation
❑	Heavy Lifting	❑	Ionizing Radiation	❑	Other:
❑	Electrical	❑	Traffic		
❑	Overhead Hazards	√	**Biological Hazards** Various wildlife may be encountered on the site		
❑	Fall (>6; Vertical)	❑	Surface Water (Immersion)		

Site hazards will be mitigated by:

1. Briefing site personnel as to identified physical hazards.
2. Traffic will be directed by site staff to well -marked traffic areas on visitor days to minimize the potential for injury to vehicular traffic.
3. Antiseptic ointment or solution will be included in the first aid kit.
4. All temporary containment facilities will be equipped with heating systems which can be utilized by the occupants as needed.

(2) SAFETY EQUIPMENT REQUIRED (check all that apply)

❑	Explosimeter	√	**Eye Wash**	❑	Confined Space Warning Signs
❑	Fall Protection Equipment	❑	Emergency Shower	√	**Communications – On Site**
√	**Barrier Tape**	√	**Emergency Air Horn**	√	**Communications – Off Site**
√	**Traffic Cones**	❑	Lights	❑	Hard Hats
❑	Stretcher	√	**Lights – emergency (Minimum 2 Flashlights)**	√	**Weather Radio**
√	**First Aid Kit**	❑	Ladder	√	**Air Horn**
√	**A-B-C- Fire Extinguisher**	❑	Tick Repellant	√	**Portable Fume Hoods with confirmed face velocity of 100 fpm +/- 20%. Vented to the outside.**
❑	Snake Bite Kit	❑	Flotation Device (USCG Type III)		

SECTION 3: PHYSICAL HAZARDS

Emergency equipment will be located at the Battelle Headquarters. See Sections 10 and 12 for communication procedures. A phone will be available on site and a cellular phone will carried by the Site Safety Officer. Due to size of the demonstration area (approximately 120 feet by 140 feet) and the close proximity of all the containment units, primary communication on site communication will be verbal. An air horn alarm system will be used for fire or other potentially dangerous situations that need site personal to immediately respond to the site muster point (Battelle Headquarters).

SECTION 4: CHEMICAL HAZARDS INFORMATION

(1) IDENTIFIED CONTAMINANTS

Known or suspected hazardous/toxic material

Media	Substances Involved	Characteristics	Estimated Concentrations	TLV
SL/SD/EX	Dioxin/Furans	OT - Low concentrations in soil, sediment or extract.	< 5 o 13,000 pg/g	See Attachment 1
SL/SD/EX	PCBs	OT - Low concentrations in soil, sediment, or extract	< 300 ppm	See Attachment 1
SL/SD/EX	PAHs	OT - Low concentrations in soil, sediment, or extract	< 100 ppm	See Attachment 1
SL/SD/EX	PCP	OT - Low concentrations in soil, sediment, or extract	< 100 ppm	See Attachment 1

Media types	GW (ground water), SW (surface water), WW (wastewater), AIR (air), SL (soil), SD (sediments), WL (waste, liquid), WS (waste, solid), WD (waste, sludge), WG (waste, gas), EX (extracts in toluene), OT (other).
Characterization	CA (corrosive, acid) CC, (corrosive, caustic), IG (ignitable), RA (radioactive), VO (volatile), TO (toxic), RE (reactive), BIO (infectious), UN (unknown), OT (other, describe)

Material Safety Data Sheets (MSDSs) for the Health and Safety contaminants of concern will be available on-site in the Battelle Headquarters. The data sheets include information on the chemical/toxicological properties of the site contaminants and signs and symptoms of over exposure.

(2) DESCRIBE POTENTIAL FOR CONTACT WITH EACH MEDIA TYPE FOR EACH OF THE BATTELLE TASKS LISTED IN SEC 2.4:

BATTELLE TASK #	ROUTE OF EXPOSURE	POTENTIAL FOR CONTACT	METHOD OF CONTROL
B1	Inhal/Contact	Low	• Latex/Nitrile Gloves, Safety Glasses, disposable lab coat • Portable Fume Hoods
B2	Inhal/Contact	Low	• Latex Nitrile Gloves, Safety Glasses, disposable lab coat, shoe covers • Portable Fume Hoods

The BATTELLE SSO will brief the participants on interpretation of the attached MSDSs and particularly on symptoms and signs of over exposure to chemical hazards.

(3) DESCRIBE POTENTIAL FOR CONTACT WITH EACH MEDIA TYPE FOR EACH OF THE OTHER TASKS LISTED IN SEC 2.4:

OTHER TASK #	ROUTE OF EXPOSURE	POTENTIAL FOR CONTACT	METHOD OF CONTROL
O1	Inhal/Contact	Low	• Latex/Nitrile Gloves, Safety Glasses, disposable lab coat, shoe covers • Portable Fume Hoods

The BATTELLE SSO will brief the participants on interpretation of the MSDSs and particularly on symptoms and signs of over exposure to chemical hazards.

SECTION 5: HAZARD COMMUNICATION PROGRAM

If chemicals are introduced to the site by Battelle (e.g., decontamination liquids, preservatives, etc.), bring a copy of the MSDSs to the site. The Battelle SSO will review this information with all field personnel prior to the start of the field activities. The current list of chemicals for this site is:

Hexane	Dimethyl sulfoxide (DMSO)
Methanol	Nitrogen
Toluene	Carbon dioxide
Sulfuric acid	

SECTION 6: ENVIRONMENTAL MONITORING

(1) The following environmental monitoring instruments shall be used on site at the specified intervals for breathing zone monitoring:

	EQUIPMENT	MONITORING PERIOD		ACTION LEVEL
❑	Combustible Gas Indicator	daily/hourly/continuous/other		
❑	O_2 Meter	daily/hourly/continuous/other		
❑	PID (Lamp 10.6 eV)	daily/hourly/continuous/other		
❑	FID	daily/hourly/continuous/other		
❑	Radiation Meter (Gamma)	daily/hourly/continuous/other		
❑	Respirable Dust Meter	daily/hourly/continuous/other		
❑	Other	daily/hourly/continuous/other		
❑		daily/hourly/continuous/other		
❑		daily/hourly/continuous/other		

NO MONITORING WILL BE CONDUCTED AS ALL HANDLING OF SAMPLES AND CHEMICALS WILL BE PERFORMED IN A FUME HOOD.

(2) Monitoring equipment is to be calibrated according to the manufacturers' instructions. Workers will record calibration data and air concentration in the Health and Safety on-site logbook. **(NOT APPLICABLE)**

(3) Action Levels are for work stoppage and on-site assessment. These are average values. Consideration should be given to the potential for release of highly toxic compounds from the waste or from reaction by-products. Levels are for persistence (> 10 min). If action levels are exceeded, work will stop until levels drop to below the action level. Personnel with respirator training may reenter the work zone with proper respirator equipment. Battelle personnel without respirator training will not reenter the work zone until levels drop below the action level. **(NOT APPLICABLE)**

	ACTION LEVEL	
Uncharacterized Airborne Vapors or Gases	>5 ppm	**Not Applicable**
Characterized Airborne Gases, Vapor, Particulates	>50% PEL, REL, TLV	**Not Applicable**
Oxygen	<19.5; > 23.5	**Not Applicable**
Flammability	>10% EL	**Not Applicable**

(4) Military and/or civilian personnel in charge of buildings adjacent to invasive monitoring activities will be notified via a health and safety kickoff meeting of site activities. A copy of this HASP will be provided. If any action levels are reached at the work area as described above or if discernible odors are released as a result of field activities, the personnel in charge or their designated representative will be notified immediately**. (NOT APPLICABLE)**

SECTION 7: HEALTH AND SAFETY TRAINING/MEDICAL MONITORING PROGRAM

The Battelle project staff is included in the Battelle Health and Safety Training and Medical Monitoring Programs in conformance with 29 CFR 1910.120(f) as applicable. As deemed necessary by the Battelle SSO, training records will be kept on file for all on-site Battelle personnel.

		HAZWOPER TRAINING		
NAME	MEDICAL (Date)	INITIAL (Hrs/Date)	REFRESHER (Date)	CPR/FA/BBP (Dates)
Mark Misita	**Not Applicable. Not a HAZWOPER site.**	**Not Applicable. Not a HAZWOPER site.**	**Not Applicable. Not a HAZWOPER site.**	CPR (4/04) FA (4/04) BBP (4/04)
Robyn Kroeger	**Not Applicable. Not a HAZWOPER site.**	**Not Applicable. Not a HAZWOPER site.**	**Not Applicable. Not a HAZWOPER site.**	BBP (4/04)
Zachary Willenberg	**Not applicable. Not a HAZWOPER site.**	**Not applicable. Not a HAZWOPER site.**	**Not applicable. Not a HAZWOPER site.**	BBP (8/03)

SECTION 8: PERSONAL MONITORING

No personal exposure monitoring or heat/cold stress monitoring will take place on site as test articles and laboratory chemicals will be handled within a portable lab hood. If the need for such monitoring is anticipated, this HASP will be modified as accordingly. Temporary containment facilities will be climate controlled.

SECTION 9: CONFINED SPACE ENTRY

No confined space and/or trench entries will take place on site. If the possibility of such entries taking place exists, this HASP will be modified accordingly.

SECTION 10: COMMUNICATION PROCEDURES

- Verbal communication will be used to communicate among staff in the mobile laboratories/trailers for routine information exchange.
- For emergencies requiring immediate muster of all site personnel, an air horn will be used.
- A land line will be available at the Green Point Environmental Learning Center. The Battelle Project Manager and the SSO will have cell phones.
- Signs will be posted to announce critical safety procedures (e.g., "Safety Glasses Must Be Worn Upon Entry To The Mobile Lab/Trailer")

Important Phone Numbers

Battelle Headquarters	(Cell) 614-893-2260
Amy Dindal Battelle Project Manager Battelle Site Safety Officer	(Cell) 561-721-5826
Gary Carlin, CIH Battelle ES&H Representative	(Office) 614-424-4929 (Cell) 614-348-5785 (Home) 614-853-2024
Green Point Environmental Learning Center	(Office) 989-759-1669
Michigan Department of Environmental Quality Contact Michael Jury	(Office) 989-686-8025, X 8311 (Cell) 989-860-6646 (Pager) 989-253-3942

SECTION 11: DECONTAMINATION PROCEDURES

Temporary Containment Facility Clearance Sampling

Each temporary containment facility (i.e., mobile laboratory or trailer) used to house the developer technologies when analyzing samples will be wipe tested after initial set-up but before sample analyses begin. The trailers and mobile laboratories will be also be cleaned and then wipe tested at the conclusion of the demonstration. Wipe test results must demonstrate that the total TEQ (dioxin/furan and PCB) are <20 pg TEQ/100cm^2 (http://www.epa.gov/wtc/wipe_samples/censuspdfs/wipe_benchmarks.htm) before a containment facility can be certified as "clean." Samples will be collected in accordance with EPA Methods 1613B and 1668A respectively. Analyses of these samples will be performed by Battelle. A letter describing the cleaning procedure and the analytical results will be provided to the suppliers of the mobile labs and trailers as soon as results are available.

Wipe Clearance Test Sampling Overview

Samples taken before analyses being will be taken on the floor near the exterior door. Samples taken after analysis by the developers within each temporary containment facility in the following places: 1 sample-fume hood, 1 sample-floor directly in front of fume hood, 1 sample-door knob, 1 blank. The wipe samples will be analyzed for dioxins/furans and the 12 World Health Organization PCBs using EPA Methods 1613B and Method 1668A, respectively.

Sampling Procedure

Each wipe test sample will be collected in the following fashion:

Step 1: A new pair of disposable nitrile gloves will be used to collect each sample.
Step 2: Each sample jar will be labeled with the sample ID.
Step 3: The sampling solvent will be poured into the jar containing the wipe test paper.
Step 3: The wipe test paper will be removed from the jar after it has been saturated with the sample solvent.
Step 4: The wipe test paper will be used to collect a surface sample (one sample per jar).
Step 5: The sample collection area will be set as a 10 cm x 10 cm area. This will determined by using a pre-cut 10 cm x 10 cm square as a reference. If the area is of irregular size or shape, the sample area will be estimated so total surface area on the wipe will equal approximately 100 cm^2.
Step 6: The wipe will be placed back into its respective jar and the lid snuggly screwed back on.
Step 7: The sampling time, sampling date, sampler's initials, location of the wipe sample, location of the wipe sample, trailer/mobile lab the sample was taken from, and any comments will be documented.
Step 8: Steps 1-7 will be repeated for all samples.

The following decontamination/spill response equipment is required (check all that apply)

√	**Shoe covers**	√	**Dry Brushes**	√	**Detergent Soap**
√	**Trash Cans/Bags**	❑	Wet Brushes	❑	Other Decontamination Solution
❑	Buckets	√	**Water**	√	**Small hand shovel**
√	**Dust Pan**	√	**Waste Tags**	√	**Barricade tape**
√	**Safety Glasses w/Side shields**	√	**Nitrile gloves**	√	**Disposable lab coat**

SECTION 12: EMERGENCY PROCEDURES

All on-site personnel will use the following standard emergency procedures. The Battelle SSO shall be notified of all on-site emergencies and be responsible for ensuring that the procedures are followed. The site SSO has ultimate authority during any emergency until relieved by a ranking responder (i.e., local fire department, police department) or until the emergency is brought under control.

Personal Injury in the demonstration test area — Designated Emergency Signal: Verbal communication as needed.

Upon notification of an injury, the Battelle SSO or alternate should evaluate the nature of the injury, and the affected person should be decontaminated to the extent possible prior to movement off site. The on-site CPR/FA personnel shall initiate the appropriate first aid, and contact should be made for an ambulance (and other emergency services as needed) and with the designated medical facility (if required).

Fire/Explosion Muster — Designated Emergency Signal: 2 short horn blasts

Upon notification of a fire or explosion on site, all site personnel will assemble at the Battelle Headquarters. The fire department shall be alerted and all personnel moved to a safe distance from the involved area.

Severe Inclement Weather — Designated Emergency Signal: Verbal communication as needed.

In severe inclement weather (i.e., thunderstorm, tornado, hail, etc.), participants will discontinue operations and go to the Green Point ELC for shelter. If the severe inclement weather occurs when Green Point ELC is closed, participants will discontinue operations and leave the site.

Equipment Failure — Designated Emergency Signal: Verbal communication as needed.

If any other equipment (i.e., portable fume hood) on-site fails to operate properly, the Battelle SSO shall be notified and then determine the effect of this failure on continuing operations on site. If the failure affects the safety of personnel or prevents completion of the demonstration plan tasks, all personnel shall leave the affected temporary containment facility until the situation is evaluated and appropriate actions taken.

In all situations, when an on-site emergency results in evacuation of an area, personnel shall not reenter the area until:

- The conditions resulting in the emergency have been corrected.
- The hazards have been reassessed by the Battelle SSO.
- The Site Safety Plan has been reviewed by the Battelle SSO and the Battelle project manager.

SECTION 13: SPILL CONTROL PROCEDURES

The following procedures will be followed in the event of a spill involving the demonstration samples or the other analytical chemicals brought on site. Spill clean-up materials will be assessed on a case-by-case basis.

Spills of Demonstration samples inside of a Temporary Containment Facility

Concentrations of contaminants in the test media will be extremely low. Primary concern is migration of trace contaminates. In case of a spill

- Barricade the spill area and contact the Battelle SSO.
- Don safety glasses and nitrile or latex gloves and obtain a spill cleanup kit from the SSO.
- Clean up the spill by first removing any bulk materials using a brush and dust pan or scraper and dust pan (Do not pick up any glass shards by hand). Next, thoroughly wipe down the spill area using a lab towel dampened with acetone. Finally, wash the spill area with a soap and water mixture.
- All waste generated from the spill will be placed into a plastic bag (or box if glass is involved) and a yellow waste tag filled out and attached to the bag. The bag will be returned to the Battelle Columbus site for proper disposal.
- The Battelle SSO shall determine if wipe test sampling of the area is required. The area should have a piece of plastic taped over it until it is determined if wipe test sampling is required.

SECTION 13: SPILL CONTROL PROCEDURES

Spills of Demonstration samples on Soil or Parking Lot

Concentrations of contaminants in the test media will be extremely low. Primary concern is migration of trace contaminates. In case of a spill

- Barricade the spill area and contact the Site Safety Officer.
- Don safety glasses and nitrile or latex gloves and obtain a spill cleanup kit.
- Clean up the spill by first removing any bulk materials using a brush and dust pan or scraper and dust pan (do not pick up any glass shards by hand). Glass should be placed into a puncture-resistant container. Next, remove any soil or debris that has come into direct contact with the spilled material.
- All waste generated from the spill will be placed into a plastic bag and a yellow waste tag filled out and attached to the bag. The bag will be returned to the Battelle Columbus site for proper disposal.
- The SSO will determine if wipe test sampling of the area is required. The area should have a piece of plastic taped over it until it is determined if wipe test sampling is required.

Spills of Lab chemicals

Spills of lab chemicals will be evaluated on a case by case basis. Small spills (involving < 50 ml) of a laboratory chemical in the fume hood can be cleaned up by staff with lab towels. For larger spills, barricade the spill area and contact the SSO.

All waste generated from a spill must be segregated and labeled. The SSO will address the disposal of the materials. Spill areas may need to be certified free of dioxin/PCB contamination depending on the type and nature of the spill. The SSO will determine if the certification analysis is necessary.

Gas Cylinder leaks

In the event of gas leak from any cylinder, the effected trailer will be evacuated until the SSO deemed it safe to reentry.

SECTION 14: EMERGENCY INFORMATION

LOCAL RESOURCES

Ambulance (name):	Mobile Medical Response	Phone:	989-758-2900
Hospital (name):	Covenant Health Care St Mary's Medical Center	Phone:	989-583-2000 989-776-8000
Police (local or state):	Saginaw Police	Phone:	989-759-1288
Fire (name):	Saginaw Fire Dept	Phone:	989-759-1376
On-Site CPR/FA(s):	Mark Misita	Phone:	614-893-2260

* For life-threatening emergencies or emergency trauma care dial 911.
The above hospital is approximately 3 miles from the furthest work area and the ambulance response time is approximately 5 minutes.

(2) DIRECTIONS TO NEAREST HOSPITALS – **See written directions (Attachment 2)**

BATTELLE RESOURCES

Project Manager Amy Dindal
ES&H Representative Gary Carlin
Hazardous Waste Coordinator Jim Walters

Important Phone Numbers

Battelle Main Number	(614) 424-6424
Legal Department	(614) 424-6585
Medical Services (Emergency)	(614) 424-4622
Amy Dindal	(561) 422-0113 (office) (561) 721-5826 (cell)
Gary Carlin	(614) 424-4929 (office) (614) 348-5785 (cell)
Jim Walters	(614) 424-4746 (office)

NOTE: All accidents/injuries/spills must be immediately reported to the SSO. The SSO will ensure any additional actions are taken (as appropriate).

SECTION 15: PERSONAL PROTECTIVE EQUIPMENT FOR ENTRY INTO TEMPORARY CONTAINMENT FACILITIES INVOLVED IN SAMPLE ANALYSIS

(In effect AFTER temporary containment facility setup is completed)

1. Respiratory protection is not anticipated to be needed on this site.

2. Any contaminated PPE will be immediately removed, cleaned or replaced (as appropriate). (Contaminated items being discarded will be placed in tagged laboratory debris containers.)

3. Shoe covers will be worn in only one trailer/mobile lab and not transferred for use in other trailers/mobile labs.

4. Safety glass must be worn upon entry into a trailer/mobile. Safety glasses will be worn at all times that work is on-going.

5. Developers will be required to wear a minimum of safety glasses when receiving/returning samples. The sample custodian will don safety glasses, gloves, and lab coat. Anyone working in the Battelle HQ trailer while samples are being distributed must wear safety glasses.

