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Innovative Technology Verification Report

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

Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit

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March 2005

Innovative Technology Verification Report

Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit Wako (for environmental)

Prepared by

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Notice

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Foreword

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

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

The EPA's Superfund Innovative Technology Evaluation (SITE) Program evaluates technologies designed for characterization and remediation of contaminated Superfund and Resource Conservation and Recovery Act (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 (MMT) Program, demonstrates and evaluates innovative technologies to meet these needs.

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

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Abstract

A demonstration of technologies for determining the presence of dioxin and dioxin-like compounds in soil and sediment was conducted under the U.S. Environmental Protection Agency's (EPA's) Superfund Innovative Technology Evaluation Program in Saginaw, Michigan, at Green Point Environmental Learning Center from April 26 to May 5, 2004. This innovative technology verification report describes the objectives and the results of that demonstration, and serves to verify the performance and cost of the Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit Wako (for environmental). Four other technologies were evaluated as part of this demonstration, and separate reports have been prepared for each technology. The objectives of the demonstration included evaluating the technology's accuracy, precision, sensitivity, sample throughput, tendency for matrix effects, and cost. The test also included an assessment of how well the technology's results compared to those generated by established laboratory methods using high-resolution mass spectrometry (HRMS). The demonstration objectives were accomplished by evaluating the results generated by the technology from 209 soil, sediment, and extract samples. The test samples included performance evaluation (PE) samples (i.e., contaminant concentrations were certified or the samples were spiked with known contaminants) and environmental samples collected from 10 different sampling locations.

The Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit Wako (for environmental) is an immunoassay technology that reports total dioxin/furan concentration in a sample. The sample units are in pg/g 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) equivalents (EQ). While the kit is most reactive to 2,3,7,8-TCDD, it is responsive to all dioxin/furans at some level. As part of the PE, the technology results were compared to toxicity equivalent (TEQ) results generated by a reference laboratory, AXYS Analytical Services, using EPA Method 1613B, which involves the use of HRMS. It should be noted that the results generated by this technology may not directly correlate to HRMS $TEQ_{D/F}$ in all cases because it is known that the congener responses and cross-reactivity of the kit are not identical to the toxicity equivalency factors that are used to convert congener HRMS concentration values to $TEQ_{D/F}$. The effect of cross-reactivities may contribute to this technology's reporting results that are biased high or low compared to HRMS $TEQ_{D/F}$ results. Therefore, this technology should not be viewed as producing an equivalent measurement value to HRMS $TEQ_{D/F}$, but as a screening value to approximate HRMS $TEQ_{D/F}$ concentration. It has been suggested that correlation between the Wako and HRMS TEQ could be improved by first characterizing a site and calibrating the Wako results to HRMS results. Subsequent analysis using the Wako kit for samples obtained from this site may then show better correlation with the HRMS TEQ result. This approach was not evaluated during this demonstration.

A summary of the performance of the Dioxin ELISA Kit Wako (for environmental) is as follows: The Wako results were biased both positively and negatively relative to the certified and reference laboratory results. No statistically significant matrix effects were observed by sample type (PE vs. environmental vs. extract), matrix type (soil vs. sediment vs. extract), or polynuclear aromatic hydrocarbon concentration. Wako completed all 209 sample analyses in the field within a nine-day period. The technology's estimated method detection limit (83 to 201 pg/g 2,3,7,8-TCDD EQ) was significantly higher than was reported by the developer (20 pg/g 2,3,7,8-TCDD EQ), but PE samples with TEQ concentrations in the precisely appropriate range for evaluation of this technology's detection limit were not available, so these calculated values should be considered a rough estimate. The kit had a false positive rate of 10% and a false negative rate of 13% around 20 pg/g TEQ. The kit had the same false positive rate around 50 pg/g (10%), but less false negatives (8%). These data suggest that the Wako kit could be an effective screening tool for determining sample results above and below 20 pg/g TEQ, and even more effective as a screen for samples above and below 50 pg/g TEQ, particularly considering that the cost to analyze the 209 demonstration samples was significantly less than that of the reference laboratory ($150,294 vs. $213,580). All samples were analyzed on-site in 9 days (in comparison to the reference laboratory, which took 8 months to report all results).

Contents

Contents (continued)

Contents (continued)

Contents (continued)

Page

Figures

Tables

Abbreviations, Acronyms, and Symbols

Ah	aryl hydrocarbon
ANOVA	analysis of variance
ASE	accelerated solvent extraction
ATSDR	Agency for Toxic Substances and Disease Registry
CIL	Cambridge Isotope Laboratories
CoA	Certificate of Analysis
COC	chain of custody
CRM	certified reference material
DER	data evaluation report
D/F	dioxin/furan
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNR	Department of Natural Resources
D/QAPP	demonstration and quality assurance project plan
ELC	Environmental Learning Center
ELISA	enzyme-linked immunosorbent assay
EMDL	estimated method detection limit
EMPC	estimated maximum possible concentration
EPA	Environmental Protection Agency
ERA	Environmental Resource Associates
EQ	equivalent
FDSC	Food and Drug Safety Center
g	gram
GC	gas chromatography
HPLC/GPC	high-performance liquid chromatography/gel permeation chromatography
HRGC	high-resolution capillary gas chromatography
HRMS	high-resolution mass spectrometry
i.d.	internal diameter
IDW	investigation-derived waste
ITVR	innovative technology verification report
kg	kilogram
L	liter
LRMS	low-resolution mass spectrometry

Abbreviations, Acronyms, and Symbols (Continued)

µm	micrometer
m	meter
MDEQ	Michigan Department of Environmental Quality
MDL	method detection limit
mg	milligram
mL	milliliter
MMT	Monitoring and Measurement Technology
MS	mass spectrometry
NERL	National Exposure Research Laboratory
ng	nanogram
NIST	National Institute for Standards and Technology
NOAA	National Oceanic and Atmospheric Administration
ORD	Office of Research and Development
PAH	polynuclear aromatic hydrocarbons
PC	positive control
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo-p-dioxin/dibenzofuran
PCP	pentachlorophenol
PE	performance evaluation
pg	picogram
POD-conjugate	peroxidase conjugated with a dioxin analog
ppb	parts per billion; nanogram/g; ng/g
ppm	parts per million; microgram/g; µg/g
ppt	parts per trillion; picogram/g; pg/g
psi	pound per square inch
QA/QC	quality assurance/quality control
RM	reference material
RPD	relative percent difference
RSD	relative standard deviation
SDL	sample-specific detection limit
SIM	selected ion monitoring
SITE	Superfund Innovative Technology Evaluation
SOP	standard operating procedure
SRM	Standard Reference Material®
TCDD	tetrachlorodibenzo-*p*-dioxin
TEF	toxicity equivalency factor
TEQ	toxicity equivalent

Abbreviations, Acronyms, and Symbols (Continued)

$TEQ_{D/F}$	total toxicity equivalents of dioxins/furans
TEQ_{PCB}	total toxicity equivalents of World Health Organization dioxin-like polychlorinated biphenyls
TOC	total organic carbon
total TEQ	total toxicity equivalents including the sum of the dioxin/furan and World Health Organization dioxin-like polychlorinated biphenyls
WHO	World Health Organization

Acknowledgments

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Chapter 1
Introduction

The U.S. Environmental Protection Agency (EPA), Office of Research and Development (ORD), National Exposure Research Laboratory (NERL) contracted with Battelle (Columbus, Ohio) to conduct a demonstration of monitoring and measurement technologies for dioxin and dioxin-like compounds in soil and sediment. A field demonstration was conducted as part of the EPA Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) Program. The purpose of this demonstration was to obtain reliable performance and cost data on the technologies to provide (1) potential users with a better understanding of the technologies' performance and operating costs under well-defined field conditions and (2) the technology developers with documented results that will help promote the acceptance and use of their technologies.