	CLOTHING		GLOVES		BOOTS		OTHER
√	**Disposable Lab Coat** ***Must be removed prior to exiting temporary containment facility***	√	**Lab gloves (*either Latex or Nitrile)***	√	**Shoe Covers (Tyvek® or equivalent)** ***Must be removed prior to exiting temporary containment facility***	√	**Safety Glasses w/Side shields**

SECTION 16: SAFE WORK PRACTICES

THE FOLLOWING PRACTICES MUST BE FOLLOWED BY PERSONNEL ON SITE

1. Smoking, eating, chewing gum or tobacco, or drinking are forbidden except in clean or designated areas.
2. Ignition of flammable liquids within or through improvised heating devices (e.g., barrels) is forbidden.
3. Contact with samples, excavated materials, or other contaminated materials must be minimized.
4. All electrical equipment used in outside locations, wet areas, or near water must be plugged into ground fault circuit interrupter (GFCI) protected outlets.
5. Good housekeeping practices are to be maintained.
6. Flammables needing refrigeration must be stored in a refrigerator rated for flammable use.
7. Where the eyes or body may be exposed to corrosive materials, water suitable for quick drenching or flushing shall be available for immediate use.

SECTION 17: WASTE DISPOSAL

Waste/Effluent/Residual Sample Handling will be handled according to the plan outlined in Attachment 4.

SECTION 18: REFERENCES

- 29 CFR 1910
- U.S. EPA Standard Operating Safety Guidelines for Hazardous Waste Operations. June 1992

SECTION 19: EMPLOYEE ACKNOWLEDGMENTS

PLAN REVIEWED BY:		DATE
Battelle Site Safety Officer	Amy Dindal	4/26/04
Battelle ES&H Representative	Gary Cox	4/26/04
Battelle Project Manager:	Amy Dindal	4/26/04

SITE HEALTH AND SAFETY PLAN FOR FIELD ACTIVITIES FOR THE SITE DEMONSTRATION OF DIOXIN MONITORING AND MEASUREMENT TECHNOLOGIES FOR SOIL AND SEDIMENT

Review Sign-Off

I acknowledge that I have read the information in this HASP form and the MSDSs. I understand the site hazards as described and agree to comply with the contents of the plan.

Date	Employee Name (Print)	Employee Name (Signature)	INDICATE: Battelle Staff = "B" Developer = "D" EPA = "E" Visitor = "V" Other (specify) = "O"
4/26/04	FERNANDO RUBIO	Fernando Rubio	D
4/26/04	Gary D. Hinshaw	Gary D. Hinshaw	D
4/26/04	Amy B Dindal	Amy B Dindal	B
4/26/04	Masako Hayakawa	Masako Hayakawa	D
4/26/04	NOBUKAZU MIYAMOTO	Nobukazu Miyamoto	D
4-26-04	Sheldon HENDERSON		D
4-26-04	Hiroyuki Hayashi		D
4-26-04	Kimihiko Sano	Kimihiko Sano	D
4-26-04	Mirora Izuokawa	Mirora Izuokawa	D
4-26-04	Tomohiro Itoh	Tomohiro Itoh	D
4-26-04	Joshua Finegold		B
4-26-04	MARK MISITA		B
4/26/04	Mary Schrock	Mary Schrock	B
04-26-04	Robyn Kroeger	Robyn Kroeger	B
4/26/04	Jeff Sterkel		D
4-26-04	Bob Harrison		D
4/26/04	John Gordon		D

SITE HEALTH AND SAFETY PLAN FOR FIELD ACTIVITIES FOR THE SITE DEMONSTRATION OF DIOXIN MONITORING AND MEASUREMENT TECHNOLOGIES FOR SOIL AND SEDIMENT

Review Sign-Off

I acknowledge that I have read the information in this HASP form and the MSDSs. I understand the site hazards as described and agree to comply with the contents of the plan.

Date	Employee Name (Print)	Employee Name (Signature)	INDICATE: Battelle Staff = "B" Developer = "D" EPA = "E" Visitor = "V" Other (specify) = "O"
26APR	MICHAEL JURY	Michael Jury	O - MDEQ
26 Apr	JEFF STURKey		D
26 APR	John Gordon		D
26Apr	Bob Harrison		D
26Apr	Randy Allen		D
26APR04	TERRY NESTRICK		D
26/9/04	John Steketee		E
26/4/04	STEVE BILLETS	Stephen Billets	E
26/4/04	Rachel Sell	Rachel M Sell	B
29/4/04	George Clark		D
4/29/04	Zachary Wilbur		B

Attachment 1
Identified or Suspected Contaminants

IDENTIFIED OR SUSPECTED CONTAMINANTS

Table 1. Known or Suspected Contaminants that may be Encountered during Analytical Activities. No employee exposures are expected as all handling of samples and laboratory chemicals will be completed in a portable fume hood.

Media	Substances Involved	Characteristics	Estimated Concentrations	American Conference of Governmental Industrial Hygienists Limits
Soil, Sediment, Toluene Extract	Dioxins (Various)	Low Concentrations in Soil, Sediment, or Extract.	< 5 to 13,000 pg/g	None Established.
Soil, Sediment, Toluene Extract	PCBs	Low Concentrations in Soil or Sediment.	< 300 ppm	42% Chlorine: 1 mg/m^3 (8 hr TWA) 54% Chlorine: 0.5 mg/m^3 (8 hr TWA)
Soil, Sediment, Toluene Extract	PAHs	Low Concentrations in Soil or Sediment.	< 100 ppm	0.2 mg/m^3 (8 hr TWA) (as coal tar pitch volatiles – benzene soluble)
Soil, Sediment, Toluene Extract	PCP	Low Concentrations in Soil or Sediment.	< 100 ppm	0.5 mg/m^3 (8 hr TWA)
Lab Chemical	Hexane	Ignitable, Volatile Organic, Toxic.	Laboratory Grade (Essentially pure)	50 ppm (8 hr TWA)
Lab Chemical	Methanol	Ignitable, Volatile Organic, Toxic.	Laboratory Grade (Essentially pure)	200 ppm (8 hr TWA) 250 ppm (STEL)
Lab Chemical	Toluene	Ignitable, Volatile Organic.	Laboratory Grade (Essentially pure)	50 ppm (8 hr TWA)
Lab Chemical	DMSO	Irritant.	Laboratory Grade (Essentially pure)	None

ppm= parts per million
hr = hour
mg/m^3 = milligrams per cubic meter
TWA= time-weighted average

Attachment 2
Written Directions to the Nearest Hospitals

EMERGENCY INFORMATION
(POST ON SITE)

EMERGENCY CONTACTS AND ROUTE TO CONVENANT HEALTHCARE
GREEN POINT ENVIRONMENTAL LEARNING CENTER

Emergency Contact	**Telephone No.**
Amy Dindal	614-893-2260

Medical Emergency

Hospital Name: Covenant Healthcare

Hospital Address: 1447 N. Harrison St.
Saginaw, MI 48602

Hospital Telephone No.: Emergency - 911
General (989) 583-0000

Ambulance Telephone No.: Mobile Medical Response
989-758-2900

Route to Hospital: (Approximate Distance: 3 miles)
From the site, start out going north on Maple Street toward Beacon Drive and turn right on S. Michigan Ave. for about 1.6 miles. S. Michigan Ave. will turn into N. Michigan Ave. Continue on N. Michigan Ave. and then turn left onto Houghton Street. Another option would be to take N. Michigan Ave all the way to Cooper St. and take a left. There are emergency entrances at both Houghton St. and Cooper St. Signs to the hospital are well-marked throughout the area.

EMERGENCY INFORMATION
(POST ON SITE)

EMERGENCY CONTACTS AND ROUTE TO ST. MARY'S
GREEN POINT ENVIRONMENTAL LEARNING CENTER

Emergency Contact	**Telephone No.**
Amy Dindal	614-893-2260

Medical Emergency

Hospital Name: St. Mary's Medical Center (http://www.saintmarys-saginaw.org/)

Hospital Address: 800 S. Washington Ave.
Saginaw MI 48601

Hospital Telephone No.:	Emergency - 911 General (989) 776-8000
Ambulance Telephone No.:	Mobile Medical Response 989-758-2900

Route to Hospital: (Approximate Distance: 3.7 miles)
From the site, start out going north on Maple Street toward Beacon Drive. Turn right onto S. Michigan Ave. for about a mile, and then take another right onto Stephens St./MI-46 E. Continue to follow MI-46 E. There is a set of one-way pairs that merge into what is known as Rust St. just before the Saginaw River. Stay on Rust, (going east) to S. Washington Ave./M-13. Turn left (going north) onto S. Washington Ave/MI-13. St. Mary's Medical Center is located on S. Washington Ave.

Attachment 3
Waste Disposal Plan

Waste/Effluent/Residual Sample Handling

Effluents (e.g., solvent/aqueous collected from analytical processes, and sample by-products), used samples, and unused sample will be collected for quantification and be reported as part of the verification of each technology. These items are necessary to aid in the complete characterization of each developer's analytical process and will be transported back to the Battelle Columbus facility for analysis and quantity confirmation/verification (see Table A-1, below). These items will be retained by Battelle until such time that they are no longer needed. At that time, they will be considered waste, characterized, and properly disposed.

Table A-1: Effluent Handling

Effluent Stream	Container Type	Quantity on Hand
Solvent effluent from analytical processes	5-gallon steel can with screw cap	6 - 5-gallon steel cans with screw cap
Used samples	5-gallon steel can with screw cap	6 - 5-gallon steel cans
Unused sample	Original container	N/A
Cell culture (non-human; non-hazardous; non-biohazard)	Double ziplock bag; 30-gallon plastic drum	1 - 30-gallon plastic drum

The miscellaneous non-RCRA hazardous wastes generated on the test site (such as booties, gloves, lab towels, and broken glass) will be taken from the site and disposed of at the Battelle – Columbus location (see Table A-2 below). The only exception to this is general refuse (e.g., coffee cups, food waste, and office waste). General refuse will be disposed of in regular public refuse containers.

Table A-2. Waste Handling

Waste Stream	Container Type	Quantity on Hand
Miscellaneous non-hazardous waste (e.g., booties, gloves, lab towels, etc.)	55-gallon drum (plastic)	2 - 55-gallon containers (A small collection container will be placed in each developer's area; the amount of times this is emptied into the central 55-gallon container will be recorded.)
Non-hazardous broken glass	Broken glass box	2 - glass boxes in central location

Chapter 11
References

1. EPA. 1996. "A Guidance Manual for the Preparation of Site Characterization and Monitoring Technology Demonstration Plans." NERL. October.

2. EPA. 1998. "Quality Assurance Project Plan Requirements for Applied Research Projects." Unpublished. National Risk Management Laboratory.

3. Kutz, F. W., Barnes, D. G., Bottimore, D. P., Greim, H., and Bretthauer, E. W. 1990. The international toxicity weighting factor (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds. *Chemosphere* 20: 751–758.

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

5. EPA. 2001. *Database of Sources of Environmental Release of Dioxin-like Compounds in the United States,* EPA/600/C-01/012, March.

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8. NOAA. 1998. Sampling and analytical methods of the national status and trends program mussel watch project: 1993-1996 update. *NOAA Technical Memorandum NOS ORCA 130.* Silver Spring, Maryland.

9. De Rosa, Christopher T., et al. 1997a. Dioxin and dioxin-like compounds in soil, Part 1: ATSDR Interim Policy Guideline. Toxicology and Industrial Health, Vol. 13, No. 6, 1997. pp. 759-768.

10. EPA. 1995. (updated March 16, 1998) "Handbook for Preparing Office of Research and Development Reports." ORD. Washington, DC, EPA/600/K-95-002. August.

ADDENDUM

Shortly before making a final commitment to participate in the field demonstration, Paracelsian decided that it did not have the resources to fulfill all of the requirements described in this document. Paracelsian committed to participate in the Visitor's Day activities, but did not plan to analyze any of the samples in the field. Subsequently, Paracelsian determined that it could only commit to analyzing about 25% of the demonstration sample set in their laboratories. It was decided that this was insufficient to draw meaningful conclusions regarding the performance of this technology. As a result, Paracelsian withdrew from further consideration as a full demonstration participant, but agreed to analyze the 15 pre-demonstration samples. The Paracelsian pre-demonstration results will be included in the Data Evaluation Report (DER, see Section 8.4), which may be obtained from the EPA. The DER also will include a limited review of the Paracelsian technology by the Battelle observers during the Visitor's Day. The DER will not be published, and no other published report regarding the performance of the Paracelsian technology will be prepared.

APPENDIX A

LIST OF DEMONSTRATION PANEL MEMBERS

Dioxin SITE Demonstration Panel

Participant	Organization	Role
Fernando Rubio	Abraxis LLC	Developer
Bob Harrison	CAPE Technologies	Developer
Randy Allen	Hybrizyme Corporation	Developer
Noriyoshi Inoue	Paracelsian Inc.	Developer
John Gordon, Jeff Sturkey, George Clark	Xenobiotic Detection Systems	Developer
Masako Hayakawa/Emmy Leung/ Hiroyuki Hayashi	Wako Pure Chemical Industries, Ltd.	Developer
Steve Billets	ORD/ESD-LV	Program Manager
Andy Beliveau	Region 1	Technical Advisor
Christopher Stitt	Region 2	Observer
William Sy	Region 2	Observer
Stevie Wilding	Region 3	Technical Advisor
Nardina Turner	Region 4	Technical Advisor
Greg Rudloff	Region 5	Technical Advisor
Allen Debus	Region 5	Technical Advisor
Barry Evans	Region 7	Observer
Craig Smith	Region 7	Technical Advisor
David Williams	Region 7	Technical Advisor
Vance Fong	Region 9	Observer
Bill Coakley	OSRTI/ERTC-NJ	Technical Advisor
Dwain Winters	OPPTS/NPCD-DC	Technical Advisor
David E. Cooper	OSWER/OERR-DC	Observer
Jon Josephs	ORD/HSTL-R2	Technical Advisor
Bob Mouringhan	ORD/HSTL-R7	Technical Advisor
Terry Smith	OSRTI/DC	Technical Advisor
Joe Ferrario	OPPTS/MS	Technical Advisor
Deana Crumbling	OSRTI/DC	Observer
Mike Jury, Sue Kaelber-Matlock, Al Taylor	Michigan Department of Environmental Quality	Technical Advisors
Becky Goche, Doug Spencer	U.S. Fish and Wildlife Service	Demonstration Site Representatives
Laurie Phillips, Coreen Hamilton, Georgina Brooks, Dale Hoover	AXYS Analytical Services	Reference Laboratory
Rosanna Buhl, Gary Carlin, Amy Dindal, Tim Pivetz, Mary Schrock, Rachel Sell, Zack Willenberg	Battelle	Demonstration Coordinator

APPENDIX B

REQUEST FOR AND OBTAINING OF SPECIAL USE PERMIT

February 3, 2004

Douglas G. Spencer
Refuge Manager
Shiawassee National Wildlife Refuge
6975 Mower Road
Saginaw, Michigan 48601

Dear Mr. Spencer:

The purpose of this letter is to request the use of the Green Point Environmental Learning Center ("Green Point") located in the Shiawassee National Wildlife Refuge managed by the U.S. Fish and Wildlife Service to serve as the host site for an upcoming demonstration of dioxin monitoring and measurement technologies.

Performance verification of innovative environmental sampling, monitoring, and measurement technologies is an integral part of the regulatory and research mission of the U.S. Environmental Protection Agency (EPA). To address this need, the Superfund Innovative Technology Evaluation (SITE) Program was established by EPA. Additional information regarding the history of this program may be found at our website: http://www.epa.gov/ORD/SITE. One component of SITE is the Monitoring and Measurement Technology (MMT) Program. This Program evaluates the performance of innovative technologies that sample, detect, monitor, or measure hazardous and toxic substances in soil, water, and sediment samples. The SITE MMT Program is currently planning a demonstration of technologies that can be deployed in the field or in a mobile laboratory for the rapid measurement of dioxins in soil and sediment. The EPA has awarded a technical support contract to the Battelle Memorial Institute to assist with this demonstration.

We have been working closely with the Michigan Department of Environmental Quality (MDEQ) in planning this demonstration, and dioxin-contaminated samples from the Saginaw area were supplied by MDEQ to be included in this study. As a result of our discussions with the MDEQ and our site selection survey, the EPA requests the use of Green Point Center's parking lot as the demonstration site. We request access for a two-week timeframe, from approximately from April 22, 2004 to May 6, 2004.

Use of Green Point's parking lot for this demonstration will entail the following:

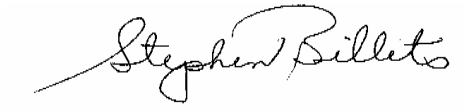
- Access to site approximately seven trailers in a remote area of the parking lot at Green Point so as not to disturb or impede normal daily activities at Green Point;
- Contain all of the analysis to within the trailers;
- Conduct a half-day Visitors Day during the demonstration period (approximately April 28), including the use of Green Point's auditorium. For the Visitors Day, the demonstration site would be open to the public so that those interested in using these technologies can learn more about them.
- Use of the Green Point building itself would be limited to bathrooms, and possibly phone use should cellular service be an issue.
- Not to disturb the grounds or parking area of Green Point in any way. We are committed to leave the area as we found it before conducting the demonstration.

This demonstration is fully funded by the SITE Program. Any infrastructure requirements that we have, such as supplying power for the trailers, will be coordinated and paid for through this contract by Battelle. All logistics for this demonstration will be coordinated by Becky Goche.

For more information about the dioxin demonstration and this request for use of Green Point's parking lot, please do not hesitate to contact me at 702-798-2232 or by e-mail at billets.stephen@epa.gov.

We look forward to working with you and would appreciate your support of this demonstration.

Sincerely,



Stephen Billets
Program Manager
U.S. EPA MMT Program

cc:
G. Rudloff, U.S. EPA, Region 5
A. Dindal and R. Sell, Battelle Memorial Institute
S. Kaelber-Matlock, Michigan Department of Environmental Quality
A. Taylor, Michigan Department of Environmental Quality

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Shiawassee NWR
6975 Mower Rd.
Saginaw, Mi. 48601

SPECIAL USE PERMIT

Station No. to be Credited Permit No.
31520 - 04006

Date March 4,2004

Period of Use (inclusive)

From April 19,2004

To May 6,2004

Permitte Name
Stephen Billets

Permittee Address
USEPA
P.O. Box 93478
Las Vegas, NV 89193

Purpose (specify in detail privilege requested, or units of products involved)

Utilized Green Point Environmental Learning Center facilites as the host site for an upcoming demonstation of dioxin monitoring and measurement technologies.

Description (specify unit numbers: metes and bounds, or other recongizable designations)

Superfund Innovative Technology Evaluation (SITE) Program and Monitoring and Measurement Technology (MMT) Program is currently planning a demonstration of technologies that can be deployed in the field or in a mobile laboratory for the rapid measurement of dioxins in soil and sediment. The USEPA has awarded a techical support contract to the Battelle Memorial Institute to assist with this demonstration to held at Green Point ELC. Michigan Department of Environmental Quality is assisting with the demonstration by supplying dioxin-contaminated samples from the local area.

Amount of fee $0 if not a fixed payment, specify rate and unit of charge:

☐ Payment Exempt - Jusification:
☐ Full Payment
☐ Partial Payment - Balance of payments to be made as follows:

Record of Payments

Special Conditions

1. Access to site for approximately seven trailers in a remote area of the parking lot at Green Point ELC so as not to disturb or impede normal daily access to the Center.
2. Contain all of the analysis to within the trailers.
3. Conduct a half-day "Visitors Day" during the demonstration period (approximately April 28), including the use of Green Point's auditorium. For the Visitor's Day, the demonstration site would be open to the public so that those interested in using these technologies can learn more about them.
4. Use of the Green Point building itself would be limited to bathrooms, and possibly phone use should cellular service be an issue. The area of use will be restored back to the way it was found before conducting the demonstration.

This permit is issued by the U.S. Fish and Wildlife Service and accepted by the undersigned, subject to the terms, covenants, oblications, and reservations, expressed or implied herein, and to the conditions and requirements appearing on the reverse side.

Permittee Signature

Issuing Officer Signature and Title Refuge Manager 3-4-04

Form 3-1383 (Rev. 6/85)

APPENDIX C

EXAMPLE COVER LETTER FOR SAMPLE COLLECTION

Battelle
The Business of Innovation

October 13, 2003

Al Taylor
Waste and Hazardous Materials Division
Michigan Department of Environmental Quality
Constitution Hall, Atrium North
525 West Allegan Street
Lansing, MI 48933

SAMPLING AND SHIPPING INSTRUCTIONS FOR SEDIMENT SAMPLES

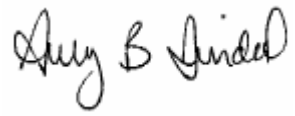
Dear Al:

Thank you so much for agreeing to provide Battelle with Saginaw River sediment samples for use in U.S. Environmental Protection Agency's Superfund Innovative Technology Evaluation (SITE) Demonstration of Monitoring and Measurement Technologies for Dioxin in Soil and Sediment. Twelve pre-cleaned one-gallon sample containers are enclosed for you to fill with sediment (6 one-gallon samples in duplicate). Please label two containers containing the same sample as "1 of 2" and "2 of 2". Please attach a description or label each container with a description of the sample including the **location where the sample was collected and the estimated concentrations of dioxin and PCBs.**

The collected samples are to be shipped back to Battelle via the provided coolers. Federal Express labels that include an account number and the shipping address are enclosed. Please notify me by phone or email once the samples have been shipped so we can expect delivery in our laboratories. We would like to have the samples returned by **December 2, 2003** if that is at all possible.

Any questions on sample shipment or receipt should be directed to Kim Andrews, andrewsk@battelle.org, 614-424-5254. Questions about the EPA project should be directed to Amy Dindal, dindala@battelle.org, 561-422-0113.

Sincerely,



Amy B. Dindal
Project Manager
Atmospheric Science and Applied Technology

ABD:llg

cc: Steve Billets, U.S. Environmental Protection Agency (letter only)

APPENDIX D

EXAMPLE CHAIN OF CUSTODY/RESULTS FORM

CHAIN-OF-CUSTODY/RESULTS FORM

SITE Demonstration for Monitoring and Measurement of Dioxin in Soil and Sediment

To: Developer **COC #: Developer 01**

From: Battelle

Sample Number/ Analysis Order	Received by Developer (T)	Result		Analyzed in Lab (L) or Field (F)
		Measured Value	Unit	
Developer 1				
Developer 2				
Developer 3				
Developer 4				
Developer 5				
Developer 6				
Developer 7				
Developer 8				
Developer 9				
Developer 10				

REMINDERS: Report results consistently (i.e., to the same number of decimal places); Non-detect results should be reported as "< reporting limits" with the reporting limits of the technology specified.

Relinquished by (signature)	*Date*	*Time*	*Received by (signature)*	*Date*	*Time*
Relinquished by (signature)	*Date*	*Time*	*Received by (signature)*	*Date*	*Time*
Relinquished by (signature)	*Date*	*Time*	*Received by (signature)*	*Date*	*Time*
Relinquished by (signature)	*Date*	*Time*	*Received by (signature)*	*Date*	*Time*

COMMENTS: _____

APPENDIX E

OBSERVER CHECKLISTS

Procedural Observations and Questionnaire
Of
ABRAXIS LLC Coplanar PCB ELISA Kit

Procedure witnessed by:

Date witnessed:

Time/Date procedure started:

Time/Date procedure ended:

Name of Kit Used:

Individuals Witnessed:

Lot Number of Kit:

Expiration Date of Kit:

Answer the following questions:

Question	**Y**	**N**	**NA**	**Comments**
Could this kit be performed in the field without a mobile lab/trailer?				
Would it take long to set up in the field before first samples could be processed? How long?				
How many samples could be prepared and analyzed in one day in the field once set up is complete? By an experienced kit user? By the novice kit user?				
Would sample throughput be faster in the lab than in the field? If so by how much?				
Are the instructions supplied with the kit the same as the operating procedure listed in the demo plan? If not, why ? If not, use the kit instructions for evaluation.				

Question	Y	N	NA	Comments
Was testing carried out at kit recommended temperature of 20° C to 25° C? How was temperature measured? Was measuring device calibrated?				Actual Temp_____
Are the following equipment and reagents supplied with the kit? (Note if item not used at all, also note grade and supplier of solvents) thermometer soil collector bottle (containing dispersion device) digital balance 30-mL high-density polyethylene (HDPE) bottle steel mixing ball anhydrous sodium sulfate acetone hexane shaker/rotater filter centrifuge extraction tube concentrated sulfuric acid nitrogen evaporator methanol water 1:10 in 50% methanol/water anti-coplanar PCB antibody solution controls standards parafilm strip holder pippetor enzyme conjugate solution waste container 1X wash solution paper towels color solution stop solution microplate reader graph paper commercial ELISA program				

Question	Y	N	NA	Comments
Were any supplies or equipment used that were not listed in the instructions? If so, please list.				
What are recommended hold times and storage conditions for: Samples? Extracts? Reagents? Standards?				
Would you know based on the instructions provided (if not how did you decide): How much sample to extract? How many sample to extract in a "batch"? How much sodium sulfate to mix with sample? Which solvent and how much to extract with? How long to extract? How many controls and standards to prepare with "batch"? How long to agitate during oxidation cleanup (acid wash) before letting phases separate and removing top layer? Maximum number of oxidation (acid wash steps) that can be complete before results are affected?				

Question	Y	N	NA	Comments
After acid wash, is sample evaporated to complete dryness during nitrogen evaporation step? Is additional cleanup ever necessary? How do you know and what additional cleanup options are there? Are all samples diluted? If not how do you know which ones to dilute? How long do you mix the wells by moving in a circular motion? (If measured what did you measure with?) How long to incubate? (If measured what did you measure with?) What temperature to incubate? (If measured, what did you measure with? Is it calibrated?) How critical is this temperature? How long do you mix the wells with the enzyme conjugate solution? How long to incubate? (If measured, what did you measure with?) What temperature to incubate?(If measured, what did you measure with? Is it calibrated?) How critical is this temperature? How dry do the wells have to be after the 1X wash step?				

Question	Y	N	NA	Comments
Do you have to mix in the color solution? How long does color solution incubate. Is its incubation temp critical- what temp is recommended? How critical is it that the plate be read within 15 minutes of adding the stop solution? How to use/measure with the microplate reader? Is it calibrated, if so, how? How much sample solution needs to be used with the microplate reader? How do you calculate PCDD/PCDF amounts from the data generated? Is it clear how to account for dilutions? For the cross-reactivity factor?				
Additional Comments				

Question	Y	N	NA	Comments
Must all procedures be completed in the same day? If not, when can procedure be stopped and how must samples be stored? Is that in the instructions?				
Were any procedural steps performed differently than you interpreted from the instructions? Were any of the instructions confusing? If so please comment:				
What QC samples are required with this approach and at what frequency? What are recommended QC acceptance criteria? Did QC samples meet acceptance criteria? If not, is it clear what corrective action to take?				
What QC samples would vendor recommend, but not require and at what frequency?				
Do you recommend that some of the data be verified by conventional methods? What method? What frequency?				
How accurate do weights and volumes used with this technique have to be?				
Were all balances, pipettes, and thermometers calibrated?				

Following the procedure you just observed, including QC requirements, how many samples do you, the observer, think you could process in a day? In a week?				
Does the vendor provide training in kit use? Is this extra charge? Video? Classes? Phone support?				
What education/experience would vendor recommend kit users have?				
What do you think would be required education/experience for successful operation of this technology?				
Additional Comments:				

Abraxis Operating Procedure

To ensure accurate and reliable results, every effort should be made to perform the coplanar PCB ELISA kit at temperatures between 20ºC and 25ºC and to allow the reagents to be at the same temperature. The following sample preparation was designed for a quick screen at 625 pg/g. Other sample preparation options are available for lower concentration.

3.4.2.1 Preparation of Sample Extracts From Soil

1. Label soil collection bottles and extract collection vials.

2. Remove the screw cap from the soil collector bottle (containing dispersion device) and collect soil by weight using a digital balance. Place the bottle in an upright position on the balance and tare weight. Weigh 2 ± 0.02 g of soil into the tube. Record the soil weight.

3. Weigh 2 g of soil in a 30-mL high-density polyethylene (HDPE) bottle.

4. Add 6 g of anhydrous sodium sulfate and mix until sample is free flowing.

5. Add 1 steel mixing ball.

6. Add 10 mL of 20% acetone in hexane. Rotate for one hour.

7. Remove the organic extract from the soil particulates by filtration, sedimentation, or centrifugation.

8. Transfer extract to a 40 mL screw cap extraction tube and oxidize using concentrated sulfuric acid (4 mL). Mix by agitation for one minute and allow phases to separate.

9. Remove the organic phase (top layer), transfer to a fresh extraction tube and add 4 mL of concentrated sulfuric acid. Mix by agitation for one minute and allow phases to separate.

10. Repeat step 9 until the acid phase is colorless.

11. Evaporate 1 mL of the organic phase using a nitrogen stream.

12. Redissolve in 0.25 mL of methanol.

13. Add 0.25 mL of water. If cloudy, centrifuge.

NOTE: This extraction procedure dilutes the sample by a factor of 2.5. Therefore, assay results need to be multiplied by 2.5 to obtain the final coplanar PCB concentration in the sample.

3.4.2.2 Dilution of Sample Extracts

Dilute sample (1:10) by adding 50 µL of extract to 950 µL of 50% methanol/water.

3.4.2.3 Assay Procedure

1. Add 50 µL of anti-coplanar PCB antibody solution successively to each well.

2. Add 50 µL of the appropriate standard, control, or sample. Using duplicates or triplicates is recommended. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents. Incubate at room temperature for 30 minutes.

3. After the incubation, remove the covering and add 50 µl of enzyme conjugate solution to the individual wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop. Be careful not to spill the contents. Incubate at room temperature for 90 minutes.

4. After the incubation, remove the covering and vigorously shake the contents of the wells into a waste container. Wash the strips 3 times using the 1X wash solution with a volume of at least 250 uL per each wash step. Any remaining buffer in the wells should be removed by patting the plate on a dry stack of paper towels.

5. Add 150 uL of color solution successively to each well. Incubate for 20 to 30 minutes.

6. Add 50 uL of stopping solution to each well in the same sequence as for the other reagents.

7. Read absorbance using a microplate reader at 450 nm within 15 minutes after adding the stopping solution.

8. Construct a standard curve by plotting the %B/Bo for each standard on a vertical logit (y axis) versus the corresponding PCB 126 standard concentration on the horizontal logarithmic axis (x) on a graph paper. Alternatively commercial ELISA programs can be used.

9. To obtain the total coplanar PCB TEQ in a sample, multiply sample assay results by the cross-reactivity factor 0.01.

Procedural Observations and Questionnaire
Of
CAPE Technologies Dioxin/Furan Immunoassay Kit

Procedure witnessed by:

Date witnessed:

Time/Date procedure started:

Time/Date procedure ended:

Name of Kit Used:

Individuals Witnessed:

Lot Number of Kit:

Expiration Date of Kit:

Answer the following questions:

Question	**Y**	**N**	**NA**	**Comments**
Could this kit be performed in the field without a mobile lab/trailer?				
Would it take to set up in the field before first samples could be processed? How long?				
How many samples could be prepared and analyzed in one day in the field once set up is complete? By an experienced kit user? By the novice kit user?				
Would sample throughput be faster in the lab than in the field? If so by how much?				
Are the instructions supplied with the kit the same as the operating procedure listed in the demo plan? If not, why? Use the kit instructions for evaluation.				

Question	Y	N	NA	Comments
Was testing carried out at kit recommended temperature of 20° C to 25° C? How was temperature measured? Was measuring device calibrated?				 Actual Temp_____
Are the following equipment and reagents supplied with the kit? (Note if item not used at all, also note grade and supplier of solvents) Thermometer Shaker DMF Sodium sulfate Hexane Fuming sulfuric acid Water-miscible organic solvent solution Carbon adsorption cleanup Toluene Triton X-100 TEG Methanol Negative control Standards Aqueous sample diluent HRP competitor conjugate Repeater pippetor Chromogenic HRP substrate/hydrogen peroxide Stop solution Tube reader or spectrophotometer				
Were any supplies or equipment used that were not listed in the instructions? If so, please list.				
What are recommended hold times and storage conditions for: Samples? Extracts? Reagents? Standards?				

Question	Y	N	NA	Comments
Would you know based on the instructions provided (if not how did you decide): How much sample to extract?				
How many samples to extract in a "batch"?				
How much sodium sulfate to mix with sample?				
Which solvent /how much to extract with?				
How long to extract?				
How many negative controls and standards to prepare with "batch"?				
How to do the chemical oxidation cleanup?				
If additional cleanup was necessary?				
How to do additional cleanup?				
How long to incubate? (If measured what did you measure with?)				
What temperature to incubate?(If measured, what did you measure with?). How critical is incubation temperature?				
What to wash tubes with after incubation?				
How to remove unbound conjugate?				
How much chromogenic HRP substrate and hydrogen peroxide to add?				
How long do you let color develop before adding stop solution? (If time was measured, what did you use to measure with?)				
How quickly must you read the results after addition of the stop solution?				
How to use/measure with the tube reader/spectrophotometer? Is it calibrated? How?				
How much sample solution needs to be used with the tube reader/spectrophotometer?				
How do you calculate PCDD/PCDF amounts from the data generated?				

Must all procedures be completed in the same day? If not, when can procedure be stopped and how must samples be stored? Is that in the instructions?				
Did samples need, or would they ever need dilution for successful analysis? Would you know when and how to do this?				
Were any procedural steps performed differently than you interpreted from the instructions? Were any of the instructions confusing? If so please comment:				
What QC samples are required with this approach and at what frequency? What are recommended QC acceptance criteria? Did QC samples meet acceptance criteria? If not, is it clear what corrective action to take?				
What QC samples would vendor recommend, but not require and at what frequency?				
Do you recommend that some of the data be verified by conventional methods? What method? What frequency?				
How accurate d weights and volumes used with this technique have to be?				
Were all balances, pipettes, and thermometers calibrated?				

Following the procedure you just observed, including QC requirements, how many samples do you think you could process in a day? In a week?				
Does the vendor provide training in kit use? Is this extra charge? Video? Classes? Phone support?				
What education/experience would vendor recommend kit users have?				
What do you think would be required education/experience for successful operation of this technology?				
Additional Comments:				

Cape Technologies Operating Procedure

PCDD/Fs are typically extracted with organic solvents that are incompatible with EIA; therefore, a solvent exchange is required. PCDD/Fs have very low volatility and are retained during this solvent exchange in a small volume of a keeper solution (Triton X-100 detergent in TEG) after evaporation of the original solvent. Methanol is added to dilute this solution, and the methanol-TEG-Triton mixture is added directly to the EIA tubes. The solubility of PCDD/Fs in methanol is augmented significantly by adding TEG and Triton X-100. During the first EIA incubation, PCDD/Fs are specifically bound by the anti-dioxin antibodies, which have been immobilized on the EIA tube surface. After washing away the unbound material, the bound PCDD/Fs remain, and a competitor-HRP conjugate is added. Bound PCDD/Fs occupy the dioxin binding sites of the antibodies in proportion to the PCDD/F content of the sample and prevent binding of the competitor-HRP conjugate. After a short incubation, unbound conjugate is removed, and the test tubes are washed thoroughly. The amount of conjugate bound by the anti-dioxin antibody is inversely related to the amount of PCDD/Fs originally present in the sample. Finally, a solution of chromogenic HRP substrate and hydrogen peroxide is added to the test tubes. Color development is directly proportional to enzyme concentration and inversely related to the PCDD/F concentration in the original sample. The test tubes are analyzed using a tube reader or spectrophotometer to measure the OD. The OD values of unknown samples are compared to the OD values of standards to determine the level of PCDD/Fs in the samples.

To ensure accurate and reliable results, every effort should be made to perform the dioxin/furan immunoassay at temperatures between 20EC (68EF) and 25EC (77°F). The following sample preparation procedure was designed for same-day analysis at 500 pg/g, using a one-step cleanup. Other sample preparation options are available for lower concentrations. All sample extraction and extract cleanup components are in a kit form and are disposable.

1. Sodium sulfate is added to a soil sample and mixed. Dimethylformamide (DMF) is added to the soil sample, and the soil is extracted by shaking for two hours. The supernatant DMF extract is removed. DMF extracts are stable for weeks to months at room temperature.

2. Interferences are removed by chemical oxidation. Hexane is added to an aliquot of the DMF extract, then treated with 15% SO_3 in concentrated H_2SO_4 (fuming sulfuric acid). The supernatant hexane is removed and exchanged to a water-miscible organic solvent solution. This hexane-based fuming sulfuric acid cleanup is sufficient for most samples; but, in certain circumstances, an additional cleanup step may be required. This is the case for samples that contain large amounts of non-volatile aliphatic oils. When the DMF extracts of such soils are cleaned using fuming sulfuric acid, the oil is not oxidized; and it remains after evaporation of the hexane, causing a biphasic system when introduced to the EIA first incubation. Such EIA samples appear opalescent or milky, and their results will be invalid because the biphasic system prevents capture of analyte by the antibody. For these samples, a new aliquot of DMF extract is cleaned by carbon adsorption. In this case, the final solvent in the cleanup procedure is toluene rather than hexane.

3. The cleaned sample in hexane or toluene is exchanged to a water-miscible organic solvent solution for EIA analysis. PCDD/Fs have very low volatility and are retained during this solvent exchange in Triton X-100 detergent in TEG after evaporation of the original solvent. Methanol is added to dilute this solution, and the methanol-TEG-Triton mixture is added directly to the EIA tubes.

4. An accurately measured volume of negative control, standard, or prepared sample is mixed with an aqueous sample diluent in test tubes with anti-dioxin antibody immobilized on the surface, and the mixture is incubated.

5. After incubation, antibody tubes are washed, and 0.5 mL of HRP competitor conjugate is added to each tube using a repeater pipettor. Bound PCDD/Fs occupy the dioxin binding sites of the antibodies in proportion to the PCDD/F content of the sample and prevent binding of the competitor-HRP conjugate. After a short incubation, unbound conjugate is removed, and the test tubes are washed thoroughly.

6. A solution of chromogenic HRP substrate and hydrogen peroxide is added to the test tubes. Color development is directly proportional to enzyme concentration and inversely related to the PCDD/F concentration in the original sample. Stop solution is added to each tube using a repeater pipettor to fix the amount of color development.

7. The test tubes are analyzed using a tube reader or spectrophotometer to measure the OD at 450 nanometers. The test is interpreted by measuring the signal produced by a sample and determining the concentration from a dose-response curve constructed from standards tested at the same time.

Procedural Observations and Questionnaire
Of
Hybrizyme Corporation AhRC PCR™ Kit

Procedure witnessed by:

Date witnessed:

Time/Date procedure started:

Time/Date procedure ended:

Name of Kit Used:

Individuals Witnessed:

Lot Number of Kit:

Expiration Date of Kit:

Answer the following questions:

Question	**Y**	**N**	**NA**	**Comments**
Could this kit be performed in the field without a mobile lab/trailer?				
Would it take long to set up in the field before first samples could be processed? How long?				
How many samples could be prepared and analyzed in one day in the field once set up is complete? By an experienced kit user? By the novice kit user?				
Would sample throughput be faster in the lab than in the field? If so by how much?				
Are the instructions supplied with the kit the same as the operating procedure listed in the demo plan? If not, why? Use the kit instructions for evaluation.				