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

1.1 Description of the SITE MMT Program

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

- MMT Program—Evaluates technologies that sample, detect, monitor, or measure hazardous and toxic substances. These technologies are expected to provide better, faster, or more cost-effective methods for producing real-time data during site characterization and remediation efforts than conventional laboratory technologies.

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

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

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

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

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

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

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

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

During the demonstration, each technology is evaluated independently and, when possible and appropriate, is compared to a reference technology. The performance and cost of one technology are not compared to those of another technology evaluated in the demonstration. Rather, demonstration data are used to evaluate the individual performance, cost, advantages, limitations, and field applicability of each technology.

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

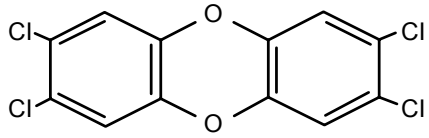
The fourth step of the verification process is to distribute demonstration information. To benefit technology developers and potential technology users, the EPA makes presentations, publishes and distributes fact sheets, newsletters, bulletins, and ITVRs through direct mailings and on the Internet. Information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

1.2 Scope of This Demonstration

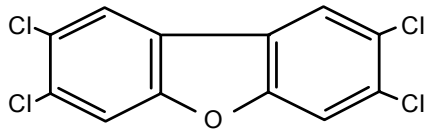
Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans, commonly referred to collectively as "dioxins," are of significant concern in site remediation projects and human health assessments because they are highly toxic. Dioxins and furans are halogenated aromatic hydrocarbons and are similar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

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

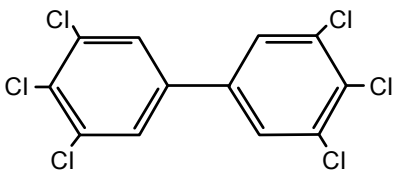
Certain polychlorinated biphenyls (PCBs) have structural and conformational similarities to dioxin compounds (Figure 1-1) and are therefore expected to exhibit toxicological similarities to dioxins as well. Currently only 12 of the total 209 PCB congeners are thought to have "dioxin-like" toxicity. These 12 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



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



2,3,7,8-Tetrachlorodibenzofuran



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

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

"dioxin-like" PCBs are often refered to as non-ortho and mono-ortho substituted coplanar PCBs.

Conventional analytical methods for determining concentrations of dioxin and dioxin-like compounds are time-consuming and costly. For example, EPA standard methods require solvent extraction of the sample, processing the extract through multiple cleanup columns, and analyzing the cleaned fraction by gas chromatography (GC)/high-resolution mass spectrometry (HRMS). The use of a simple, rapid, cost-effective analytical method would allow field personnel to quickly assess the extent of contamination at a site and could be used to direct or monitor remediation or risk assessment activities. This data could be used to provide immediate feedback on potential health risks associated with the site and permit the development of a more focused and cost-effective sampling strategy. At this time, more affordable and quicker analytical techniques will not replace HRMS. However, before adopting an alternative to traditional laboratory-based methods, a thorough assessment of how commercially available technologies compare to conventional laboratory-based analytical methods using certified, spiked, and environmental samples is warranted. A summary of the demonstration activities to evaluate measurement technologies for dioxin and dioxin-like compounds in soil and sediment is provided below. The experimental design and demonstration approach are described in greater detail in Chapter 4 and was published in the Demonstration and Quality Assurance Project Plan (D/QAPP).[2]

1.2.1 Organization of Demonstration

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

1.2.2 Sample Descriptions and Experimental Design

Soil and sediment samples with a variety of distinguishing characteristics such as high levels of PCBs and

PAHs were analyzed by each participant. Samples were collected from a variety of dioxin-contaminated soil and sediment sampling locations around the country. Samples were identified and supplied through EPA Regions 2, 3, 4, 5, and 7 and the MDEQ. The samples were homogenized and characterized by the characterization laboratory prior to use in the demonstration to ensure a variety of homogeneous, environmentally derived samples with concentrations over a large dynamic range (< 50 to > 10,000 picogram/gram [pg/g]) were included. The environmental samples comprised 128 of the 209 samples included in the demonstration (61%). Performance evaluation (PE) samples were obtained from five commercial sources. PE samples consisted of known quantities of dioxin and dioxin-like compounds. Fifty-eight of the 209 demonstration samples (28%) were PE samples. A suite of solvent extracts was included in the demonstration to minimize the impact of sample homogenization and to provide a uniform matrix for evaluation. A total of 23 extracts (11% of the total number of samples) was included in the demonstration. The demonstration samples are described in greater detail in Section 4.3.

1.2.3 Overview of Field Demonstration

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

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

Chapter 2
Description of Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit

This technology description is based on information provided by Wako and only editorial changes were made to ensure document consistency. Actual cost and performance data, as reported and observed during the demonstration, will be provided later in this document. The Dioxin ELISA Kit Wako (for environmental) 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.

2.1 Company History

1922 The Chemicals Department of Takeda Chobei Shoten (the present Takeda Chemical Industries, Ltd.) separated and established as an independent company, Takeda Pure Chemicals Ltd. in June.
1935 Tokyo Office opened
1940 Osaka Plant opened
1944 Tokyo Plant opened
1947 Company name changed to Wako Pure Chemical Industries, Ltd.
1952 Head office moved to the current address
1964 Tokyo Plant moved to the current site
1967 Tokyo and Osaka Research Laboratories completed
1968 Harima Plant opened
Paris Liaison Office opened (until 1970)
1970 Dusseldorf Liaison Office opened (until 1983)
1972 Head Office building completed
1974 Wako Chemicals GmbH established in Dusseldorf under 100% capital ownership
1978 Dallas Liaison Office opened (until 1989)
1981 Wako Chemicals USA, Inc. founded in Dallas, Texas, under 100% capital ownership
1983 Uedesheim Plant, Wako Chemicals GmbH, completed
Neuss Liaison Office opened
1988 Mie Plant opened
1989 Tobu Distribution Center opened
Wako Chemicals USA, Inc. moved to Richmond, Virginia
Richmond Liaison Office opened
1990 Miyazaki Plant opened
Richmond Plant, Wako Chemicals USA, Inc. completed
1991 Seibu Distribution Center opened
Matumoto Plant opened
1994 Hafen Plant, Wako Chemicals GmbH completed
1995 All domestic plants completed registration of ISO-9002 certification
1998 Wako obtained ISO-9002 certificate of registration for the total quality system of the domestic operation
2001 Wako obtained Environment Management System ISO-14001 certificate of registration
Tokyo and Osaka plants were approved as official testing laboratories based on ISO/IEC-17025

2.2 Product History

The kit was developed by Wako and the Food and Drug Safety Center (FDSC) Research Institute in Japan. FDSC received research funding from the Ministry of Health, Labour and Welfare to develop the kit. FDSC developed the monoclonal antibody used for the analysis of dioxins, and Wako developed the Dioxin ELISA Kit Wako (for environmental) using the monoclonal antibody.

2.3 Technology Description

The extraction procedure is described in Figure 2-1. The process involves accelerated solvent extraction (ASE) followed by cleanup. Once extracted, a monoclonal antibody specific to dioxin is mixed with a sample solution or the positive control (PC) provided with the

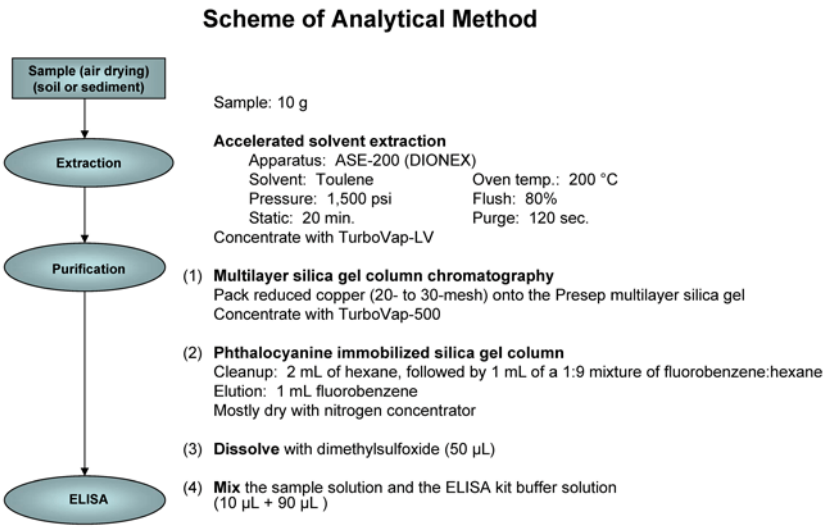


Figure 2-1. Dioxin ELISA Kit Wako (for environmental) extraction procedure.

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

The monoclonal antibodies used for the Dioxin ELISA Kit Wako (for environmental) 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 toxicity equivalent (TEQ). (See Table 2-1.)

The Dioxin ELISA Kit Wako (for environmental) sensitivity is claimed by Wako to be from 1.6 to 100 pgs per assay, and 96 samples can be assayed in two days. The assay procedure is summarized in Figure 2-2.

Wako reported its data in pg/g 2,3,7,8-TCDD equivalent (EQ). Although the Wako units specifically include 2,3,7,8-TCDD and the kit is most sensitive to this congener, the technology represents a total D/F toxicity equivalent value. Wako reported results in the approximate range of 20 to 2,000 pg/g. Concentrations measured to be below or above these concentrations were reported semiquantitatively (e.g.,<20 pg/g or >2,000pg/g). Wako notes that results can be reported below 20 pg/g by taking a larger sample size (than 10 g) and can be reported above 2,000 pg/g by performing dilutions. This is the developer method that was implemented during the field demonstration. A photo of the technology in operation during the field demonstration is presented in Figure 2-3. Wako provided supplemental information about the performance of their technology during the demonstration and it is presented in Appendix B.

Table 2-1. Dioxin ELISA Kit Wako (for environmental) Cross-Reactivity

No.	Class.	Number of Chlorines	Article name	Toxicity Equivalency Factor	ELISA Kit Cross-Reactivity Data
1	Dioxins	Dichloride	2,7-DiCDD		0.05
2		Trichloride	2,3,7-TriCDD		0.16
3		Tetrachloride	1,2,3,4-TeCDD		0.0002
4			1,3,6,8-TeCDD		0.0004
5			2,3,7,8-TeCDD	1.0	1.00
6		Pentachloride	1,2,4,6,8/1,2,4,7,9-PeCDD		0.008
7			1,2,3,7,8-PeCDD	1.0	0.48
8		Hexachloride	1,2,3,4,6,7-HxCDD		0.003
9			1,2,3,4,7,8-HxCDD	0.1	0.07
10			1,2,3,6,7,8-HxCDD	0.1	0.04
11			1,2,3,7,8,9-HxCDD	0.1	0.06
12		Heptachloride	1,2,3,4,6,7,8-HpCDD	0.01	0.006
13		Octachloride	1,2,3,4,6,7,8,9-OCDD	0.0001	0.00004
14	Furans	Tetrachloride	2,3,7,8-TeCDF	0.1	0.14
15		Pentachloride	1,2,3,7,8-PeCDF	0.05	0.03
16			2,3,4,7,8-PeCDF	0.5	0.17
17		Hexachloride	1,2,3,4,7,8-HxCDF	0.1	0.02
18			1,2,3,6,7,8-HxCDF	0.1	0.08
19			1,2,3,7,8,9-HxCDF	0.1	0.07
20			2,3,4,6,7,8-HxCDF	0.1	0.06
21		Heptachloride	1,2,3,4,6,7,8-HpCDF	0.01	0.002
22			1,2,3,4,7,8,9-HpCDF	0.01	0.002
23		Octachloride	1,2,3,4,6,7,8,9-OCDF	0.0001	0.00004
24	Coplanar-PCB	Tetrachloride	3,3',4,4'-TeCB(#77)	0.0001	0.0008
25			3,4,4',5-TeCB(#81)	0.0001	0.0009
26		Pentachloride	2,3,3',4,4'-PeCB(#105)	0.0001	0.00003
27			2,3,4,4',5-PeCB(#114)	0.0005	0.00004
28			2,3',4,4',5-PeCB(#118)	0.0001	0.00005
29			2',3,4,4',5-PeCB(#123)	0.0001	0.00004
30			3,3',4,4',5-PeCB(#126)	0.1	0.0005
31		Hexachloride	2,3,3',4,4'5-HxCB(#156)	0.0005	0.00001
32			2,3,3',4,4',5'-HxCB(#157)	0.0005	0.00002
33			2,3',4,4',5,5'-HxCB(#167)	0.00001	0.00003
34			3,3',4,4',5,5'-HxCB(#169)	0.01	0.00003
35		Heptachloride	2,2',3,3',4,4',5-HpCB(#170)	0.0001	0.00002
36			2,2',3,4,4',5,5'-HpCB(#180)	0.00001	0.00002
37			2,3,3',4,4'5,5'-HpCB(#189)	0.0001	0.00006
38	Brominated dioxin	Tetrabromide	2,3,7,8-TeBrDD		0.39
39		Pentabromide	1,2,3,7,8-PeBrDD		0.04
40		Hexabromide	1,2,3,6,7,8-HxBrDD		0.003
41		Octabromide	1,2,3,4,6,7,8,9-OBrDD		0.0007
42	Brominated furan	Pentachloride	2,3,4,7,8-PeBrDF		0.11
43	Brominated/ chlorinated dioxin	Monobromine trichloride	2-Br-3,7,8-TriCDD		0.40
44		Monobromine tetrachloride	1-Br-2,3,7,8-TeCDD		0.19