Question	Y	N	NA	Comments
Is there a recommended temperature for processing the test kits? If so, what? How was temperature measured? Was measuring device calibrated?				Actual Temp_____
Are the following equipment and reagents supplied with the kit? (Note if item not used at all, also note grade and supplier of solvents) Thermometer Methanol Activation solution Shaker Capture strips Assay buffer Capture reagent Primer/probe PCR wash concentrate Glass vials (rack with 96 vials) Distilled or DI water Automated plate washer Standards Multichannel pippetter 2x Universal Master Mix adhesive tape compression pads thermocycler				
Were any supplies or equipment used that were not listed in the instructions? If so, please list.				
What are recommended hold times and storage conditions for: Samples? Extracts? Reagents? Standards?				

Question	Y	N	NA	Comments
Would you know based on the instructions provided (if not how did you decide): How much sample to extract? How many samples to extract in a "batch"? How to extract and with what solvent? How long to extract? How many controls and standards to prepare with "batch"? Is extract cleanup necessary? If so what cleanups are a must and what are optional? How do you know when to use optional cleanups? Is the automated plate washer difficult to use? How much does an automated plate washer cost? Can the plates be washed manually? Would that be difficult? At what temperature does step 14 need to be completed? How long were samples shaken for step 14? (If measured what did you measure with?) At what temperature does step 17 need to be completed?				

Question	Y	N	NA	Comments
How long were samples shaken for step 17? (If measured what did you measure with?) At what temperature does step 18 need to be completed? How long were samples shaken for step 18? (If measured what did you measure with?) How long did the PCR wash step take? Did strips need to incubate before analyzing on thermocycler? If so how long and at what temperature? How quickly must you read the results after addition of the 1 X Master Mix? How to use/measure with the thermocycler? Is it calibrated? How? How much sample solution needs to be used with the thermocycler? How do you calculate PCDD/PCDF amounts from the data generated?				
Must all procedures be completed in the same day? If not, when can procedure be stopped and how must samples be stored? Is that in the instructions?				

Question	Y	N	NA	Comments
Did samples need, or would they ever need dilution for successful analysis? Would you know when and how to do this?				
Were any procedural steps performed differently than you interpreted from the instructions? Were any of the instructions confusing? If so please comment:				
What QC samples are required with this approach and at what frequency? What are recommended QC acceptance criteria? Did QC samples meet acceptance criteria? If not, is it clear what corrective action to take?				
What QC samples would vendor recommend, but not require and at what frequency?				
Do you recommend that some of the data be verified by conventional methods? What method? What frequency?				
How accurate d weights and volumes used with this technique have to be?				
Were all balances, pipettes, and thermometers calibrated?				
Following the procedure you just observed, including QC requirements, how many samples do you think you could process in a day? In a week?				

Question	Y	N	NA	Comments
Does the vendor provide training in kit use? Is this extra charge? Video? Classes? Phone support?				
What education/experience would vendor recommend kit users have?				
What do you think would be required education/experience for successful operation of this technology?				
Additional Comments:				

Hybrizyme Corporation Operating Procedure

The general steps for analyzing sample extracts are as follows:

1. Add 5 µL of sample extract in methanol to the activation solution, mix, and shake for 1 hour at room temperature.

2. Transfer to a capture strip Amicrowell@ and shake for 30 minutes at room temperature.

3. Wash the capture strip.

4. Add PCR mix.

5. PCR amplify.

Real-time PCR is analyzed by examining "primary growth curves" generated by fluorescent probes within newly synthesized DNA. For quantitative and comparative purposes, a threshold cycle (Ct) is defined for each sample. The threshold may be an arbitrary signal above background, or a certain number of standard deviations above background.

The approximate detection limit of the AhRC is less than 0.2 pg TCDD in the assay, according to Hybrizyme.

The AhRC PCR™ Kit includes

- Activation solution (12 vials, 0.5 mL each): Source of Ah receptor and DNA probe (store at -80oC).

- Capture strips (1 plate, 8 x 12 wells): Keep unused strips sealed and in the pouch (store at room temperature). Strip rack included.

- Assay buffer (1 bottle, 20 mL): Ready to use (store at room temperature for up to 2 weeks or at 4oC for extended periods of time).

- Capture reagent (red cap) (1 vial, 0.60 mL): A 16-fold concentrated solution (store at 4oC). Prepare for use by adding 40 L to 600 L of assay buffer.

- Primer/probe (white cap) (1 vial, 600 µL): Forward and reverse primers containing Taqman MGB FAM probe (aliquot, store at 20oC when not in use).

- PCR wash concentrate (25X) (2 bottles, 40 mL each): A 25-fold concentrated solution (store at 4oC). Prepare for use by mixing entire contents of one bottle with 960 milliliter (mL) of deionized water.

- Glass vials: Rack with 96 vials (flat-bottomed 0.5-mL glass vials with plate).

3.2.2 Operating Procedure

12. Reconstitute PCR wash solution to 1 X with distilled or deionized water for use with an automated plate washer. Prime the plate washer with PCR wash solution.

13. Prepare standards and unknowns in methanol.

14. Prepare the 1 X capture reagent by diluting 40 µL of stock reagent into 600 µL of assay buffer for each strip used. Place desired number of strips in the strip frame and re-seal the remainder in the foil pouch. Wash the strips using the "3XWASH" program of the plate washer. Using the multichannel pipetter, dispense 50 µL to each well in the strip. Shake 60 to 90 minutes.

15. Thaw the activation solution, mixing gently during the process. Do not allow the activation solution to remain at room temperature for more than 20 minutes prior to use. For best results, mix the activation solution vials together prior to dispensing when performing multiple strips.

16. Dispense 50 µL of the assay buffer into each glass vial using a multichannel pipette. Assay buffer should be at room temperature prior to use. Add 5 µL of standard or sample. The use of a pipetter (i.e., P-10 Pipetman) with a filter tip is preferred. After adding sample to the entire row, tap gently to mix. Repeat the process for each row of glass vials.

17. Add 50 µL of activation solution to each glass vial using a multichannel pipette. Shake for 1 hour at room temperature.

18. Wash the strips using the "3XWASH" program of the plate washer. Using a multichannel pipetter, transfer 30 µL of each reaction to each well. Shake 30 minutes.

19. 8. Wash the strips using the "PCRWash" program of the plate washer. This series of soaks and washes takes about 15 minutes. Thaw the primer/probe solution at this time.

20. Add 100 µL of primer/probe solution, 400 µL of water, and 500 µL of 2 X Universal Master Mix to make each 1,000 µL of 1 X Master Mix. Dispense 40 µL into each well.

21. Seal the wells with adhesive tape and cover with two compression pads. Insert the strips into thermocycler and run the "PCR" template.

22. Analyze the data.

Procedural Observations and Questionnaire
Of
Paracelsian, Inc., Ah-IMMUNOASSAY®

Procedure witnessed by:

Date witnessed:

Time/Date procedure started:

Time/Date procedure ended:

Name of Kit Used:

Individuals Witnessed:

Lot Number of Kit:

Expiration Date of Kit:

Answer the following questions:

Question	**Y**	**N**	**NA**	**Comments**
Could this kit be performed in the field without a mobile lab/trailer?				
Would it take long to set up in the field before first samples could be processed? How long?				
How many samples could be prepared and analyzed in one day in the field once set up is complete? By an experienced kit user? By the novice kit user?				
Would sample throughput be faster in the lab than in the field? If so by how much?				
Are the instructions supplied with the kit the same as the operating procedure listed in the demo plan? If not, why? Use the kit instructions for evaluation.				

Question	Y	N	NA	Comments
Is there a recommended temperature for using the kit? If so, what is it? How was temperature measured? Was measuring device calibrated?				Actual Temp_____
Are the following equipment and reagents supplied with the kit? (Note if item not used at all, also note grade and supplier of solvents) Refrigerator/Freezer Thermometer Cytosol DRE Oligo ARNT extract Activator Beakers Icebath 50-mL mixing tube ice DMSO Tubes ELISA plates TCDD reference standard NAP standard Multichannel pippettor 20X wash buffer reagent grade water AB1 AB diluent AB2 Detection tablets Detection buffer Paper towels Automated plate washer Plate reader Graph paper Curve fitting statistical software				

Question	Y	N	NA	Comments
Were any supplies or equipment used that were not listed in the instructions? If so, please list.				
What are recommended hold times and storage conditions for: Samples? Extracts? Reagents? Standards?				
Would you know based on the instructions provided (if not how did you decide): Which kit components are to be stored refrigerated and which are frozen? How many of the cytosol vials need to be thawed at once? Which mixtures are intermediates and need to be kept on an ice bath? How to decide how much DRE Oligo is needed for the amount of cytsol thawed? How long the does tube need to be rocked in step 5 for sufficient mixing? Is this done by hand or automated shaking? How to decide how much ARNT to add in step 6?				

Question	Y	N	NA	Comments
How long the does tube need to be rocked in step 6 for sufficient mixing? Is this done by hand or automated shaking?				
How to decide how much activator is needed for step 8?				
How long the does tube need to be rocked in step 9 for sufficient mixing? Is this done by hand or automated shaking?				
How are samples extracted?				
How much sample is used to extract?				
How many samples to extract in a "batch"?				
Which solvent and how much to extract with?				
How long to extract?				
Does the extract require any cleanup? If so, what?				
Does extract have to be solvent exchanged to DMSO?				
How many negative controls and standards to prepare with "batch"?				
When should the TCDD reference standard be used and when should the NAP standard be used?				

Question	Y	N	NA	Comments
Steps 11 through 14 says you can use four dilutions of 10 samples. What dilution levels would you use and why?				
How long were samples incubated for step 16? (If measured what did you measure with?)				
What temperature to incubate?(If measured, what did you measure with?). How critical is incubation temperature? How was temp controlled?				
How to prepare the 1X wash buffer?				
How to wash tubes after incubation?				
Was wash done by hand or with an automated washer? Which is easier to use? Faster?				
How to prepare the primary antibody stock?				
How long was plate incubated after adding primary antibody stock? What temperature was used?				
How to prepare the secondary antibody stock?				
How long was plate incubated after adding secondary antibody stock? What temperature was used?				

Question	Y	N	NA	Comments
How to prepare the detection reagent stock? How do you know how many tablets to dissolve and volume of detection buffer to use? How was this protected from light? Does it have to be stored protected from light? How long was plate incubated after adding detection reagent stock? What temperature was used? How was this protected from light? How critical is it that readings be taken at 15, 30, 45, and 60 minutes? Must these times be exact? Is the plate kept incubating between each reading? How to use/measure with the plate reader? Is it calibrated? How? How much sample solution needs to be used with the plate reader? How do you calculate PCDD/PCDF amounts from the data generated?				
Must all procedures be completed in the same day? If not, when can procedure be stopped and how must samples be stored? Is that in the instructions?				

Question	Y	N	NA	Comments
Did samples need, or would they ever need dilution for successful analysis? Would you know when and how to do this?				
Were any procedural steps performed differently than you interpreted from the instructions? Were any of the instructions confusing? If so please comment:				
What QC samples are required with this approach and at what frequency? What are recommended QC acceptance criteria? Did QC samples meet acceptance criteria? If not, is it clear what corrective action to take?				
What QC samples would vendor recommend, but not require and at what frequency?				
Do you recommend that some of the data be verified by conventional methods? What method? What frequency?				
How accurate do weights and volumes used with this technique have to be?				
Were all balances, pipettes, and thermometers calibrated?				
Following the procedure you just observed, including QC requirements, how many samples do you think you could process in a day? In a week?				

Question	Y	N	NA	Comments
Does the vendor provide training in kit use? Is this extra charge? Video? Classes? Phone support?				
What education/experience would vendor recommend kit users have?				
What do you think would be required education/experience for successful operation of this technology?				
Additional Comments:				

Paracelsian Operating Procedure

3.3.2 Operating Procedure

The following protocol is for an assay that uses all 96 wells in the 96-well version of the kit. Steps 18 through 21, describing the procedure for the three replicate washes following each incubation, can be automated by a commercial plate washer. Likewise, any residual fluid remaining in the plate wells can be completely removed by a properly adjusted plate washer, thereby eliminating the need for the manual blotting in step 31.

1. Remove the refrigerated components of the kit from cold storage and warm to room temperature.

2. Remove the cytosol components (cytosol, DRE Oligo, ARNT extract, activator) from frozen storage. Thaw up to four 7.5-mL vials of cytosol in a beaker of tepid water. Steps 3 through 9 will generate the activated cytosol. Keep all intermediate mixtures in an ice bath as much as possible.

3. Pool the thawed tubes of cytosol into the 50-mL mixing tube.

4. Add the required volume of DRE Oligo to the pooled cytosol. Return unused DRE Oligo to the freezer.

5. Mix by rocking the tube gently.

6. Add the required volume of ARNT to the cytosol, DRE Oligo mixture. Return unused ARNT to the freezer.

7. Mix by rocking the tube gently.

8. Add the required volume of activator to the cytosol, DRE Oligo, ARNT mixture. Return unused activator to the freezer.

9. Mix immediately (to avoid high local concentrations of salt) by rocking the tube gently. This mixture is the activated cytosol and must be kept on ice.

10. Add 10 L each of one reference standard, one negative control, and up to 10 samples in DMSO (the negative control) to 1.0-mL aliquots of the activated cytosol and mix gently. Tubes for these preparations are supplied by the user. These mixtures compose the treated stock for each type of determination.

11. The 96-well ELISA plate layout will accommodate one negative control, seven dilutions of the TCDD reference standard (or a single dilution of the NAP Standard), and four dilutions of 10 samples, all in duplicate. If less than 96 wells are used, return the unused ELISA strips to 4 C storage in the foil bag with desiccant.

12. Remove the ELISA plate from the desiccated plastic bag and add 400 L of each treated stock to the top row of the wells to give duplicate runs of each of the 10 samples and the single reference standard.

13. With a multichannel pipettor, add 200 L of activated cytosol to all the remaining wells. One of the kit reservoirs will facilitate loading the pipettor.

14. Beginning with the first sets of duplicate wells, make serial dilutions (seven dilutions for the TCDD reference or the single dilution of the NAP, four dilutions for each sample) down the respective plate columns. Individual dilutions are mixed by a minimum of six repetitive aspiration and redispensing cycles of the well contents. The residual waste following the final dilution must be collected for proper disposal.

15. Attach the lid to the plate.

16. Incubate the plate for two hours at 30 C. Incubation at room temperature (i.e., 20 C) is satisfactory, but will result in a slightly lower response. Incubation at a higher temperature (i.e., 37 C) will also result in a lower response.

17. Prepare the 1 X wash buffer by diluting the contents of the 20 X wash buffer bottle to 500 mL with reagent-grade water (250 mL for the 48-well kit). Store unused 1 X wash buffer at 4 C.

18. After the two-hour incubation, remove the well contents by aspiration into a waste receptacle for proper disposal.

19. Add 400 L 1 X wash buffer to all wells, loading the multichannel pipette from a kit reservoir.

20. Wait two minutes. Remove the well contents by aspiration into the waste receptacle for proper disposal.

21. Repeat Steps 19 and 20 twice for a total of three separate washes.

22. Prepare the primary antibody stock by aliquoting the required amount of AB 1 into the appropriate volume of AB diluent. Mix gently. Store unused AB 1 and AB diluent at 4 C.

23. Deliver 200 L AB 1 stock to all wells of the plate, loading the multichannel pipette from a kit reservoir.

24. Incubate the plate for one hour at 30 C.

25. After the one hour incubation, repeat steps 18 through 21.

26. Prepare the secondary antibody stock by aliquoting the required amount of AB 2 into the appropriate volume of AB diluent. Mix gently. Store unused AB 2 and AB diluent at 4 C.

27. Deliver 200 L AB 2 stock to all wells of the plate, loading the multichannel pipette from a kit reservoir.
28. Incubate the plate for one hour at 30 C.

29. After the one-hour incubation, repeat Steps 18 through 21.

30. Prepare the detection reagent stock by dissolving the required number of detection tablets in the appropriate volume of detection buffer with gentle mixing while protected from light. Allow a minimum of 15 minutes for the tablets to completely dissolve. Unused detection buffer and tablets are kept at 4 C.

31. Following the incubation of the final wash, strike the inverted plate against paper toweling to remove residual fluid before proceeding to the next step.

32. Deliver 200 L of the detection reagent stock from Step 30 to all wells of the plate, loading the multichannel pipette from a kit reservoir.

33. Incubate the plate at 30 C while protected from light. Residual moisture from condensation and/or from contact with incubator water must be carefully blotted from the plate before reading. The plate is read at 405 nanometers (nm) after 15, 30, 45, and 60 minutes. Variations in incubation temperatures and plate reader characteristics may dictate the best incubation times for individual conditions.

A standard curve is used to determine the amount of TCDD equivalents present in a sample. The standard curve is generated by plotting the average absorbance (OD) measured at 405 nm for each of the TCDD reference standards on the vertical (Y) axis versus the corresponding quantity (pg) of TCDD on the horizontal (X) axis. Results are calculated manually using graph paper or a curve-fitting statistical software package. The TCDD equivalents for the samples are determined from the standard curve by interpolating from the absorbance value (Y axis) to the quantity (pg) of TCDD (X axis). The initial TCDD concentration in the sample is found by correcting for the sample dilution. The supplied NAP solution, at 2 L per test well, may optionally be used as a single-point positive control that is equivalent to 32 pg TCDD.

Procedural Observations and Questionnaire
Of
WAKO Dioxin ELISA Kit

Procedure witnessed by:

Date witnessed:

Time/Date procedure started:

Time/Date procedure ended:

Name of Kit Used:

Individuals Witnessed:

Lot Number of Kit:

Expiration Date of Kit:

Answer the following questions:

Question	**Y**	**N**	**NA**	**Comments**
Could this kit be performed in the field without a mobile lab/trailer?				
How long would it take to set up in the field before first samples could be processed?				
How many samples could be prepared and analyzed in one day in the field once set up is complete? By an experienced kit user? By the novice kit user?				
Would sample throughput be faster in the lab than in the field? If so by how much?				
Are the instructions supplied with the kit the same as the operating procedure listed in the demo plan? If not, why? Use the kit instructions for evaluation.				

Question	Y	N	NA	Comments
Was testing carried out at kit recommended temperature of 20° C to 25° C? How was temperature measured? Was measuring device calibrated?				Actual Temp_____
Instructions say kit is to be stored at 2 to 10°C. How long can it be kept at room temperature before the kit is compromised?				
Are the following equipment and reagents supplied with the kit? How many of each are needed? What grade and supplier of solvents are used? Thermometer PC tube Methanol Stirring rod Sample solubilizer Tubes Tube Rack Acetone Buffer B Vortex mixer Centrifuge Primary antibody tube Purified water Paper towels Buffer A POD conjugate solution tube Scotch tape Refrigerator Parafilm Reaction mixture Wash solution concentrate Color developing solution Citrate buffer Aluminum foil Stop solution Pipettes Microplate reader				

Question	Y	N	NA	Comments
Were any supplies or equipment used that were not listed in the instructions? If so, please list.				
What are recommended hold times and storage conditions for: Samples? Extracts? Reagents? Standards?				
Would you know based on the instructions provided (if not how did you decide): How much sample to extract? How to extract/what sample pretreatment to use? Is any extract cleanup was necessary? If so, what? How many samples to include in a "batch"? How many controls and standards to prepare with "batch"? How to prepare the PC solutions?				