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

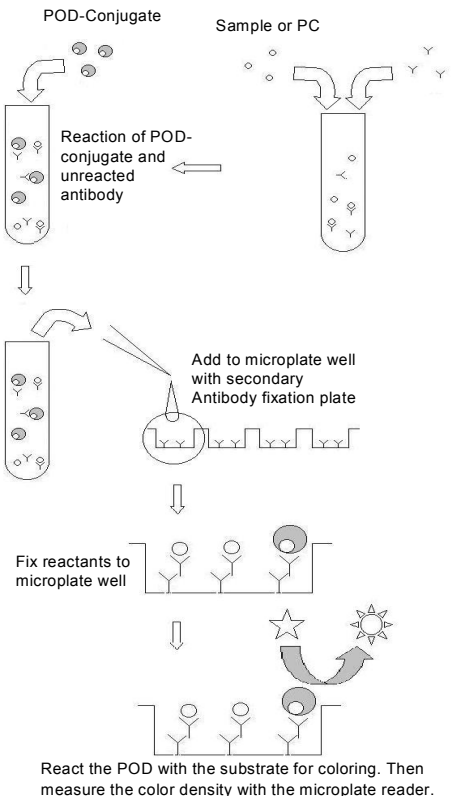


Figure 2-2. Dioxin ELISA Kit Wako (for environmental) assay procedure.

Figure 2-3. Dioxin ELISA Kit Wako (for environmental) in operation during the field demonstration.

2.4 Developer Contact Information

Additional information about this technology can be obtained by contacting:

Wako Pure Chemical Industries, Ltd.
1-2 Doshomachi 3-Chome Chuo-ku
Osaka 540-8605 Japan
Telephone: +81-6-6203-3841
Web site: http://www.wako-chem.co.jp
E-mail: cservice@wako-chem.co.jp

U.S. Subsidiary:
Wako Chemicals USA, Inc.
1600 Bellwood Road
Richmond, Virginia 23237-1326 USA
Telephone: (804) 271-7677
Web site: http://www.wakousa.com
E-mail: emmy@wakousa.com

2.5 Product Information

Code No.: 295-59601
Product Name: Dioxin ELISA Kit Wako (for environmental)
Quantity: for 96 tests

Chapter 3
Demonstration and Environmental Site Descriptions

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

3.1 Demonstration Site Description and Selection Process

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

After review of the information available, the site selected for the demonstration was the Green Point 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 Green Point ELC is situated within the Tittabawassee River flood plain. The MDEQ found higher than normal levels of dioxins in soil and sediment samples taken from the flood plain of the Tittabawassee River. The flood plain is not heavily laden with PCBs; however, low levels of PCBs have been detected in some areas. Soil samples taken from areas outside the flood plain were at typical background levels. The source of the contamination was speculated to be attributed to legacy contamination from chemical manufacturing.

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

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

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

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

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

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

3.2 Description of Sampling Locations

This section provides an overview of the 10 sampling sites and methods of selection. Table 3-1 summarizes each of the locations, what type of sample (soil or sediment) was provided, the number of samples submitted from each location, and the number of samples included in the demonstration from each location. Samples were collected from multiple sampling sites so that a wide variety of matrix conditions could be used to evaluate the performance of the technologies in addressing monitoring needs at a diverse range of Superfund sites.

Samples consisted of either soil or sediment and are described below based on this distinction. It should be noted that it was not an objective of the demonstration to accurately characterize the concentration of dioxins, furans, and PCBs from a specific sampling site. It was, however, an objective to ensure comparability between technology samples and the reference laboratory samples. This was accomplished by homogenizing each matrix, such that all sub-samples of a given matrix had consistent contaminant concentrations. As a result, homogenized samples were not necessarily representative of original concentrations at the site.

3.2.1 Soil Sampling Locations

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

3.2.1.1 Warren County, North Carolina

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

3.2.1.2 Tittabawassee River Flood Plain

The MDEQ sampled the Tittabawassee River flood plain soils from three sites in the flood plain. The source of the contamination was speculated to be attributed to legacy contamination from chemical manufacturing. Two samples were collected from two locations at Imerman Park in Saginaw Township. The first sample was taken near the boat launch, and the second sample was taken in a grassy area near the river bank. Previous analysis from these areas of this park indicated a range of PCDD/F concentrations from 600 to 2,500 pg/g. Total PCBs from these previous measurements were in the low parts-per-trillion range. Two samples were collected from two locations at Freeland Festival Park in Freeland, MI. The first sample was taken above the river bank, and the second sample was taken near a brushy forested area within the park complex. Previous PCDD/F concentrations were from 300 to 3,400 pg/g, and total PCBs were in the low part per trillion (ppt) range. The final two samples were collected from Department of Natural Resources (DNR)-owned property in Saginaw,

Table 3-1. Summary of Environmental Sampling Locations

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

which was formerly a farming area located almost at the end of the Tittabawassee River where it meets the Shiawassee River to form the Saginaw River. Previous PCDD/F concentrations ranged from 450 to 1,150 pg/g. Total PCBs were not previously analyzed, but concentrations were expected to be less than 1 ppm. The DNR property is approximately a 10-minute walk from where the demonstration was conducted at the Green Point ELC.

3.2.1.3 Midland, Michigan

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

3.2.1.4 Winona Post

The Winona Post site in Winona, Missouri, was a Superfund cleanup of a wood treatment facility. Contaminants at the site included PCP, dioxin, diesel fuel, and PAHs. Over a period of at least 40 years, these contaminants were deposited into an on-site drainage ditch and sinkhole. Areas of contaminant deposition (approximately 8,500 cubic yards of soils/sludge) were excavated in late 2001/early 2002. This material was placed into an approximate 2½-acre treatment cell located on facility property. During 2002/2003, material at the treatment cell was treated through addition of amendments (high-ammonia fertilizer and manure) and tilling. Final concentrations achieved in the treatment cell averaged 26 milligram per kilogram (mg/kg) for PCP and from 8,000 to 10,000 for pg/g dioxin equivalents. Samples obtained for this study from this site were obtained from the treatment cell after these concentrations had been achieved.