Question	Y	N	NA	Comments
How to prepare the primary antibody working solution?				
How to prepare the POD-conjugate solution?				
How long to leave tubes in ice water in step 15?				
What is the temperature of the refrigerator used to store the microplates in step 17?				
How long were the microplates actually refrigerated in step 17?				
How to prepare wash solution?				
How to prepare color developing solution?				
How long do you let color develop before adding stop solution? (If time was measured, what did you use to measure with?)				
How critical is it that reading be taken within 15 minutes of adding stop solution?				
How to use/measure with the tube reader/spectrophotometer?				
How much sample solution needs to be used with the tube reader/spectrophotometer?				
How do you calculate PCDD/PCDF amounts from the data generated?				

Question	Y	N	NA	Comments
Must all procedures be completed in the same day? If not, when can procedure be stopped and how must samples be stored? Is that in the instructions?				
Did samples need, or would they ever need dilution for successful analysis? Would you know when and how to do this?				
Were any procedural steps performed differently than you interpreted from the instructions? Were any of the instructions confusing? If so please comment:				
What QC samples are required with this approach and at what frequency? What are recommended QC acceptance criteria? Did QC samples meet acceptance criteria? If not, is it clear what corrective action to take?				
What QC samples would vendor recommend, but not require and at what frequency?				
How accurate do weights and volumes used with this technique have to be?				
Were all balances, pipettes, and thermometers calibrated?				

Question	Y	N	NA	Comments
Following the procedure you just observed, including QC requirements, how many samples do you think you could process in a day? In a week?				
Do you recommend that some of the data be verified by conventional methods? What method? What frequency?				
Does the vendor provide training in kit use? Is this extra charge? Video? Classes? Phone support?				
What education/experience would vendor recommend kit users have?				
What do you think would be required education/experience for successful operation of this technology?				
Additional Comments:				

WAKO Dioxin ELISA Kit Operating Procedure

The Dioxin ELISA Kit should be used at room temperature (20E to 25EC) and stored at 2E to 10EC.

1. To prepare the PC solution (5,000 pg/mL), add 2.0 mL of methanol to the tube containing the PC. Then, gently stir the tube four or five times, and leave it at room temperature for 10 minutes. Gentle stirring is repeated once more before use. Do not vigorously shake or vortex the solution to avoid PC material adhering to the tube wall. The solution can be used within four weeks at 2E to 10EC after reconstitution.

2. Prepare the dilution solution by dispensing 2 mL of sample solubilizer in a tube and adding 2 mL of methanol.

3. Prepare 1/10-fold diluted PC solution (500 nanograms/mL) by adding 900 µL of dilution solution to a disposable culture tube rinsed with acetone and dried. Then add 100 µL of concentrated PC solution and gently stir the tube two or three times. Leave the tube at room temperature. The solution can be used after pipetting it up and down two or three times.

4. Prepare PC dilution solutions for the standard curve using the 1/10-fold diluted PC solution described above as follows:

Tube Number:	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Concentration	0 pg/mL	40 pg/mL	100 pg/mL	250 pg/mL	500 pg/mL	1,000 pg/mL	2,500 pg/mL
Dilution solution	500 µL	460 µL	400 µL	250 µL	450 µL	400 µL	250 µL
Concentrated PC solution	-	-	-	-	50 µL	100 µL	250 µL
1/10-fold diluted PC solution	0 µL	40µL	100µL	250µL	-	-	-
Total volume	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL

5. Dry the contents of each tube.

6. To prepare a dilution series of PC solutions, add 500 µL of Buffer B to each tube, lightly stir it with a vortex mixer, and centrifuge it at 2,000 revolutions per minute (rpm) at room temperature to collect the solution at the bottom.

7. To prepare the primary antibody stock solution, add 200 µL of purified water to the tube and gently stir it, then leave the tube for 10 minutes at room temperature. The solution can be used within four weeks.

8. Rinse a beaker or a 5- to 10-mL vial with acetone and air dry on clean paper towels.

9. Prepare the primary antibody working solution using a 100-fold dilution of the primary antibody stock solution described above with Buffer A according to assay sample numbers and volumes. Stir the diluted solution gently several times, and leave it at room temperature for 10 minutes. Stir the solution several times just before use. The solution cannot be stored prior to use.

10. Prepare the POD-conjugate solution by adding 4 mL of Buffer A to the tube and gently stir several times. Leave the tube at room temperature for 10 minutes. Gently stir the tube just before use. The solution can be used within two weeks when stored at 2E to 10EC.

11. Fix the necessary number of microplate wells (coated with the secondary antibody) to a microplate holder with Scotch tape. Place the holder in a closed box, and store it in a refrigerator for at least 30 minutes before use.

12. Add 100 µL of Buffer B to the tube containing the material obtained by sample pretreatment, and gently stir the solution along the inner surface of the tube several times with vortex mixer. Briefly centrifuge the tube at 2,000 rpm for five minutes to collect the solution at the bottom.

13. Line up the tubes containing the dilution series of PC solutions and the samples resolved in Buffer B in a tube rack. Dispense an equal volume of the primary antibody working solution to each tube.

14. Immediately, agitate the solutions several times, then seal the tubes with Parafilm and leave them at room temperature for 30 minutes.

15. During the incubation, prepare chilled water containing ice in a water bath and sink the tubes under the water in the bath. After the 30 minute-incubation at room temperature, add 250 µL and 50 µL of POD-conjugate solution to the tubes containing the diluted PC solutions and those containing sample material, respectively.

16. Gently agitate the tubes several times, and leave them for 10 minutes. Avoid vigorous shaking with the vortex because dioxins may adsorb to the tube wall.

17. Take the microplate holder from the box stored in a refrigerator. Dispense 100 µL of the reaction mixture to the wells. Briefly agitate the solutions in the wells. Seal the wells with Parafilm, return the holder to the box, and store the box in a refrigerator at 2E to 10EC for 18 to 20 hours.

18. On the second day, prepare the wash solution by diluting the wash solution concentrate (x 6) with five volumes of purified water. The solution volume prepared can be adjusted depending upon the number of wells used. Usually, one well needs 1.2 mL of the wash solution. The solution can be used within two weeks after dilution when stored at 2E to 10EC.

19. Prepare the necessary volume of color-developing solution by mixing the substrate and the citrate buffer in a ratio of 1:50 just before use. (The tube used in this preparation should be cleaned with purified water.) Add the citrate buffer to the tube, then the substrate. Avoid vigorous agitation of the mixed solution because it may cause crystalization.

20. Take the well holder from the box stored in a refrigerator, and remove the seal from the wells. Remove the reaction mixture from the wells, being careful not to spread the solution to the holder. Lightly tap the holder upside down on sheets of paper to remove the residual mixture in the wells. Dispense approximately 0.35 mL of wash solution to each well, and then drain the solution, being careful not to spread the solution to the holder. Remove the solution from the well walls by tapping the holder upside down on the papers. Repeat washing twice. At the last washing, remove the solution by tapping as much as possible.

21. For the enzyme reaction, dispense 100 µL of the color-developing solution to all the wells. Seal with Parafilm, cover the holder with aluminum foil to interrupt light, and leave the holder at room temperature for 30 minutes. Stop the reaction by adding 100 µL of stop solution in the same order as the color-developing solution was added. Tap the side of the holder to mix the solution. Read the signal with a microplate reader at 450 nm or at 450/650 nm within 15 minutes after stopping the reaction. Construct the standard curve obtained with the diluted PC solutions and estimate the dioxin concentrations of samples as 3,7,8-TCCD. The primary antibody used in this kit reacts with 2,3,7,8-TCCD and 3,7,8-TCCD with equal intensity.

22. In estimating the dioxin concentration, it is useful to plot the obtained values with a regression of polynomial quadratic equation or four-parameter logic after log-logit conversion.

Procedural Observations and Questionnaire
Of
Xenobiotics Detection Systems, Inc. CALUX®

Procedure witnessed by:

Date witnessed:

Time/Date procedure started:

Time/Date procedure ended:

Name of Kit Used:

Individuals Witnessed:

Lot Number of Kit:

Expiration Date of Kit:

Answer the following questions:

Question	**Y**	**N**	**NA**	**Comments**
Is this product intended for use by anyone other than XDS?				
Could this kit be performed in the field without a mobile lab/trailer?				
Would it take long to set up in the field before first samples could be processed? How long?				
How many samples could be prepared and analyzed in one day in the field once set up is complete? By an experienced kit user? By the novice kit user?				
Would sample throughput be faster in the lab than in the field? If so by how much?				

Question	Y	N	NA	Comments
Are the instructions supplied with the kit the same as the operating procedure listed in the demo plan? If not, why? Use the kit instructions for evaluation.				
Is there a recommended temperature for carrying out these analyses? If so, what? How was temperature measured? Was measuring device calibrated?				Actual Temp_____
Are the following equipment and reagents supplied with the kit? (Note if item not used at all, also note grade and supplier of solvents) Thermometer Glass vials with PTFE-lined caps Methanol Toluene Ultrasonic water bath Filter Vacuum centrifuge Hexane DMSO Cell culture medium 96-well culture plates 2,3,7,8-TCDD standard curve humidified CO2 incubator microscope Promega luciferase assay kit				

Question	Y	N	NA	Comments
Were any supplies or equipment used that were not listed in the instructions? If so, please list.				
What are recommended hold times and storage conditions for: Samples? Extracts? Reagents? Standards?				
Would you know based on the instructions provided (if not how did you decide): How much sample to extract? How many samples to extract in a "batch"? How to dry the sample? Which solvent and how much to extract with? How long to extract? How many controls and standards to prepare with "batch"?				

During extraction is there a recommended temperature for the ultrasonic water bath? How are extracts filtered? How much are they concentrated by vacuum centrifugation? If cleanup is proprietary, how does kit user know what to do? After cleanup, how is extract concentrated into DMSO? How do you suspend the DMSO/extract concentrate in the cell culture medium? How do you prepare the 96-well culture plates? How long do the plates incubate in the humidified CO2 incubator? Is this step temperature sensitive? What humidity range is acceptable? How do you tell if the cells are microscopically viable? How do you use the Promega luciferase assay kit to quanitify? Is it clear how this converts to dioxin and PCB TEQ? Do cells have to be read within a certain amount of time from incubation? How critical is this? How much sample solution needs to be used with the detection technique?				

Must all procedures be completed in the same day? If not, when can procedure be stopped and how must samples be stored? Is that in the instructions?				
Did samples need, or would they ever need dilution for successful analysis? Would you know when and how to do this?				
Were any procedural steps performed differently than you interpreted from the instructions? Were any of the instructions confusing? If so please comment:				
What QC samples are required with this approach and at what frequency? What are recommended QC acceptance criteria? Did QC samples meet acceptance criteria? If not, is it clear what corrective action to take?				
What QC samples would vendor recommend, but not require and at what frequency?				
Do you recommend that some of the data be verified by conventional methods? What method? What frequency?				
How accurate do weights and volumes used with this technique have to be?				

Were all balances, pipettes, and thermometers calibrated?				
Following the procedure you just observed, including QC requirements, how many samples do you think you could process in a day? In a week?				
Does the vendor provide training in kit use? Is this extra charge? Video? Classes? Phone support?				
What education/experience would vendor recommend kit users have?				
What do you think would be required education/experience for successful operation of this technology?				
Additional Comments:				

Xenobiotics Detection Systems, Inc. CALUX ® Operating Procedure

3.6.2 Operating Procedure

Xenobiotic Detection Systems, Inc. has a patented genetically engineered cell line (mouse hepatoma H1L1) that contains the gene for firefly luciferase under transactivational control of the AhR. This cell line can be used for the detection and relative quantification of a sample's total dioxin I-TEQ. Using a patent pending sample processing procedure, it is also possible to use the CALUX® by Xenobiotic Detection Systems assay to estimate the I-TEQ contributions of PCDDs/Fs or the I-TEQ contributions of the coplanar PCBs. The assay that uses this cell line is called the Chemical-Activated Luciferase Expression or CALUX® by Xenobiotic Detection Systems assay.

The samples are extracted using a modification of the EPA 8290 extraction method. Briefly, the dried samples are ground and 1-g aliquots are placed in solvent-cleaned glass vials with polytetrafluoroethylene-lined caps. The sample is extracted with a 20% solution of methanol in toluene then twice with toluene. During each extraction step, the samples are incubated in an ultrasonic water bath. The three extracts from each sample are filtered, pooled, and concentrated by vacuum centrifugation. The sample extract is suspended in hexane and prepared for the bioassay by a proprietary cleanup method. The eluate from the cleanup method is concentrated under vacuum into DMSO. The DMSO solution is used to dose the genetically engineered cells in the CALUX® by Xenobiotic Detection Systems assay.

Prior to dosing the cells, the sample extracts in DMSO are suspended in cell culture medium. This medium is then used to expose monolayers of the H1L1 cell line grown in 96-well culture plates. In addition to the samples, a standard curve of 2,3,7,8-TCDD is assayed (161, 80.5, 40.2, 20.1, 10.1, 5.0, 2.5, 1.2, and 0.6 ppt TCDD). The plates are incubated for a time to produce optimal expression of the luciferase activity in a humidified CO2 incubator. Following incubation, the medium is removed and the cells are examined microscopically for viability. The induction of luciferase activity is quantified using the luciferase assay kit from Promega.

APPENDIX F

AXYS ANALYTICAL SERVICES STATEMENT OF WORK

Statement of Work
AXYS Analytical Services Ltd.
SITE Demonstration for Dioxin Monitoring and Measurement Technologies

AXYS Project Manager: Georgina Brooks
Phone: 250-655-5800
Email: gbrooks@axys.com

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Battelle Memorial Institute is conducting a field demonstration of dioxin monitoring and measurement technologies under the U.S. Environmental Protection Agency's (EPA) Superfund Innovative Technology Evaluation (SITE) Program. In April 2004, developers of innovative dioxin measurement technologies will gather at a field demonstration site in Saginaw, MI to analyze dioxin-contaminated soil, sediment, and extract samples. A reference laboratory will be concurrently analyzing replicate splits of the samples by high resolution mass spectrometry (HRMS). The reference laboratory, AXYS Analytical Services, Ltd. (Sidney, British Columbia, Canada), will analyze samples for 17 dioxin/furans and the 12 World Health Organization dioxin-like polychlorinated biphenyl (WHO PCB) congeners in 13 soil/sediment samples and 2 extracts for the pre-demonstration phase of the project and 186 soil/sediment samples and 23 extracts in the demonstration phase of the project. AXYS will also be responsible for the preparation of the extract samples.

The purpose of this statement of work is to describe in detail the analytical services to be procured from AXYS for this project. AXYS will provide reference analytical measurements for dioxin/furans (D/F) using EPA Method 1613B and for the 12 WHO PCBs using EPA Method 1668A. The congeners to be reported and their associated toxic equivalency factors (TEFs) are provided in Table 1. The following is a list of the major tasks that will be completed by AXYS.

Task 1: D/F and PCB Analysis. Approximately 200 soil/sediment samples will be provided by Battelle to AXYS for analysis by both 1613B and 1668A. AXYS will receive ~13 samples as soon as this purchase order is in place (pre-demonstration phase), and the remaining 186 samples in mid-April (demonstration phase). AXYS will also generate and analyze 25 extracts in Tasks 2 and 3 below for 1613B and 1668A analysis. Two extracts will be prepared and analyzed immediately after this purchase order is in place (pre-demonstration phase). Twenty-three extracts will be prepared and analyzed in late April (demonstration phase).

Task 2: Soil Extract Preparation. Battelle will provide AXYS with approximately nine100 g soil samples. AXYS will extract each 100 g sample according to Dean Stark extraction procedure for 1613B, with the exception of surrogate spiking which will be spiked (after aliquot splitting step) into the AXYS aliquot only. AXYS will split the extract into 11 equal aliquots. Each aliquot will be concentrated to 10 mL and put in a heat-sealed ampoule. Each ampoule will be labeled per Battelle's instructions. Six of the 11 ampoules will be shipped by Federal Express according to Battelle's directions. One

Table 1. Congeners to be Reported and Toxicity Equivalency Factors (TEF) Values

Compound[(a)]	TEF Value		Compound	TEF Value	
	I-TEF	WHO		I-TEF	WHO
PCDDs			**PCDFs**		
2,3,7,8-TCDD	1	1	2,3,7,8-TCDF	0.1	0.1
1,2,3,7,8-PeCDD	0.5	1	1,2,3,7,8-PeCDF	0.05	0.05
			2,3,4,7,8-PeCDF	0.5	0.5
1,2,3,4,7,8-HxCDD	0.1	0.1	1,2,3,4,7,8-HxCDF	0.1	0.1
1,2,3,6,7,8-HxCDD	0.1	0.1	1,2,3,7,8,9-HxCDF	0.1	0.1
1,2,3,7,8,9-HxCDD	0.1	0.1	1,2,3,6,7,8-HxCDF	0.1	0.1
			2,3,4,6,7,8-HxCDF	0.1	0.1
1,2,3,4,6,7,8-HpCDD	0.01	0.01	1,2,3,4,6,7,8-HpCDF	0.01	0.01
			1,2,3,4,7,8,9-HpCDF	0.01	0.01
OCDD	0.001	0.0001	OCDF	0.001	0.0001
Dioxins-like PCBs					
co-planar			**mono-*ortho***		
3,3',4,4'-TCB (PCB 77)	0.0005	0.0001	2,3,3',4,4'-PeCB (PCB 105)	0.0001	0.0001
3,4,4',5-TCB (PCB 81)	-	0.0001	2,3,4,4',5-PeCB (PCB 114)	0.0005	0.0005
3,3',4,4',5-PeCB (PCB 126)	0.1	0.1	2,3,'4,4',5-PeCB (PCB 118)	0.0001	0.0001
3,3',4,4',5,5'-HxCB (PCB 169)	0.01	0.01	2,3,4,4',5-PeCB (PCB 123)	0.0001	0.0001
			2,3,3',4,4',5-HxCB (PCB 156)	0.0005	0.0005
			2,3,3',4,4',5-HxCB (PCB 157)	0.0005	0.0005
			2,3',4,4',5,5'-HxCB (PCB 167)	0.00001	0.00001
			2,3,3',4,4'5,5'-HpCB (PCB 189)	0.0001	0.0001

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

ampoule each will be analyzed by AXYS by 1613B and 1668A. Three ampoules will be archived. AXYS will repeat this procedure for all nine soil samples. One soil will be extracted for the pre-demonstration phase and eight soils will be extracted for the demonstration phase.

Task 3: Spiked Extract Preparation. AXYS will provide spiked extracts prepared in the same solvent (toluene) as the soil extracts in Task 2. AXYS will prepare four sets of spiked extracts. The details for these samples are described in Table 2. Spike #0 will be

prepared for the pre-demonstration phase (by ~March 23), while Spike #1, 2, and 3 will be prepared for the demonstration phase (by April 21).

Table 2. Summary of spiked extracts

Spike #	No. of ampoules	Sample Description	No. to be analyzed by AXYS using 1613B and 1668A	No. to be shipped by AXYS according to Battelle-provided address(es)	No. to be archived by AXYS
0	11	100 pg/mL TCDD	1, 1	6	3
1	60	0.5 pg/mL TCDD	7	42	11
2	35	100 pg/mL TCDD and 1,000 pg/mL all 12 WHO PCBs	4	24	7
3	35	10,000 pg/mL all 12 WHO PCBs	4	24	7

Task 4: Technical Support/Project Management. AXYS will provide written text describing the extraction, clean-up, analysis, quality control checks, data reporting, data review, and data packaging procedures for the project demonstration plan. AXYS will also participate in project conference calls as requested. AXYS will also host an on-site audit while the demonstration samples are being analyzed. Battelle anticipates that this audit will occur in mid-May, around the time when the first batch of data are being finalized and reported.

Project Requirements

The following are technical requirements for this project that AXYS is expected to follow. Any deviation from these requirements should be discussed in advance with Battelle's project manager.

- AXYS's standard electronic data deliverable (EDD) will be delivered to Battelle's project manager by email, at no additional charge, by the due date committed by AXYS in Table 3. A complete, auditable data package is also required for each sample. The paginated data package will be delivered to the Battelle project manager by hard copy and CD within 5 days of the EDD being delivered. The narrative for the data package will include the sample extraction weight for each sample.

- Final reporting deliverable turn around time for the pre-demonstration samples will be 21 days. Data packages for the demonstration samples will be delivered to Battelle according to the schedule in Table 3 (at 1-week intervals starting with Week 4 of the project). Final reporting deliverable turn around time for the demonstration samples will be no more than 90 days from receipt of samples.

Table 3. AXYS Anticipated Reporting Schedule

Date [a]	Start D/F	Start WHO PCB	Report D/F	Report WHO PCB
Mar-22	One soil extract and one spiked extract prepared and distributed to participants			
Apr-7	Pre-demo results reported for D/F and PCBs for 13 soils, one soil extract, one spiked extract			
Apr-19	Group1 20 samples	group1 20 samples		
Apr-21	Soil and spiked extracts distributed to location specified by Battelle			
Apr-26	Group2 20 samples	group2 20 samples		
May-03	Group3 20 samples	group3 20 samples		
May-10	Group4 20 samples	group4 20 samples		
May-17	Group5 20 samples	group5 20 samples	group1	group1
May-24	Group6 20 samples	group6 20 samples	group2	group2
May-31	Group7 20 samples	group7 20 samples	group3	group3
Jun-07	Group8 20 samples	group8 20 samples	group4	group4
Jun-14	Group9 20 samples	group9 20 samples	group5	group5
Jun-21	group 10 20 samples	group 10 20 samples	group6	group6
Jun-28			group7	group7
Jul-05			group8	group8
Jul-12			group9	group9
Jul-19			group 10	group 10

[a] *Date may shift, depending on when samples are received at AXYS.*

- Dean Stark extraction will be used for the dioxin/furan analysis. Regular soxhlet extraction will be used for the PCB analysis.
- Percent moisture measurements do not need to be performed.
- A six-point initial calibration, including a low level calibration point (at 0.2 ng/mL), will be performed for 1613B. The standard five-point calibration will be performed for 1668A.
- All sample processing, clean-up, analysis, and reporting will follow EPA Method 1613B and 1668A except for the method modifications defined in the AXYS's standard operating procedures (SOPs).
- The data will be reported as both concentration (for each target analyte), total concentration, TEQ (for each target analyte), and total TEQ. TEQ will be calculated and reported two ways: using both the WHO TEFs (listed in Table 1) and using both a value of zero and ½ the detection limit when the analyte is not detected. The extract samples are to be reported in pg/mL TEQ.
- The static mass resolution check will be demonstrated every 12 hours.

- The following will be standard with each analytical batch. Results for quality control (QC) samples must meet method/SOP requirements.
    - No more than 20 samples will be included in each batch.
    - One procedural blank will be included in each batch.
    - One demonstration sample, of AXYS's choosing, will be analyzed in duplicate with each batch.
    - Surrogates will be spiked in each sample, except for the soil and spiked extracts that are prepared for the participants.
    - A spiked matrix (OPR or SRM) will be analyzed and reported with each batch.
    - Daily calibration check will occur every 12 hours.
    - A column carryover solvent blank will be run after each calibration standard to demonstrate instrumental carry over rate. Sample responses will be evaluated using the demonstrated instrumental carryover information to ensure that results are not significantly affected by carryover from a previously injected sample of higher concentration. Where the data indicates this may have occurred the sample in question will be re-injected.
- The pre-demonstration samples will be analyzed completely blind by AXYS. For the demonstration samples, Battelle will identify which sites the samples are from, and AXYS can refer to the pre-demonstration results so that congener patterns and approximate dilutions can be estimated. Battelle will also identify which samples are anticipated to contain high levels (> 10,000 pg/g) so that AXYS can take pre-cautionary measures to deal with these samples.
- AXYS will report sample specific detection limits, as specified in their response to the SITE demonstration questionnaire. AXYS will flag any low level detections that are lower than the lowest calibration standard.
- A carbon column cleanup step to isolate toxic PCBs will be performed and the DB-1 column will be used as the primary column.
- Secondary column confirmation for 2,3,7,8-TCDF will be performed for every sample.
- Analytical data generated by AXYS for this project will be backed-up on a weekly basis. The data shall remain on the instruments (or, at a minimum, readily accessible) until the reports for this project are finalized in December 2004.
- All project files and supporting data will be archived for a minimum of 5 years.

APPENDIX G

QUESTIONNAIRE FOR REFERENCE LABORATORY SELECTION

Inquiry Regarding Participation in EPA SITE Demonstration Program

Statement of Work

The purpose of this inquiry is to identify laboratories that are interested in providing dioxin analyses in support of the US Environmental Protection Agency (EPA) Superfund Innovative Technology Evaluation (SITE) Program. In April 2004, developers of innovative dioxin monitoring and measurement technologies will gather at a field demonstration site in Saginaw, MI to analyze approximately 200 dioxin-contaminated soil and sediment samples. A reference laboratory will be concurrently analyzing a replicate split of the samples by Method 1613B for the 17 dioxin/furan congeners and by EPA Method 1668A for the 12 World Health Organization PCBs, with concentrations ranging from < 1 pg/g to > 10,000 pg/g. The data generated during the field demonstration will be used to prepare Innovative Technology Evaluation Reports for each of the participating technologies. Each report will be a comprehensive evaluation of the technology's ability to measure dioxin in contaminated soil and sediment, including a comparison to the HRMS method results, so the selection of a laboratory to perform these analyses is a critical step in the process.

If you are interested in being considered for selection, please answer the following questions and provide your answers to Battelle Memorial Institute, who is the contractor that is conducting the demonstration for EPA. Responses are to be submitted by email or fax no later than **Tuesday, December 23, 2003** to Amy Dindal, dindala@battelle.org, phone: 561-422-0113, fax: 561-258-0777.

1) List all dioxin and PCB methods that you routinely perform. State whether you are running modifications of methods and note what the modifications are. List any certifications that you hold for performing each analysis, as applicable. List the approximate number of analyses your lab performed by each method in 2003. Also provide a quote on a per sample basis if you were to receive 15 soil samples in one batch in February 2004 and 200 soil samples in one batch in March 2004 for analysis by those methods.

EPA Method	Modifications (Y/N - if Y explain on separate sheet)	Certifications (Y/N - if Y please attach)	Number of analyses in 2003	Quote for 215 sample analyses

2) List the make, model, age, and number of analytical instruments that would be available for use in this study.

3) Describe how many samples will constitute a sample batch and the associated quality control samples that will be analyzed with each batch.

4) Define the congeners and concentrations in the lowest calibration standard typically analyzed in your lab.

5) Describe how you determine the method detection limit with and without background present. Be sure to include the size of sample required and list the detection limits on a congener specific basis.

6) List the most recent EPA contracts you held for the analysis of dioxin in soil or sediment and how many samples were analyzed for each contract.

7) Will you accept high level samples (> 10,000 pg/g D/F; > 100 ppm PCB)? If so, does the quote provided in the table include dilution/re-analysis or re-extraction with smaller amounts to accommodate higher level samples, or will there be an additional fee?

8) Are you willing to have a representative of your laboratory serve on the project's advisory panel? This would include participating in conference calls and reviewing documents (10-20 hours). Would there be an additional charge for this service or would this be included as part of the project participation?

9) Describe your process for data review from the time the data is generated until it is reported to the client (e.g., what percentage is reviewed by the analyst, QA officer, manager, etc.).

10) What would you need/want to know about a sample prior to analysis?

11) What is your current sample load for these methods? Could you complete the 15 samples received in February within 21 days? Could you complete 200 samples received in March within 160 days of receipt?

12) Are you willing to run three PE samples and host an onsite audit of your laboratory in January? Can you provide the results and a data package for these samples within14 days of receipt? Would you be willing to do these analyses free of charge and, if not, what would be the cost per sample?

13) Would you provide a representative data package for review prior to receiving the PE samples?

NAME OF PERSON COMPLETING THE FORM:

TITLE:

DATE:

APPENDIX H

METHOD 1613B and METHOD 1668A TABLES

Note: All tables are reproduced verbatim from the methods and do not represent AXYS modifications.

TABLE 3. CONCENTRATION OF STOCK AND SPIKING SOLUTIONS CONTAINING CDDS/CDFS AND LABELED COMPOUNDS

CDD/CDF	Labeled Compound Stock Solution [1] (ng/mL)	Labeled Compound Spiking Solution [2] (ng/mL)	PAR Stock Solution [3] (ng/mL)	PAR Spiking Solution [4] (ng/mL)
2,3,7,8-TCDD	—	—	40	0.8
2,3,7,8-TCDF	—	—	40	0.8
1,2,3,7,8-PeCDD	—	—	200	4
1,2,3,7,8-PeCDF	—	—	200	4
2,3,4,7,8-PeCDF	—	—	200	4
1,2,3,4,7,8-HxCDD	—	—	200	4
1,2,3,6,7,8-HxCDD	—	—	200	4
1,2,3,7,8,9-HxCDD	—	—	200	4
1,2,3,4,7,8-HxCDF	—	—	200	4
1,2,3,6,7,8-HxCDF	—	—	200	4
1,2,3,7,8,9-HxCDF	—	—	200	4
2,3,4,6,7,8-HxCDF	—	—	200	4
1,2,3,4,6,7,8-HpCDD	—	—	200	4
1,2,3,4,6,7,8-HpCDF	—	—	200	4
1,2,3,4,7,8,9-HpCDF	—	—	200	4
OCDD	—	—	400	8
OCDF	—	—	400	8
$^{13}C_{12}$-2,3,7,8-TCDD	100	2	—	—
$^{13}C_{12}$-2,3,7,8-TCDF	100	2	—	—
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	2	—	—
$^{13}C_{12}$-1,2,3,7,8-PeCDF	100	2	—	—
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	2	—	—
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	2	—	—
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	2	—	—
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	2	—	—
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	2	—	—
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	2	—	—
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	100	2	—	—
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	2	—	—
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	2	—	—
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	2	—	—
$^{13}C_{12}$-OCDD	200	4	—	—

TABLE 3. CONCENTRATION OF STOCK AND SPIKING SOLUTIONS CONTAINING CDDS/CDFS AND LABELED COMPOUNDS

CDD/CDF	Labeled Compound Stock Solution [1] (ng/mL)	Labeled Compound Spiking Solution [2] (ng/mL)	PAR Stock Solution [3] (ng/mL)	PAR Spiking Solution [4] (ng/mL)
	Concentration (ng/mL)			
Cleanup Standard [5]				
$^{37}Cl_4$-2,3,7,8-TCDD	0.8			
Internal Standards [6]				
$^{13}C_{12}$-1,2,3,4-TCDD	200			
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	200			

[1] Section 7.10—prepared in nonane and diluted to prepare spiking solution.
[2] Section 7.10.3—prepared in acetone from stock solution daily.
[3] Section 7.9—prepared in nonane and diluted to prepare spiking solution.
[4] Section 7.14—prepared in acetone from stock solution daily.
[5] Section 7.11—prepared in nonane and added to extract prior to cleanup.
[6] Section 7.12—prepared in nonane and added to the concentrated extract immediately prior to injection into the GC (Section 14.2).

TABLE 4. CONCENTRATION OF CDDS/CDFS IN CALIBRATION AND CALIBRATION VERIFICATION SOLUTIONS [1] (section 15.3)

	CDD/CDF	CS2 (ng/mL)	CS3 (ng/mL)	CS4 (ng/mL)	CS5 (ng/mL)
2,3,7,8-TCDD	0.5	2	10	40	200
2,3,7,8-TCDF	0.5	2	10	40	200
1,2,3,7,8-PeCDD	2.5	10	50	200	1000
1,2,3,7,8-PeCDF	2.5	10	50	200	1000
2,3,4,7,8-PeCDF	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDD	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDD	2.5	10	50	200	1000
1,2,3,4,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,7,8,9-HxCDF	2.5	10	50	200	1000
2,3,4,6,7,8-HxCDF	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDD	2.5	10	50	200	1000
1,2,3,4,6,7,8-HpCDF	2.5	10	50	200	1000
1,2,3,4,7,8,9-HpCDF	2.5	10	50	200	1000
OCDD	5.0	20	100	400	2000
OCDF	5.0	20	100	400	2000
$^{13}C_{12}$-2,3,7,8-TCDD	100	100	100	100	100
$^{13}C_{12}$-2,3,7,8-TCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8-PeCDD	100	100	100	100	100
$^{13}C_{12}$-PeCDF	100	100	100	100	100
$^{13}C_{12}$-2,3,4,7,8-PeCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	100	100	100	100	100
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	100	100	100	100	100
$^{13}C_{12}$-OCDD	200	200	200	200	200
Cleanup Standard					
$^{37}Cl_4$-2,3,7,8-TCDD	0.5	2	10	40	200
Internal Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	100	100	100	100	100
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	100	100	100	100	100

TABLE 8. DESCRIPTORS, EXACT M/Z's, M/Z TYPES, AND ELEMENTAL COMPOSITIONS OF THE CDDs AND CDFs

Descriptor	Exact M/Z [1]	M/Z Type	Elemental Composition	Substance [2]
	392.9760	Lock	C_9F_{15}	PFK
	401.8559	M+2	$^{13}C_{12}H_2{}^{35}Cl_5{}^{37}ClO_2$	HxCDD [3]
	403.8529	M+4	$^{13}C_{12}H_2{}^{35}Cl_4{}^{37}Cl_2O_2$	HxCDD [3]
	430.9729	QC	C_9F_{17}	PFK
	445.7555	M+4	$C_{12}H_2{}^{35}Cl_6{}^{37}Cl_2O$	OCDPE
4	407.7818	M+2	$C_{12}H{}^{35}Cl_6{}^{37}ClO$	HpCDF
	409.7789	M+4	$C_{12}H{}^{35}Cl_5{}^{37}Cl_2O$	HpCDF
	417.8253	M	$^{13}C_{12}H{}^{35}Cl_7O$	HpCDF [3]
	419.8220	M+2	$^{13}C_{12}H{}^{35}Cl_6{}^{37}ClO$	HpCDF [3]
	423.7766	M+2	$C_{12}H{}^{35}Cl_6{}^{37}ClO_2$	HpCDD
	425.7737	M+4	$C_{12}H{}^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD
	430.9729	Lock	C_9F_{17}	PFK
	435.8169	M+2	$^{13}C_{12}H{}^{35}Cl_6{}^{37}ClO_2$	HpCDD [3]
	437.8140	M+4	$^{13}C_{12}H{}^{35}Cl_5{}^{37}Cl_2O_2$	HpCDD [3]
	479.7165	M+4	$C_{12}H{}^{35}Cl_7{}^{37}Cl_2O$	NCDPE
5	441.7428	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO$	OCDF
	442.9728	Lock	$C_{10}F_{17}$	PFK
	443.7399	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O$	OCDF
	457.7377	M+2	$C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD
	459.7348	M+4	$C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD
	469.7779	M+2	$^{13}C_{12}{}^{35}Cl_7{}^{37}ClO_2$	OCDD [3]
	471.7750	M+4	$^{13}C_{12}{}^{35}Cl_6{}^{37}Cl_2O_2$	OCDD [3]
	513.6775	M+4	$C_{12}{}^{35}Cl_8{}^{37}Cl_2O$	DCDPE

[1] Nuclidic masses used:

H = 1.007825 C = 12.00000 ^{13}C = 13.003355 F = 18.9984
O = 15.994915 ^{35}Cl = 34.968853 ^{37}Cl = 36.965903

[2]
TCDD = Tetrachlorodibenzo-*p*-dioxin
PeCDD = Pentachlorodibenzo-*p*-dioxin
HxCDD = Hexachlorodibenzo-*p*-dioxin
HpCDD = Heptachlorodibenzo-*p*-dioxin
OCDD = Octachlorodibenzo-*p*-dioxin
HxCDPE = Hexachlorodiphenyl ether
OCDPE = Octachlorodiphenyl ether
DCDPE = Decachlorodiphenyl ether
TCDF = Tetrachlorodibenzofuran
PeCDF = Pentachlorodibenzofuran
HxCDF = Hexachlorodibenzofuran
HpCDF = Heptachlorodibenzofuran
OCDF = Octachlorodibenzofuran
HpCDPE = Heptachlorodiphenyl ether
NCDPE = Nonachlorodiphenyl ether
PFK = Perfluorokerosene

[3] Labeled compound.

[4] There is only one m/z for $^{37}Cl_4$-2,3,7,8,-TCDD (cleanup standard).

TABLE 9. THEORETICAL ION ABUNDANCE RATIOS AND QC LIMITS

Number of Chlorine Atoms	M/Z's Forming Ratio	Theoretical Ratio	QC Limit [1]	
			Lower	Upper
4 [2]	M/(M+2)	0.77	0.65	0.89
5	(M+2)/(M+4)	1.55	1.32	1.78
6	(M+2)/(M+4)	1.24	1.05	1.43
6 [3]	M/(M+2)	0.51	0.43	0.59
7	(M+2)/(M+4)	1.05	0.88	1.20
7 [4]	M/(M+2)	0.44	0.37	0.51
8	(M+2)/(M+4)	0.89	0.76	1.02

[1] QC limits represent ±15% windows around the theoretical ion abundance ratios.
[2] Does not apply to $^{37}Cl_4$-2,3,7,8-TCDD (cleanup standard).
[3] Used for $^{13}C_{12}$-HxCDF only.
[4] Used for $^{13}C_{12}$-HpCDF only.