3.2.1.5 Solutia

The chemical production facility at the Solutia site in Nitro, West Virginia, is located along the eastern bank of the Kanawha River, in Putnam County, West Virginia. The site has been used for chemical production since the early 1910s. The initial production facility was developed by the U.S. government for the production of military munitions during the World War I era between 1918 and 1921. The facility was then purchased by a small private chemical company, which began manufacturing chloride, phosphate, and phenol compounds at the site. A major chemical manufacturer purchased the facility in 1929 from Rubber Services Company. The company continued to expand operations and accelerated its growth in the 1940s. A variety of raw materials has been used at the facility over the years, including inorganic compounds, organic solvents, and other organic compounds, including Agent Orange. Agent Orange is a mixture of chemicals containing equal amounts of two herbicides: 2,4-D (2,4 dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5 trichlorophenoxyacetic acid). Manufacture of this chemical herbicide began at the site in 1948 and ceased in 1969. The source of the

dioxin contamination in the site soils was associated with the manufacture of 2,4,5-T, where dioxins are an unintentional by-product. The site has a dioxin profile from ppt to low parts per billion (ppb) range. No PCBs or PAHs were identified in the soil.

3.2.2 Sediment Sampling Sites

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

3.2.2.1 New York/New Jersey Harbors

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

3.2.2.1.1 Newark Bay

Surrounded by manufacturing industries, Newark Bay is a highly contaminated area with numerous sources (sewage treatment plants, National Pollutant Discharge Elimination System discharges, and nonpoint sources). This bay is downstream from a dioxin Superfund site that contains some of the highest dioxin concentrations in the United States and also is downstream from a mercury Superfund site. The dioxin concentration in the area sampled for this demonstration was approximately 450 pg/g. Average PCB concentrations ranged from 300 to 740 ppb. Fine-grained sediments make up 50% to 90% of the dredged material. Average total organic carbon (TOC) was about 4%.

3.2.2.1.2 Raritan Bay

Surrounded by industry and residential discharges, Raritan Bay has dioxin contamination in the area, but it is not to the degree of Newark Bay. No major Superfund sites are located in the vicinity. Dioxin concentration should be significantly less than in Newark Bay. PCB concentrations are around 250 ppb. The fine-grained sediment and TOC values were similar to percentages in Newark Bay.

3.2.2.2 Tittabawassee River

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

3.2.2.3 Saginaw River

Saginaw River samples were collected at six locations. The first sampling location was in the Saginaw River just downstream of Green Point Island. Samples were collected near the middle of the river in about 21 feet of water. The sample was granular with some organic material. The estimated TEQ concentration was 100 ppt. Another Saginaw River sample was taken upstream of Genesee Bridge on the right side of the river. The sample

was a brown fine sand from about 15 feet of water. The estimated TEQ concentration was 100 ppt. The third location was in the Saginaw River downstream of the Saginaw wastewater treatment plant in about eight feet of water. The sample was gray silty clay with an unknown TEQ concentration. The fourth location was in the Saginaw River in about eight feet of water. The sample was a black sandy material. The estimated TEQ concentration for this location was unknown. The fifth location was downstream of a petroleum pipeline crossing upstream of the Detroit and Mackinaw railroad bridge crossing. This location was selected because of its proximity to a former PCB dredging location. The sediment sample consisted of dark black silt with some sand. The estimated TEQ concentration was unknown, but PCB concentrations are expected to be high. The sixth and final sampling location was near the mouth of the Saginaw River in about five feet of water. The sediment was a mix of fine black silt and layers of sand and shells. The estimated TEQ concentration for this location was also unknown.

3.2.2.4 Brunswick Wood Preserving Site

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

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

Chapter 4
Demonstration Approach

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

4.1 Demonstration Objectives

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

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

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

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

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

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

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

4.2 Toxicity Equivalents

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

$$TEQ = C_C * TEF$$

where C_C is the concentration of the congener. The TEF (see Table 4-1) provides an equivalency factor for each

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

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

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

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

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

This demonstration was designed with these limitations of the TEQ concept in mind. The samples chosen contained a variety of combinations of dioxins, furans,

and PCBs and at a wide range of concentration levels. Some samples were high in analytes with better understood TEFs, while others were high in analytes with TEFs that have more uncertainty. Some were high in other Ah-receptor binding compounds such as PAHs, while still others were free of these possible TEQ contributing compounds. The purpose was to evaluate each of the technologies under a variety of conditions and assess the comparability of the $TEQ_{D/F}$ and TEQ_{PCB} values determined by the reference laboratory.

4.3 Overview of Demonstration Samples

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

4.3.1 PE Samples

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

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

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

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

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

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

4.3.1.1 Cambridge Isotopes Laboratories

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

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

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

Table 4-4. Summary of Performance Evaluation Samples

Sample Type ID	Source	PE Type	Product No.	Certified Concentration			Correlation to Environ. Sample Type ID[a]	No. of Replicates Per Sample
				$TEQ_{D/F}$ (pg/g)	TEQ_{PCB} (pg/g)	PAH (mg/kg)		
PE # 1	CIL	Certified	RM 5183	3.9	5.0	0.18	6	7[b]
PE #2	LGC Promochem	Certified	CRM 529	6,583	424[c]	NA[d]	5	4
PE #3	Wellington	Certified	WMS-01	62	10.5	NA	6	7[b]
PE #4	CIL	Certified	RM 5184	171	941	27	2, 8, 9	4
PE #5	NIST	Certified	SRM 1944	251	41[c]	2.4[e]	3, 4	4
PE #6	ERA	Spiked	custom	11	NS[f]	<0.33	10	4
PE #7	ERA	Spiked	custom	33	NS	< 0.33	10	4
PE #8	ERA	Spiked	custom	NS	NS	61[g]	5, 7	4
PE #9	ERA	Spiked	custom	NS	11	< 0.33	1	4
PE #10	ERA	Spiked	custom	NS	1121	< 0.33	1	4
PE #11	ERA	Spiked	custom	11	3,760[c]	< 0.33	1	4
PE #12	ERA	Organic, Semivolatile, Blank Soil	056 (lot 56011)	0.046	0.01	< 0.33	not applicable	8
Total Number of PE samples								***58***

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

laboratories used a variety of sample preparation and analytical techniques.

4.3.1.2 LGC Promochem

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

4.3.1.3 Wellington

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

on the package size available from Wellington. Certified values for the 17 D/F congeners and the 12 WHO PCB congeners are provided on the CoA.

4.3.1.4 National Institute for Standards and Technology

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

4.3.1.5 Environmental Resource Associates

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

The manufacturing techniques that ERA used to prepare the PE samples for this demonstration were consistent with those used for typical semivolatile soil products by ERA. These techniques have been validated through hundreds of round robin performance test studies over ERA's more than 25 years in business. The D/F stock solutions used in the manufacture of these PE samples were purchases from CIL. The PCB and PAH stock solutions were purchased from ChemService. For each PE sample, a spiking concentrate was prepared by combining appropriate weight/volume aliquots of stock materials required for that PE sample. Typically, additional solvent was added to this concentrate to yield sufficient volume of solution, appropriate for the mass of soil to be spiked. Based on a soil mass of 1,600 g, the volume of spike concentrate was approximately 10 to 30 milliliter (mL). For each PE sample, the blank soil matrix was weighed into a 2-liter (L) wide mouth glass jar, the spike concentrate was distributed onto the soil, and the soil was allowed to air-dry for 30 to 60 minutes. The PE samples were then capped and mixed in a rotary tumbler for 30 minutes. Each PE sample was certified as the concentration of target analytes present in the blank matrix, plus the amount added during manufacture, based on volumetric and gravimetric measurements. CoAs were provided by ERA for all six ERA-provided PE samples. The certified values provided by ERA were different from the commercially available certified

samples since the data were not based on analytically derived results. Further confirmation of the concentrations was conducted by the reference laboratory.

4.3.2 Environmental Samples

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

4.3.2.1 Environmental Sample Collection

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

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

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

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

4.3.2.2 Homogenization of Environmental Samples

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

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

4.3.2.3 Selection of Environmental Samples

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

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

4.3.3 Extracts

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

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

where DF = dilution factor.

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

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

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

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

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

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

Table 4-6. Distribution of Extract Samples

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

[a] Prepared in toluene.

[b] Seven replicates were analyzed for EMDL evaluation.

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

4.4 Sample Handling

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

The samples were randomized in two ways. First, the order in which the filled jars were distributed was randomized. All jars had two labels. The label on the top of the jar was the analysis order and contained sample numbers 1 through 209. A second label placed on the side of the jar contained a coded identifier including a series of 10 numbers coded to include the site, replicate, developer, and matrix. All samples believed to have at least one D/F or PCB congener greater than 10,000 pg/g were marked with an asterisk for safety purposes. This was consistent for both the developer and reference laboratory samples. The developer was given the option of knowing which environmental site the samples came from and whether the sample was a soil or sediment. Wako elected to have the samples identified as soil or sediment. As described in the D/QAPP, AXYS was informed of which environmental site that the samples came from so it could use congener profiles and dilution schemes determined during the pre-demonstration phase as a guide, along with the concentration range data that was provided in the D/QAPP. This information was supplied to the reference laboratory with the samples, along with which samples contained high (i.e., a sample with at least one congener with concentration > 120,000 pg/g) or ultrahigh (i.e., a sample with at least one congener with concentration > 1,200,000 pg/g) PCB levels. Using this information, AXYS regrouped the samples in batches so that, to the extent possible, samples from the same site would be analyzed within the same analytical batch. Because an analytical laboratory might know at least what site samples came from, and because it is reasonable from an analytical standpoint to group samples that might require similar dilution schemes and which have similar congener patterns in an analytical batch, this approach was an acceptable deviation from the original intention of having the samples run by the reference laboratory completely blind and in the prescribed analytical order. Wako analyzed the samples in the order received. The extracts were the first 23 samples in the Wako analysis order.

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

4.5 Pre-Demonstration Study

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

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

4.6 Execution of Field Demonstration

Wako arrived on-site on Thursday, April 22, and spent several hours on four consecutive days setting up two facilities (a mobile laboratory and a support trailer). The demonstration officially commenced on Monday, April 26 after 1.5 hours of safety and logistical training. During this meeting, the health and safety plan was reviewed to ensure participants understood the safety requirements for the demonstration. Logistics, such as how samples would be distributed and results reported, were also reviewed during this meeting. After the safety and site-specific training meeting and prior to samples being received by the developers, each trailer and mobile laboratory was surface wipe sampled on the floor to the entrance of the developer work area to establish the background level of D/F and PCB contamination. The wipe sampling procedure was followed as described in the D/QAPP. Following demobilization by the developers, all of the trailers and mobile laboratories were cleaned and surface wipe sampled. Analysis of the pre- and post-deployment wipe samples indicated that all trailers and mobile laboratories met the acceptable clearance criteria that were outlined in the D/QAPP. Only one fume hood had to be re-cleaned and re-sampled before receiving final clearance.

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

Wako received its first batch of samples by midmorning on April 26. Wako completed analysis of all 209 demonstration samples (23 extracts and 186 soil/sediment samples) in 9 working days (on May 4). It should be noted that the morning of April 28 was dedicated to a Visitor's Day, so minimal work on sample analyses was performed. Once the complete data set was submitted, Wako was offered the opportunity to re-analyze any samples before reporting final results. Wako elected to re-analyze 36 samples. The samples for re-analysis were received by Wako representatives on June 18. Wako reported the reanalysis results on August 4. It was at the developer's discretion whether to keep the originally reported results or to replace with re-analysis data. Of the 36 reanalysis samples, 12 of the original sample results were retained and 24 were reported with new results based on the re-analysis.

4.7 Assessment of Primary and Secondary Objectives

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

Wako reported its data in pg/g 2,3,7,8-TCDD equivalent (EQ). The Wako results were compared to the reference laboratory results and certified values relative to $TEQ_{D/F}$. Although the Wako units specifically include 2,3,7,8-TCDD and the kit is most sensitive to this congener, the technology represents a total D/F toxicity equivalent value, so it was compared to the HRMS total $TEQ_{D/F}$ concentration in all cases.

Wako reported results in the approximate range of 20 to 2,000 pg/g. Concentrations measured to be below or above these concentrations were reported semiquantitatively (e.g.,<20 pg/g or >2,000 pg/g). Treatment of semiquantitative data is described in each data analysis section. Wako notes that results can be reported below 20 pg/g by taking a larger sample size (than 10 g) and can be reported above 2,000 pg/g by performing dilutions.

4.7.1 Primary Objective P1: Accuracy

The determination of accuracy was based on agreement with certified or spiked levels of PE samples. PE samples containing concentrations from across the analytical range of interest were analyzed. Measurements from the 58 PE samples were evaluated to determine whether there was a statistically significant difference between the measurements and the certified value or spiked level. Percent recovery values relative to the certified or spiked concentrations were also calculated. The PE samples were analyzed by the laboratory reference method for confirmation of certified and spiked values.

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

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

where $\overline{C}$ is the average concentration value (in pg/g 2,3,7,8-TCDD EQ) calculated from the technology replicate measurements and C_R is the certified value (in pg/g $TEQ_{D/F}$). Nondetects and values reported as "> (value)" were not included in the accuracy assessment. Mean concentration values were determined when at least three of four replicates were reported as actual values [i.e., were not reported as, "< (value)" or "> (value)"]. The mean, median, minimum, and maximum R values are reported as an assessment of overall accuracy. An ideal R value would be 100%.

4.7.2 Primary Objective P2: Precision

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

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

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

where *SD* is the standard deviation and $\overline{C}$ is the average measurement. Both are reported in pg/g 2,3,7,8-TCDD EQ.

The equation used to calculate RSD between replicate measurements was:

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

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

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

4.7.3 Primary Objective P3: Comparability

Data comparability was maximized by using the homogenization procedures and applying criteria for acceptable results prior to a sample being included in the demonstration. (See Section 4.3.2.3 for additional information.)