Table 3. Concentrations of native and labeled chlorinated biphenyls in stock solutions, spiking solutions, and final extracts

	Solution concentrations		
CB congener	**Stock (μg/mL)**	**Spiking (ng/mL)**	**Extract (ng/mL)**
Native Toxics/LOC[1]			
1	20	1.0	50
3	20	1.0	50
4	20	1.0	50
15	20	1.0	50
19	20	1.0	50
37	20	1.0	50
54	20	1.0	50
77	20	1.0	50
81	20	1.0	50
104	20	1.0	50
105	20	1.0	50
114	20	1.0	50
118	20	1.0	50
123	20	1.0	50
126	20	1.0	50
155	20	1.0	50
156	20	1.0	50
157	20	1.0	50
167	20	1.0	50
169	20	1.0	50
188	20	1.0	50
189	20	1.0	50
202	20	1.0	50
205	20	1.0	50
206	20	1.0	50
208	20	1.0	50
209	20	1.0	50
Native congener mix stock solutions[2]			
MoCB thru TrCB	2.5		
TeCB thru HpCB	5.0		
OcCB thru DeCB	7.5		
Labeled Toxics/LOC/window-defining[3]			
1L	1.0	2.0	100
3L	1.0	2.0	100
4L	1.0	2.0	100

Table 6. QC acceptance crtiteria for chlorinated biphenyls in VER, IPR, OPR, and samples[1]

Congener	IUPAC number[2]	Test conc (ng/mL)[3]	VER[4] (%)	IPR		OPR (%)	Labeled compound recovery in samples (%)
				RSD (%)	X (%)		
2-MoCB	1	50	70-130	40	60-140	50-150	
4-MoCB	3	50	70-130	40	60-140	50-150	
2,2'-DiCB	4	50	70-130	40	60-140	50-150	
4,4'-DiCB	15	50	70-130	40	60-140	50-150	
2,2'6-TrCB	19	50	70-130	40	60-140	50-150	
3,4,4'-TrCB	37	50	70-130	40	60-140	50-150	
2,2'6,6'TeCB	54	50	70-130	40	60-140	50-150	
3,3',4,4'-TeCB	77	50	70-130	40	60-140	50-150	
3,4,4',5-TeCB	81	50	70-130	40	60-140	50-150	
2,2',4,6,6'-PeCB	104	50	70-130	40	60-140	50-150	
2,3,3',4,4'-PeCB	105	50	70-130	40	60-140	50-150	
2,3,4,4',5-PeCB	114	50	70-130	40	60-140	50-150	
2,3',4,4',5-PeCB	118	50	70-130	40	60-140	50-150	
2',3,4,4',5-PeCB	123	50	70-130	40	60-140	50-150	
3,3',4,4',5-PeCB	126	50	70-130	40	60-140	50-150	
2,2',4,4',6,6'-HxCB	155	50	70-130	40	60-140	50-150	
2,3,3',4,4',5-HxCB[5]	156	50	70-130	40	60-140	50-150	
2,3,3',4,4',5'-HxCB[5]	157	50	70-130	40	60-140	50-150	
2,3',4,4',5,5'-HxCB	167	50	70-130	40	60-140	50-150	
3,3',4,4',5,5'-HxCB	169	50	70-130	40	60-140	50-150	
2,2',3,4',5,6,6'-HpCB	188	50	70-130	40	60-140	50-150	
2,3,3',4,4',5,5'-HpCB	189	50	70-130	40	60-140	50-150	
2,2',3,3',5,5',6,6'-OcCB	202	50	70-130	40	60-140	50-150	
2,3,3',4,4',5,5',6-OcCB	205	50	70-130	40	60-140	50-150	
2,2',3,3',4,4',5,5',6-NoCB	206	50	70-130	40	60-140	50-150	
2,2',3,3,'4,5,5',6,6'-NoCB	208	50	70-130	40	60-140	50-150	
DeCB	209	50	70-130	40	60-140	50-150	
$^{13}C_{12}$-2-MoCB	1L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-4-MoCB	3L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2'-DiCB	4L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-4,4'-DiCB	15L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',6-TrCB	19L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,4,4'-TrCB	37L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',6,6'-TeCB	54L	100	50-150	50	35-135	30-140	25-150
13C12-3,3',4,4'-TCB	77L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,4,4',5-TeCB	81L	100	50-150	50	35-135	30-140	25-150

Congener	IUPAC number[2]	Test conc (ng/mL)[3]	VER[4] (%)	IPR		OPR (%)	Labeled compound recovery in samples (%)
				RSD (%)	X (%)		
$^{13}C_{12}$-2,2',4,6,6'-PeCB	104L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4'-PeCB	105L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,4,4',5-PeCB	114L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3',4,4',5-PeCB	118L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2',3,4,4',5-PeCB	123L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,3',4,4',5-PeCB	126L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',4,4',6,6'-HxCB	155L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4',5 -HxCB[5]	156L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4',5'-HxCB[5]	157L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3',4,4',5,5'-HxCB	167L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-3,3',4,4',5,5'-HxCB	169L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,4',5,6,6'-HpCB	188L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2',3,3',4,4',5,5'-HpCB	189L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',5,5',6,6'-OcCB	202L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,3,3',4,4',5,5',6-OcCB	205L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6-NoCB	206L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',4,5,5',6,6'-NoCB	208L	100	50-150	50	35-135	30-140	25-150
$^{13}C_{12}$-2,2',3,3',4,4',5,5',6,6'-DeCB	209L	100	50-150	50	35-135	30-140	25-150
Cleanup standard							
$^{13}C_{12}$-2,4,4'-TrCB	28L	100	60-130	45	45-120	40-125	30-135
$^{13}C_{12}$-2,3,3',5,5'-PeCB	111L	100	60-130	45	45-120	40-125	30-135
$^{13}C_{12}$-2,2',3,3',5,5',6-HpCB	178L	100	60-130	45	45-120	40-125	30-135

1. QC acceptance criteria for IPR, OPR, and samples based on a 20 μL extract final volume
2. Suffix "L" indicates labeled compound.
3. See Table 5.
4. Section 15.3.
5. PCBs 156 and 157 are tested as the sum of two concentrations

	Solution concentrations		
CB congener	**Stock (μg/mL)**	**Spiking (ng/mL)**	**Extract (ng/mL)**
15L	1.0	2.0	100
19L	1.0	2.0	100
37L	1.0	2.0	100
54L	1.0	2.0	100
77L	1.0	2.0	100
81L	1.0	2.0	100
104L	1.0	2.0	100
105L	1.0	2.0	100
114L	1.0	2.0	100
118L	1.0	2.0	100
123L	1.0	2.0	100
126L	1.0	2.0	100
155L	1.0	2.0	100
156L	1.0	2.0	100
157L	1.0	2.0	100
167L	1.0	2.0	100
169L	1.0	2.0	100
188L	1.0	2.0	100
189L	1.0	2.0	100
202L	1.0	2.0	100
205L	1.0	2.0	100
206L	1.0	2.0	100
208L	1.0	2.0	100
209L	1.0	2.0	100
Labeled clean-up[4]			
28L	1.0	2.0	100
111L	1.0	2.0	100
178L	1.0	2.0	100
Labeled injection internal[5]			
9L	5.0	1000	100
52L	5.0	1000	100
101L	5.0	1000	100
138L	5.0	1000	100
194L	5.0	1000	100

Diluted combined 209 congener[6]		
	Solution concentration (μg/mL)	
Standard	Native	Labeled
Native congeners		
MoCB thru TrCB	50	
TeCB thru HpCB	100	
OcCB thru DeCB	150	
Labeled Toxics/LOC/window-defining		100
Labeled Cleanup		100
Labeled Injection internal		100

1. Stock solution: Section 7.8.1; Spiking solution: Section 7.11
2. Section 7.8.1.2
3. Stock solution: Section 7.9.1; Spiking solution: Section 7.12
4. Stock solution: Section 7.9.2; Spiking solution: Section 7.13
5. Stock solution: Section 7.9.3; Spiking solution: Section 7.14
6. Section 7.10.2.2.2

APPENDIX I

REFERENCE LABORATORY PROCEDURES FOR DATA REVIEW

Axys Analytical Services Ltd.
Standard Operating Procedure

Purpose

This document describes specific protocols for quality review of all PCDD/F results and data packages from application of USEPA Method 1613B to environmental samples.

Scope

Axys SOP # QAQC-01 describes the general procedures for conducting a final quality review of analytical data and should be consulted along with this document which contains complimentary method specific details for review of USEPA 1613B results.

Requirement and Authority

This review must be completed for all data reports and data packages prior to release to clients. The review is conducted by designated QA Chemists fully trained in application of Method 1613B who report to the QA Manager. QA Chemists assess the acceptability of results and have the authority to request corrective actions to bring any results not meeting specifications into conformance. The QA Manager, or the designee, has final responsibility for ensuring that data reviews are conducted properly and for final decisions on data acceptability.

Definitions of Terms Used

Terminology used in this document is consistent with that of Method 1613B. The following additional terms are used:

PCDD – Polychlorinated dibenzo-p-dioxins
PCDF - Polychlorinated dibenzofurans
TCDD -Tetrachlorinated dibenzo-p-dioxin
TCDF - Tetrachlorinated dibenzofurans

Sample Specific Detection Limit (SDL) – detection limits calculated from an estimation of instrumental noise converted to a final concentration in the same manner that target compound peak responses are converted to final concentrations.

Procedures

Sample Receipt, Holding Times, Preservation

Sample Receipt forms must be completed fully including USEPA Sample Login Sheet DC-1 where required.

Method 1613B requires that aqueous samples be stored at 0-4 °C and that solids/semi-solids/ oily/mixed phase/tissue samples be stored at <4 °C from time of collection until receipt at the laboratory. Contract specific guidelines may also apply. USEPA contract guidelines require that the client be informed if the shipping cooler temperature exceeds 10 °C upon receipt at laboratory.

Method 1613B requires that aqueous samples be stored at 0-4 °C and solids/semi-solids/ oily/mixed phase/tissue samples be stored at <10 °C in the laboratory.

There are no demonstrated storage times for PCDD/F and Method 1613B defaults to a one-year holding time.

Method 1613B specifies that if the PH of aqueous samples exceeds 9.0 they be adjusted to PH 7-9 with sulphuric acid and that any free chlorine be neutralized with 80 mg/l of sodium thiosulfate. These procedures are to be done at the sampling stage. Some clients may choose to waive this requirement or arrange for it to be done upon receipt at the lab. Note any departures from the 1613B method protocol in the narrative.

Method Performance Documentation Inspection

- Annual Initial Performance and Recovery study results fall within limits in Method 1613B Table 6.
- Annual Method Detection Limit study results fall below one-third the minimum levels in Method 1613B Table 2.

Laboratory Work-up Documentation Inspection

- Consistency of client and laboratory sample identifiers on laboratory work-sheets with those on instrumental run file listings, LIMS listings, sample receiving records, and client supplied documentation, verified every sample.
- Sample size, moisture and lipid content determinations – verified every sample.
- Amount, time, and analysts' initials documenting addition of labelled compounds, verified every sample.
- Correct extraction and clean-up procedures, verified every sample.

Instrumental Performance and Calibration Inspection

- Mass resolution demonstration >10,000, verified at the beginning and end of 12 hour periods

- Mass calibration software routine ensures <5 ppm mass drift, verified every 12 hours.
- S:N > 10:1 for target PCDD/F in CS1 calibration standard, verified every initial calibration.
- Retention time of $^{13}C_{12}$-1234-TCDD > 25 minutes on DB-5 column and >15 minutes on DB-225, verified every initial calibration.
- First/last eluting target compounds as per Table 5 of Method 1613B within acquisition window for window defining mixture run, verified with every initial calibration sequence and at the beginning of every 12 hour period during which samples are analysed.
- Isomer specificity test valley height <25 % as per Figure 6 and 7 of Method, every initial calibration and at the beginning of every 12 hour period during which samples are analysed.
- Instrument carryover test <0.3 % for 2378-TCDD/F, verified every 12 hours.
- Initial calibration RSD of response factors <20 % for all target compounds except 123789-HxCDD, OCDF and <35 % for labelled standards, 123789-HxCDD, OCDF; all ion ratios within the acceptance ranges in Table 9, verified every 30 days, as required to maintain calibration verification specifications, or after instrumental changes (i.e. new GC column)
- Calibration verification concentrations and ion ratios within acceptance limits in Method 1613B Table 6 and 9 respectively, S:N > 10:1 for all compounds, verified at the beginning and end of 12 hour sample brackets.
- Calibration verification SICP areas for labelled internal standards within 50–200 % of initial calibration CS3 standard run, verified every 12 hours.
- Lock mass variation < ±20 % for all lock mass ions over the acquired RT range, verified every injection.

Batch Quality Control Sample Inspection

- Sample batch size maximum 20 samples, verified every batch.
- Laboratory blank concentrations less than one-tenth the minimum levels in Method 1613B Table 2, except for OCDD and OCDF which can be up to one-fifth the minimum levels in Method 1613B Table 2; labelled compound recoveries within acceptance limits in Method 1613B Table 7, verified every blank.
- OPR concentrations within acceptance ranges in Method 1613B Table 6, verified every OPR.
- QC samples and samples were processed identically in the same analysis batch- this to ensure that any systematic errors in sample data do not go undetected

Sample Data Inspection

- Target compound responses within calibration range, verified every sample.
- Labelled compound recoveries within Method 1613B limits in Table 7, verified every sample.
- Responses for target compounds detected meet all RT, ion ratio, and S:N identification criteria specified in Method 1613B section 16.0, verified every sample.
- Concentrations calculated in accordance with Method 1613B section 17.0 using correct input data and scaling factors, verified very sample.
- DB-5 column concentration of 2378-TCDF above the reporting limit is confirmed on a DB-225 column, verified every sample.
- Sample results are not affected by instrumental sample to sample carryover, verified every sample.
- Absence of diphenylether interferences, verified every sample
- General chromatography meets Axys quality standard, verified every sample.

General Documentation Inspection

- Record keeping practices consistent with good laboratory protocols – (ink, revisions made neatly and initialled and dated, no obscuring of original entries, no use of corrective fluid or impermanent attachments).
- Sign-off on sample worksheets, instrument acquisition lists, instrument data reports, final reports as per Axys Document SQA-019, 'General Documentation Policies'
- Data package complete and assembled in accordance with Axys Document SAD-023, 'Data Package Preparation'.

Electronic Data Submissions:

- Electronic file submissions compiled in accordance with Axys Document SAD-023, 'Electronic Data Template Management'
- hard copy printouts of electronic data file submissions initialled to document verification against hard copy client reports

Remedial Actions

Quality Assurance Chemists must initiate remedial action to correct any deficiencies noted in results during the final data review. These instructions are documented on the laboratory worksheets used for the original sample analysis. They may include re-calculations, re-coding of

chromatograms, additional extract clean-up, instrumental re-injections or complete sample re-analysis.

Reporting results which fall outside the specifications in Method 1613B and this document requires the approval of the Quality Manager. Non-conformances to Method 1613B must be documented in the data package narrative with an explanation of the cause and the impact on final data interpretation.

References and Related Documents

1. USEPA Method 1613B
2. Axys Document SQA-001 Final Data Checking
3. Axys Document SCO-004 Determination of Carryover
4. Axys Document SCO-009 Hand Calculation of Relative Response Factors, Concentrations, and Detection Limits of Target Analytes
5. Axys Document SCO-011 Code a Chromatogram
6. Axys Document SAD-025 'Electronic Data Template Management'
7. Axys Document SQA-019 'General Documentation Policies'
8. Axys Document SAD-023 Data Package Preparation

Approval: _____

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Axys Analytical Services Ltd.
Standard Operating Procedure

Title:	Final Data Checking	**SOP #:**	QAQC-1
Area:	QA/QC	**Rev. No.:**	4
		Date:	01 May 2001
		Page:	1 of 4

Purpose:

To review analytical data prior to release to ensure reported results satisfy all quality and contract specific criteria

Scope:

Analytical raw data are converted to sample concentrations and final data reports by a team of data interpretation and report preparation specialists in the Data Production Group. In addition to the data quality checks performed during that process, a final data quality review is conducted by the Quality Assurance Group prior to release of results. This SOP describes the general procedures for conducting the final data review.

Requirement and Authority

All final data reports are subjected to a comprehensive review prior to release and the results of the review are documented. The review is conducted by designated QA Chemists reporting to the QA Manager. QA Chemists judge the acceptability of results and are responsible for ensuring the accuracy and completeness of work. They have the authority to request confirmation or corrective action such as additional documentation, re-injections, or analysis repeats. QA chemists provide the Supervisors, Lab Manager, Chemists, and QA Manager with information and advice regarding work quality, corrective action and special job requirements. Feedback is provided on the quality of work-up, instrumental analysis, interpretation, coding, calculation, reporting, tracking, interlab communication, and documentation. The QA Manager, or the designee, has final responsibility for ensuring that data reviews are conducted properly and for the accuracy of final reports.

Definitions:

Surrogate standard – isotopically labeled compound added at the beginning of the analysis for calculation of targets by isotope dilution
LIMS – Laboratory Information Management System
Instrument Carryover – elevation of instrument response for a target compound due to residual contamination of the injection system from a proceeding sample

General Procedure and Corrective Actions:

1. Review raw data, including calculations and chromatograms, and final analytical reports to ensure that all results are correctly interpreted and accurately coded, calculated, and reported. The data review must cover the following as a minimum:

a) header information on final reports must be confirmed, especially sample identification which must match that on client custody forms, Axys receiving forms, LIMS entry, sample work-up sheets, and instrument run file listings;
b) the demonstration of instrument carryover (instrument blank following calibration standard) meets the required standard;
c) the demonstration of instrument sensitivity, linearity, and stability meets the required standard;
d) sample to sample carryover has not affected results;
e) chromatography quality and GC resolution meet standard;
f) analyte responses are within calibration range;
g) surrogate recoveries meet method, contract and internal control specifications;
h) correct addition of quantification standards;
i) correct completion of worksheet;
j) absence of sample contamination by inspection of laboratory and instrumental blanks, replicate analysis and injection data;
k) calibration data meet specifications;
l) correct quantification procedure used – manual check required;
m) accuracy of all coding, transcription, and typing;
n) blanks, duplicate tests and reference spikes/CRMs meet quality specifications;
o) detection limit criteria are met;
p) data pass reasonableness test;
q) documentation is complete, accurate and documentation practices are consistent with good laboratory protocols (all records in permanent ink form, revisions made by a line through original entry, new entry initialled; no use of correction fluid or 'sticky' labels);
r) data packages are complete and contain all contract required supporting quality documentation such as method detection limit and initial performance/recovery studies;
s) inspection for systematic errors that could be undetected by QC sample results – verify that samples and QC samples have been processed in an identical manner and within the same analysis batch;
t) electronic data file submissions compiled in accordance with Axys SOP# ADMIN-24 'Electronic Data Template Management' and hardcopy printouts of electronic data file submissions initialled to document verification against hard copy client reports;
u) any departures from sample acceptance criteria (containers, storage, preservation, labelling, handling) have been authorized and are properly documented in the narrative and/or data reports.

The frequency of checks, general quality criteria, and recommended corrective actions are listed in Specification Chart/04 attached to this SOP.

Specific quality criteria for particular analyses are listed in the Method Specification Table

attached to each method in the 'Axys Analytical Methods Manual' and in analyte specific Quality Review SOPs.

Detailed procedures covering the complete analytical procedure from sample receipt to final report are documented in the 'Axys Analytical Standard Operating Procedures Manual' and should be consulted as necessary.

2. Document the acceptability of results by initialling the QA/QC sections of the Batch Summary List. Clearly flag any problem data and alert the QA Manager. If it is necessary to report results for which all QA/QC parameters have not been satisfied, clearly flag the data and specify the parameters affected. Describe the probable impact on the results.

3. Initiate corrective action by completing the 'Additional Work' section of the Sample Worksheet. Inform the Production Manager, who will up-date the sample tracking records, and the supervisor of the appropriate group, who will assign the work to an analyst.

4. Review results from sub-contracting laboratories. Ensure that the sub-contracting lab is authorized to perform the work as listed on the "List of Approved Contracting Laboratories". Verify that all requested QC information has been supplied and meets accepted standards. Inform the QA Manager of any concerns regarding quality of sub-contracted work.

5. Prepare Letters of Transmittal for final data reports to present results completely, clearly, and unambiguously. Avoid the use of acronyms that may mislead or confuse the client. Explain any shortfalls in data quality or completeness, and recommend further action where required.

6. Document any client questions or complaints regarding data quality on Client Complaint Form (Client Services/03), according to standard operating procedure ADMIN-11. Provide a copy to the QA Manager and the contract administration file.

7. Routine requests for additional information or for help in understanding results may be handled directly by the QA Chemist who authorized release of the final data report.