Technology results reported by Wako Pure Chemical Industries, Ltd. were compared to the corresponding reference laboratory results by calculating relative percent difference (RPD). The equation for RPD, reported in percent, is as follows:

$$\text{RPD} = \frac{\left(\text{M}_\text{R} - \text{M}_\text{D}\right)}{\text{average}\left(\text{M}_\text{R}, \text{M}_\text{D}\right)} \times 100\%$$

where M_R is the reference laboratory measurement (in pg/g) and M_D is the developer measurement (in pg/g 2,3,7,8-TCDD EQ). Nondetects were not included in this evaluation. For PE samples, $TEQ_{D/F}$ RPD calculations were only performed for the analyte classes that the PE sample contained. For example, PE sample #9 was only spiked with PCBs. Consequently, $TEQ_{D/F}$ RPD calculations were not performed.

The absolute value of the difference between the reference and developer measurements in the equation above was not taken so that the RPD would indicate whether the technology measurements were greater than the reference laboratory measurements (negative RPD values) or less than the reference laboratory measurements (positive RPD). Because negative values for RPD could be obtained with this approach, the median RPD of all individual RPDs was calculated rather than the average RPD in calculation of comparability between the Wako results and reference laboratory measurements. The median, minimum, and maximum RPD values were reported as an assessment of overall comparability. RPD values between positive and negative 25% indicated good agreement between the two measurements.

As another measure of comparability, the reference data were grouped into four TEQ concentration ranges. The ranges were $\leq$ 50 pg/g, 50 to 500 pg/g, 500 to 5,000 pg/g, and $\geq$ 5,000 pg/g. The intervals were determined by the Demonstration Panel and were based on current guidance for cleanup levels. The percentage of developer results that agreed with those ranges of values was reported. It should be noted that developer results that correctly agreed with the reference laboratory result, but fell in a different interval, were counted as "in agreement." For example, a Wako result as reported < 51 pg/g 2,3,7,8-TCDD EQ (which fell into the second interval) agreed with a reference laboratory result reported as 25.8 pg/g $TEQ_{D/F}$ (which fell into the first interval). The same rule applied to concentrations that were reported as > 2,000 pg/g 2,3,7,8-TCDD EQ.

The accuracy of reporting blank samples was assessed. The blanks included eight replicate samples that contained levels of D/Fs that were below the reporting limits of the developer technology but contained levels that could be detected by the reference methods (see Table 4-4). If the reference laboratory result was in the nondetect interval reported by the developer technology reporting limit, this result was considered accurately reported by the developer. The accuracy of the blank samples was reported in terms of % agreement. Ideal % agreement values would be 100%.

4.7.4 Primary Objective P4: Estimated Method Detection Limit

The method detection limit (MDL) calculation procedure described in the demonstration plan was 40 CFR Part 136, Appendix B, Revision 1.11. This procedure is based on an assumption that the replicates are homogeneous enough to allow proper measurement of the analytical precision and that the concentration is in the appropriate range for evaluation of the technology's sensitivity. For this evaluation, Wako analyzed seven aliquots each of a low-level PE soil, PE sediment, and a toluene-spiked extract. MDL-designated samples are indicated in Tables 4-4 and 4-6. PE samples in the precisely appropriate range for evaluation of this technology's detection limit were not available. The developer reported nondetect values for some of the replicates, so provisions had to be made for the treatment of nondetects. As such, the results from these samples were used to calculate an estimated MDL (EMDL) for the technology.

A Student's t-value and the standard deviation of seven replicates were used to calculate the EMDL in pg/g TEQ as shown in the following equation:

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

where $t_{(n-1,1-\infty=0.99)}$ = Student's t-value appropriate for a 99 percent confidence level and a standard deviation estimate with n-1 degrees of freedom. Nondetect values were assigned the reported value (i.e., "< 1" was assigned as value of 1), half of the reported value (i.e., "< 1" was assigned 0.5), or zero. The various treatments of nondetect values were performed to see the impact that reduced statistical power (i.e., lower degrees of freedom) had on the EMDL calculation. The lower the EMDL value, the more sensitive the technology is at detecting contamination.

4.7.5 Primary Objective P5: False Positive/False Negative Results

The tendency for the Wako kit to return false positive results (e.g., results reported above a specified level for the field technology but below a specified level by the reference laboratory) was evaluated. The frequency of false positive results was reported as a fraction of results available for false positive analysis. Similarly, the frequency of false negatives results was examined. For this purpose, the results were evaluated for samples reported as having concentrations above and below 20 pg/g TEQ and 50 pg/g TEQ. As such, the samples that were reported as ≤ 20 (or 50) pg/g TEQ by the reference laboratory but > 20 (or 50) pg/g TEQ by Wako were considered false positive. Conversely, those samples that were reported as ≤ 20 (or 50) pg/g TEQ by Wako, but reported as > 20 (or 50) pg/g by the reference laboratory, were considered false negatives. In the case of semiquantitative results (reported as < or >), if the laboratory result was within the interval reported by the developer, it was not considered a false positive or false negative result. Ideal false positive and negative percentages would be equal to zero.

4.7.6 Primary Objective P6: Matrix Effects

The likelihood of matrix-dependent effects on performance was investigated by grouping the data by matrix type (i.e., soil, sediment, extract), by sample type (i.e., PE, environmental, and extract), by varying levels of PAHs, by environmental site, and by known interferences. Precision (RSD) data were summarized by soil, sediment, and extract (matrix type); by environmental, PE, and extract (sample type); and by PAH concentration. Analysis of variance (ANOVA) tests were performed to determine if there was a dependence on matrix type or sample type. Only the environmental samples were included in the matrix effect assessment based on PAH concentration, because only the environmental samples were analyzed for PAHs during the characterization analysis (described in Section 5.2.3). Some PAH data were available for the PE samples, but data were not available for all of the same analytes that were determined during the characterization analysis. The environmental samples were segregated into four ranges of total PAH concentrations: < 1,000 nanogram/g (ng/g), 1,000 to 10,000 ng/g, 10,000 to 100,000 ng/g, and > 100,000 ng/g. The precision (RSD) data were summarized for samples within these PAH concentration ranges. ANOVA tests were used to determine if the summary values for RSD were statistically different, indicating performance dependent upon PAH concentration. For the environmental site evaluation, the comparability (RPD) values from each of the 10 environmental sites were compared to see if the developer results were more or less comparable to the reference laboratory for a particular site. For known interferences, the developer's reported results for PE samples were summarized for samples where the PE samples did not contain the target analyte (e.g., did the developer report D/F detections for a sample only spiked with PCBs).

This objective also evaluated whether performance was affected by measurement location (i.e., in-field versus laboratory conducted measurements), although this is not a traditional matrix effect. However, Wako analyzed all 209 samples during the field demonstration, and only a few were rerun in their laboratories and rereported, so measurement location was not evaluated for Wako.