References and Related Documents

1. Axys Analytical QA/QC Policies and Procedures Manual
2. Axys Analytical Standard Operating Procedures Manual
3. Axys Analytical Methods Manual
4. SOP CODE-1 Visual Inspection of Chromatogram
5. SOP CODE-4 Determination of Carryover
6. SOP CODE-9 Hand Calculation of Relative Response Factors, Concentrations, and

Detection Limits of Target Analytes
7. SOP CODE-11 Code a Chromatogram
8. SOP ADMIN-23 Data Package Preparation

Attachments

1. Specification Chart /04.

Approval: _____

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APPENDIX J

DATA VALIDATION CHECKLISTS FOR REFERENCE LABORATORY DATA REVIEW

Dioxin/Furan Data Review Checklist

Data Package:
Sample ID Nos:

Initials/Date	Activity	Y	N	NA	Comments
	Were storage and holding times met? • Held at <-10C after receipt • Analyzed within 1 yr of receipt				
	Track chain-of-custody from sample receipt to analytical injection.				
	Confirm correct concentration of standards spiked into samples.				
	6-Point Initial Calibration meets criteria of <20% RSD for items quantified by isotope dilution, <35% for items quantified by internal standard.				
	Column performance checked at beginning of each 12 hr period. • <25% valley from 2,3,7,8-TCDD (2,3,7,8-TCDF for confirms) and closest eluters. • 1,2,3,4 TCDD$^{13}C_{12}$ elutes >25 minutes on DB5 and> 15 minutes on DB225				
	10K resolution documented every 12 hours.				
	Continuing calibration every 12 hours. • Results within Demonstration Plan Table 9-2 CAL/VER criteria • S:N of concal peaks at least 10:1				
	Method blanks with every 20 test samples • less than 0.5 pg/sample for tetras, 1 pg/sample for penta-hepta, 5 pg/sample for octas or 20x lower than the sample concentration.				
	OPR with every 20 test samples • Within Demonstration Plan Table 9-2 for OPR				
	Duplicate with every 20 test samples • <20% difference between duplicates for total TEQ. Note in comments if any individual congeners >10 x DL are >20% difference.				
	All 2,3,7,8-isomers are accounted for: concentration reported, flagged for reason not used, or obviously not present.				
	Ether masses were monitored and there are no ether contributions to furans.				
	Lock masses were monitored and there were no variations which could affect reported results.				
	Internal standard recoveries 40-120% except OCDD$^{13}C_{12}$ at 25-120%?				
	Review 10% data transfer into final report spreadsheets.				
	If confirmations, check separation of column, calibration, COC, and transfer of 10% of confirmation data to final spreadsheet.				
	Review report narrative for accurately reflecting raw data and resulting spreadsheets.				
	Were TEQs reported two ways? • All ND and K values assigned zero • ND = ½ the detect limit, K = EMPC concentration				
	Have data flags been applied appropriately? • J for values between the detection limit and the low calibration level • K for estimated maximum possible concentration • TEQs with 10% or greater contribution from J or K flagged data also flagged J or K as appropriate				

PCB Data Review Checklist

Data Package:
Sample ID Nos:

Initials/Date	Activity	Y	N	NA	Comments
	Were storage and holding times met? • Held at <-10C after receipt • Analyzed within 1 yr of receipt				
	Track chain-of-custody from sample receipt to analytical injection.				
	Confirm correct concentration of standards spiked into samples.				
	5-Point Initial Calibration meets criteria of <20% RSD for all compounds and CS-1 has S:N of 10:1				
	Column performance checked at beginning of each 12 hr period. • For SPB Octyl: 34 resolved from 23, 187 resolved from 182, 156 and 157 coelute. • For SPB Octyl: PCB 209 elutes >55 minutes • For DB1: PCB elutes > 55 minutes, PCB 156/157 resolution < 20% valley high.				
	10K resolution documented every 12 hours.				
	Continuing calibration every 12 hours. • Results within Demonstration Plan Table 9-4 CAL/VER criteria • S:N of concal peaks at least 10:1				
	Method blanks with every 20 test samples • PCB 77, 81,114,123,126, and 169 < 2 pg/congener • PCB 156, 157, 167, 189 < 10 pg/congener • All other PCB < 50 pg/ congener • Total PCB < 200 pg or 20x below sample concentration.				
	OPR with every 20 test samples • Within Demonstration Plan Table 9-4 for OPR				
	Duplicate with every 20 test samples • <20% difference between duplicates for total TEQ. Note in comments if any each congener >10 x DL are > 20% difference.				
	Each 12 WHO PCB accounted for: concentration reported, flagged for reason not used, or obviously not present.				
	Lock masses were monitored and there were no variations which could affect reported results.				
	Internal standard recoveries within Demonstration Plan Table 9-4 Labeled Compound Recovery criteria				
	Review 10% data transfer into final report spreadsheets.				
	Review report narrative for accurately reflecting raw data and resulting spreadsheets.				
	Were TEQs reported two ways? • All ND and K values assigned zero • ND = ½ the detect limit, K = EMPC concentration				
	Have data flags been applied appropriately? • J for values between the detection limit and the low calibration level • K for estimated maximum possible concentration • TEQs with 10% or greater contribution from J or K flagged data also flagged J or K as appropriate				

APPENDIX K

REFERENCE LABORATORY PROCEDURES FOR DATA PACKAGE PREPARATION

AXYS Analytical Services Ltd.
Standard Operating Procedure

Purpose:

To assemble and submit to a client a detailed data package that includes all final reports, raw data and supporting documentation such that an independent data reviewer can validate all final results through the analytical process.

Scope:

The following procedures apply to all samples for which a data package style of report has been requested, unless the client has specified an alternate format. Data packages may be in a paper format or in CD format. Data packages are assembled and prepared for shipment by a Data Packager.

Abbreviations and Definitions:

Data package (DP) – A compilation of final data reports, raw data, and supporting documentation for a pre-defined set of samples.

Instrument Run List– A sequential listing of instrumental run files including sample IDs, data filenames and acquisition times.

Instrument Run Table – A subset of the instrumental run list, listing sample data file names directly below the calibration data used to quantify them.

Analysis Worksheets - AXYS forms containing the information on sample extraction and cleanup, one form per sample and QC sample. Other worksheets with data may be included as specified by the Project Chemist.

Workgroup (WG) – A laboratory analysis batch including QC samples. The composition of a workgroup is listed on a Batch List

PC – Project Chemist

Procedures:

Paper Data Packages

1. Refer to the Project Notes to determine which samples are to be included in the data package. Depending upon the client request, this may be by AXYS log-in ("L" number), by workgroup or by the client's Sample Delivery Group (SDG). Determine what type of data package is required

(hard copy or CD ROM).

2. To prepare a paper data package, assemble all the appropriate records for the data package. Use the originals of the analysis worksheets. For all other records use photocopies or copies of scanned originals. For information recorded in bound logbooks include page photocopies. In some cases the client may request originals of all records or may specify which records are to be included in the data package. Refer to the Project Notes for details of required records. Each data package must be 'self-contained' and include all records relevant to the samples included in the data package.

3. Ensure all photocopies are legible and are an accurate reproduction of the original copy. Poor copies of the custody seals should be excluded from the data package.

4. Where documents relate to more than one data package and original records have been included, place the originals in the data package with the lowest AXYS log-in number. Place a photocopy in all other data packages .

6. To assemble the data package, compile the records listed below. Note that it may be convenient to first compile the sample data, QC sample date, instrumental QC and raw data prior to assembling the initial records, provided the records are ordered in the data package in sequence below.

   i. Hardcover title page
   ii. Title page
   iii. Narrative
   iv. Method Summary (as available)
   v. GC Column Temperature Program
   vi. Cover page(s) with signature line
   vii. AXYS Correlation Table (correlates AXYS ID with client ID)
   viii. Individual Sample Receiving Forms (use copies unless Project Notes specify otherwise)

   - Client Chain of Custody
   - FedEx waybill
   - AXYS Sample Receiving Record
   - Custody Seals
   - Field Notes (documentation about the samples taken in the field-if available)

- AXYS Login Chain of Custody (do not include Sample Acknowledgement Form
- Selective Documentation Between AXYS and Client- only as requested. Documents for inclusion will be stamped with 'Data Package'.

ix. Sample Preparation Records (if applicable, order numerically by AXYS ID number)
x. Sample Pretreatment Records (if applicable, order numerically by AXYS ID number)
xi. Analysis Workup Sheets (order numerically by workgroup and AXYS ID number)
xii. Sample Data (order numerically by AXYS ID number and then by analysis (ordered by date for multiple analyses, including dilutions) – refer to the example at the end of this document for guidance.

NOTE: in a paper data package, place coloured sheets between samples, so complete reports for a given sample are visible at a glance

xiii. QC Sample Data including the following:

- Procedural Blank (order numerically by Workgroup Number)
- OPRs/SPMs (order numerically by WG number)
- MS/MSDs (order numerically by WG number)
- CRMs (order numerically by WG number)

xiv. Instrument QC (order chronologically by Analysis Run Date, using the date and time of the starting Run List or Injection Log to order each set). Start with run list for each "set" or bundle of related QC. Each set or bundle may include:

- Run List
- Mass resolutions, opening and closing (HRMS PCBs and PCDD/F only, opening mass resolution only for pesticides)
- Run Table
- Linearity (Form 3s, if present in run)
- Calibration Verification. Forms 4 and 6, opening only unless closing required.
- Isomer Specificity (Form 5 (Dx) or single page from PCB chromatogram)
- Full Calibration form 3A (PCB)
- Client Standard (if applicable)

xv. Raw Data

- Sample Raw Data (order in the same sequence as the Sample Data Reports above, usually by AXYS sample ID). Bind the chromatograms with an elastic band, but do

not bind in the final paper copy.

- Laboratory blank chromatograms (order in the same sequence as Blank reports).
- OPR/SPM (or MS/MSD, CRM) chromatograms (order in same sequence as data reports).
- Instrumental chromatograms (order chronologically by Analysis Run Date and Time- to mirror the order of the Instrument QC hardcopy reports
  - -Run List
  - -Linearity, with OpusQuan RRF summary sheet
  - -Calibration Verification
  - -Window
  - -Client Standards (if applicable)

5. Prepare a title page, a cover page and a correlation table.

6. Submit the data package to the Project Chemist for review and for a narrative. Track the progress of the data package on the Data package and Shipment Log (FSA-023).

7. When the completed, reviewed data package has been returned, make any necessary corrections as directed.

8. Stamp each page with a sequential number.

9. If additional copies of the data package are required, make the appropriate number of photocopies.

10. Bind the hardcopy data package(s) using AXYS cover pages. Unless otherwise instructed by the client (refer to Project Notes).

11. Scan the data package as AXYS' backup copy.

CD Data Packages

1. To prepare a CD from a paper data package, reorder the paper data package so that the chromatogram for each sample appears after the sample analysis report. Otherwise, the sample data are ordered numerically by AXYS ID, as described for the paper data package. Scan the Sample Data, QC Data, Instrument QC and Raw Data (items 6.xii to 6.xv above).

2. If a paper data package has not been prepared, assemble the required documents, (as specified in 6.ii to 6.xv above) either as hardcopy or as PDF files. Order the files such that the chromatogram for each sample appears after the sample analysis report. Scan the documents as appropriate and import the chromatograms. All scanned data is located AXYS' network at G:/PDF_Data.

3. Create links to the Sample Data, Lab Blanks, QC Samples (a separate link for each type i.e. OPR, MS/MSD) and Instrument QC. Within each section, bookmark each sample as it appears in the data package. Notify the Project Chemist that the Data Package is ready for the narrative and checking.

4. When the checked data package is returned with the narrative, scan the narrative and client information (steps 6.ii to 6.xi above) and create a bookmark to the file.

5. Arrange for another Data Packager who has not been involved in the preparation to check the scanned data package.

6. Burn the final checked data package onto a non-rewriteable CD.

Quality Measures and Remedial Actions

1. Data Packages are prepared and stored in a secure area that is locked during non-working hours.

2. A secure back-up copy of all data in electronic medium is stored as described in AXYS SOP SAD-024 "Data Management and Handling".

3. Completeness and consistency of assembled data packages are verified by the Project Chemist who writes and a narrative.

4. Procedural deficiencies detected in assembled data packages are returned to the Data Packager for correction.

5. A re-submission of an entire data package must contain a revised narrative documenting the reasons for re-submission.

6. Minor corrections to a data package may be resubmitted with a covering letter describing

the correction(s). If the original data package was a paper copy, the relevant revised pages accompany the letter. These pages are reported with a revision date. If the data package was originally issued as a CD, then the entire data package with revisions is sent on a CD with the covering letter.

7. Client complaints and/or requests for further explanations are handled as per AXYS SOP# SAD-011 “Settlement of Client Complaints”.

Transmission Procedures:

1. Prepare four copies of a covering letter to the client indicating the contents of the package. A Project Chemist must sign the original covering letter.

2. Package up the data packages(s) in an appropriate sized box or envelope.

3. Place the original letter in the box and tape the second copy on the outside of the box. Do a check to verify that the two letters accompanying the data package are the same. Seal the box for shipping.

4. Give the package to the Receptionist for shipping.

5. Complete the shipping information on the Data Package and Shipment Log (FSA-023).

6. Prior to shipment, the Receptionist verifies the shipping address on the letter with previous shipping information for the client and reports any discrepancies to a Data Packager. If necessary, the Data Packager prepares a new covering letter. The Receptionist places a waybill on the box and checks that the name and address on the waybill are the same as on the letter taped to the box. When the waybill is in place, the letter is removed from the outside of the box.

7. The Receptionist tracks the package with the courier and maintains files of the shipping information.

8. The Receptionist sends original copies of shipping records to the accounting department.

9. File the third copy of the covering letter in the Data Package binder and give the fourth copy to the Accounts Receivable Technician.

AXYS Analytical Services Ltd., PO Box 2219, Sidney, BC, Canada V8L 3S8 Tel (250) 655-5800 FAX (250) 655-5811

AXYS Analytical Services Ltd.
Standard Operating Procedure

References

SAD-024	Data Management and Handling
FSA-023	Data Package And Shipment Log
SAD-011	Settlement of Client Complaints

Approval:

_____ _____
Coreen Hamilton, Technical Director Date

_____ _____
Dale Hoover, Quality Manager Date

AXYS Analytical Services Ltd.
Standard Operating Procedure

Example of Sequence for Sample Data Reports, Method EPA 1668A – ordered by instrumental analysis date

- Initial Analysis Data (target reports, surrogate reports). Forms 1A,2
- Extra Work Analysis Data: i.e. dilution, recolumn, reinjection (target reports, surrogate reports). Forms 1A, 2 (organize multiple dilutions by run date)
- Client Standards, if applicable. Form 1A
- Homologue Totals – if required or not on initial analysis report
- TEQ – if required or not on initial analysis report. Form 1A
- Aroclors – if required
- If there is Lipid weight basis analysis results, organize similarly (do not worry about surrogate pages being with reports)- For a paper data package all wet wt basis data is organized by L number, Samples -1 through -x and all lipid wt data is in a separate section also organized by L number samples –1 through –x. In the paper DP this is separated by a coloured cover page. ON A CD DP, the lipid data comes immediately after the chromatogram for the wet wt data for a given L number.

APPENDIX L

WORK PLAN FOR REFERENCE LABORATORY IN-PROCESS AUDIT

AUDIT PLAN

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

Subtask: In-Process Laboratory Audit

Client: EPA NERL, SITE Program (FEATS contract)

Project Manager: Amy Dindal (Battelle Columbus Laboratories)

Assessee Organizations: AXYS Analytical Services, LTD

Location: 2045 Mills Rd.
PO Box 2219
Sidney, BC
Canada V8L 3S8

Senior Official/Title: Ms. Laurie Phillips

QA Manager: Mr. Dale Hoover

Authorizing Entity: US EPA

Review and Concurrence by *Amy Dindal* (signature on file)

Assessment Team: Ms. Rosanna Buhl and Mr. Mark Misita

Lead Assessor: Ms. Rosanna Buhl

Anticipated Dates of Assessment: May 26, 2004

Authority to Conduct Assessment:

The US EPA Task Order Manager (TOM) has authorized Battelle to conduct an independent assessment of AXYS, the reference laboratory for the *Technologies for Monitoring and Measurement of Dioxin in Soil and Sediment* demonstration.

Criteria for Assessment: Compliance with the following documents:

- MLA-017 Rev 09 (To be completed) (*Analytical method for the determination of dioxins and furans by EPA Method 1613B)*
- MLA-010 Rev 5 24-Sept-2003 (*Analytical method for the determination of 209 PCB congeners by EPA Method 1668A)*
- *Quality Assurance/Quality Control (QA/QC) Policies and Procedures Manual* Rev 8 (17-Jul-2003)
- Second Draft Demonstration Plan (April 2004)

Purpose and Scope of Assessment:

The purpose of this audit is to verify the compliance of AXYS Analytical Services, LTD vs. their SOPs, QA/QC Manual, the Project Notes directives, and the project Demonstration/Quality Assurance Plan. The scope will specifically include a review of dioxin and PCB congener sample processing, analysis, and data reduction; sample receipt, handling, and tracking; supporting

laboratory systems; and responses to the report generated as a result of the February 11, 2004 audit.

Issues Selected:

- Sample and standards tracking
- Sample and standards preparation
- Instrument calibration
- Sample analysis
- Sample integration and data reduction
- Acceptability of quality control data

Personnel to Be Interviewed:

- Laboratory Point of Contact
- Analyst
- Sample prep personnel
- Data management personnel
- Sample custodian

Documents to be Reviewed:

☑ SOP MLA 010 (*Analytical method for the determination of 209 PCB congeners by EPA Method 1668A)*

☑ *Quality Assurance/Quality Control (QA/QC) Policies and Procedures Manual* Rev 8 (17-Jul-2003)

☑ Demonstration and Quality Assurance Project Plan (April 2004)

☐ MLA-017 (*Analytical method for the determination of dioxins and furans by EPA Method 1613B)*

☐ Current Organization Chart (if revised after QDO-005 Rev 24 (23 January 2004)

☐ Q-Pulse Project Notes

☐ Results of current sample analysis

Anticipated Date for Receipt of Records:

The auditors have three of the seven documents needed to prepare for the audit. The auditors have requested the unchecked documents above be supplied by May 19th.

Records to be Reviewed:

- Data generated to date
- Sample receipt records and holding location/conditions
- Standard (spiking and calibration solution) receipt records and certificates of analysis
- Standard preparation records
- Support equipment records (Balances, refrigerators)
- Sample preparation and spiking records
- Preparation of instrument standards
- Preparation of spiking solutions
- Instrument calibration and maintenance records
- Instrument sample run logs

- Instrument set-up conditions, temperature programs, calibration and acquisition methods
- Sample chromatograms
- Instrument reports
- Quantification techniques
- QC sample results
- Data management
- Data validation and verification

Anticipated Opening Meeting: May 26, 2004 (8:00 AM)

Opening Meeting Participants:

- Audit team
- Laboratory Point of Contact
- Analyst
- Laboratory QA Officer
- Others at the discretion of the laboratory

Anticipated Assessment Schedule:

May 26, 2004 (times are approximate)	Quality Systems Audit (Rosanna Buhl)	Technical System Audit (Mark Misita)
8 AM	Opening Meeting	
8:30 AM	Sample Receiving & Storage	Dioxin/Furan Sample Preparation
9:00	Dioxin/Furan Sample Preparation	
9:30	Support equipment calibration and maintenance	D/F Instrument calibration and operation
10:00	Calibration standard tracking Spiking solution tracking	D/F Sample analysis, Integration, and Chromatograms
11:00	Reagent and solvent tracking	D/F Data reduction and reporting
Noon	Lunch – The audit team would like to order a simple lunch to eat in.	
1:00 pm	Instrument maintenance	PCB Sample Preparation
2:00 PM	Data validation	PCB Instrument calibration and operation
3:00 PM	Quality Control data and corrective actions	PCB Sample analysis, Integration, and Chromatograms
3:45 PM	Audit team prepares for the debriefing	
4:15 PM	Debriefing	
5 PM	Audit ends	

Anticipated Closing Meeting: May 26, 2004 (4:15 PM)

Closing Meeting Participants:

- Audit team
- Laboratory Point of Contact
- Analyst
- Laboratory QA Officer
- Others at the discretion of the laboratory

AUDIT PLAN

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

Anticipated Reporting Schedule: See above

Report Routing Pathway:

The Lead Auditor will submit the audit reports to the Battelle Project Manager. Comments from the Battelle Project Manager and the EPA TOM will be provided to the Lead Auditor by the Battelle Project Manager. The audit is not closed until the laboratory has responded to each finding.

Confidentiality of Findings Report:

Dissemination of the report to anyone besides the Battelle Project Manager, the EPA TOM, and the laboratory QA Manager is at the discretion of the EPA TOM.

Dissemination of Findings Report: See Confidentiality of Findings Report.