4.7.7 Primary Objective P7: Technology Costs

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

4.7.8 Secondary Objective S1: Skill Level of Operator

Based on observations during the field demonstration, the type of background and training required to properly operate the Dioxin ELISA Kit Wako (for environmental) was assessed and documented. The skill required of an operator was also evaluated. The evaluation of this secondary objective also included user-friendliness of the technology.

4.7.9 Secondary Objective S2: Health and Safety Aspects

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

4.7.10 Secondary Objective S3: Portability

Observers documented whether the Dioxin ELISA Kit Wako (for environmental) could be readily transported to the field and how easy it was to operate in the field. This included an assessment of what infrastructure requirements were provided to Wako Pure Chemical Industries, Ltd. (e.g., a mobile laboratory), and an assessment of whether the infrastructure was adequate (or more than adequate) for the technology's operation. Limitations of operating the technology in the field are also discussed.

4.7.11 Secondary Objective S4: Sample Throughput

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

Chapter 5
Confirmatory Process

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

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

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

5.1.1 High-Resolution Mass Spectrometry

EPA Method 1613B[3] and SW-846 Method 8290[8] are both appropriate for low and trace-level analysis of dioxins and furans in a variety of matrices. They involve matrix-specific extraction, analyte-specific cleanup, and high-resolution capillary GC (HRGC)/HRMS analysis. The main differences between the two methods are that EPA Method 1613B has an expanded calibration range and requires use of additional $^{13}C_{12}$-labeled internal standards resulting in more accurate identifications and quantitations. The calibration ranges for the HRMS methods based on a typical 10-g sample and 20-microliter (µL) final sample volume are presented in Table 5-1.

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

Compound	EPA Method 1613B	SW-846 Method 8290
Tetra Compounds	1–400 pg/g	2–400 pg/g
Penta-Hepta Compounds	5–2,000 pg/g	5–1,000 pg/g
Octa Compounds	10–4,000 pg/g	10–2,000 pg/g

5.1.2 Low-Resolution Mass Spectrometry

SW-846 Method 8280 is appropriate for determining dioxins and furans in samples with relatively high concentrations, such as still bottoms, fuel oils, sludges, fly ash, and contaminated soils and waters. This method involves matrix specific extraction, analyte-specific cleanup, and HRGC/LRMS analysis. The calibration ranges in Table 5-2 are based on a typical 10-g sample size and 100-µL final volume.

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

Compound	SW-846 Method 8280
Tetra-Penta Compounds	1,000–20,000 pg/g
Hexa-Hepta Compounds	2,500–50,000 pg/g
Octa Compounds	5,000–100,000 pg/g

5.1.3 PCB Methods

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

5.1.4 Reference Method Selection

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

5.2 Characterization of Environmental Samples

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

5.2.1 Dioxins and Furans

Four aliquots of homogenized material and one unhomogenized (i.e., "as received") aliquot were prepared and analyzed for seventeen 2,3,7,8-substituted dioxins and furans following procedures in EPA Method 1613B. The homogenized and unhomogenized aliquots were each approximately 200 g. Depending on the anticipated levels of dioxins from preliminary information received from each sampling location, approximately 1 to 10 g of material were taken for analysis from each aliquot, and spiked with $^{13}C_{12}$-labeled internal standards, and extracted with methylene chloride using 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; however, extracts were not rigorously evaluated to ensure that all peaks were below the peak area of the highest calibration standard.

Each extract was analyzed by HRGC/HRMS in the selected ion monitoring (SIM) mode at a resolution of 10,000 or greater. A DB-5 column was used for analysis of the seventeen 2,3,7,8-PCDD/F congeners. The instrument was calibrated for PCDD/F at levels specified in Method 1613B with one additional calibration standard at concentrations equivalent to one-half the level of Method 1613B's lowest calibration point. Using a DB5 column, 2,3,7,8-TCDF is not separated from other 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/F data were reported as both concentration (pg/g dry) and TEQs (pg TEQ/g dry).

5.2.2 PCBs

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

5.2.3 PAHs

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

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

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

5.3 Reference Laboratory Selection

Based on a preliminary evaluation of performance and credibility, 10 laboratories were contacted and were sent a questionnaire geared toward understanding the capabilities of the laboratories, their experience with analyzing dioxin samples for EPA, and their ability to meet the needs of this demonstration. Two laboratories were selected for the next phase of the selection process and were sent three blind audit samples. Each laboratory went through a daylong audit that included a technical systems audit and a quality systems audit. At each laboratory, the audit consisted of a short opening conference; a full day of observation of laboratory procedures, records, interviews with laboratory staff; and a brief closing meeting. Auditors submitted followup questions to each laboratory to address gaps in the observations.

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

5.4 Reference Laboratory Sample Preparation and Analytical Methods

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

5.4.1 Dioxin/Furan Analysis

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

5.4.2 PCB Analysis

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

5.4.3 TEQ Calculations

For the reference laboratory data, D/F and PCB congener concentrations were converted to TEQ and subsequently summed to determine total TEQ, using the TEFs established by WHO in 1998 (see Table 4-1).[5] Detection limits were reported as sample-specific

detection limits (SDLs). SDLs were determined from 2.5 times the noise in the chromatogram for D/F and 3.0 times the noise for PCBs, converted to an area, and then converted to a concentration using the same calculation procedure as for detected peaks. Any value that met all quantification criteria (>SDL and isotope ratio) was reported as a concentration. A "J" flag was applied to any reported value between the SDL and the lowest level calibration. The concentration of any detected congener that did not meet all quantification criteria (such as isotope ratio or peak shape) was reported but given a "K" flag to indicate estimated maximum possible concentration (EMPC).[8] TEQs were reported in two ways to cover the range of possible TEQ values:

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

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

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

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

Chapter 6
Assessment of Reference Method Data Quality

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

6.1 QA Audits

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

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

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

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

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

6.2 QC Results

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

6.2.1 Holding Times and Storage Conditions

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

6.2.2 Chain of Custody

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

6.2.3 Standard Concentrations

The concentration of all calibration and spiking standards was verified.

6.2.4 Initial and Continuing Calibration

All initial calibrations met the criteria for response factor RSD and minimal signal-to-noise ratio requirements for the lowest calibration point.

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

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

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

6.2.5 Column Performance and Instrument Resolution

Column performance was checked at the beginning of each 12-hour analytical period and met method criteria.

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

6.2.6 Method Blanks

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

6.2.7 Internal Standard Recovery

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

6.2.8 Laboratory Control Spikes

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

6.2.9 Sample Batch Duplicates

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

6.3 Evaluation of Primary Objective P1: Accuracy

Accuracy was assessed through the analysis of PE samples consisting of certified standard reference materials, certified spikes, and certified blanks. A summary of reference method percent recovery (R) values is presented in Table 6-1. The R values are presented for TEQ_{PCB}, $TEQ_{D/F}$, and total TEQ. The minimum, maximum, mean, and median R values are presented for each set of TEQ results. The reference method values were in best agreement with the certified values for the TEQ_{PCB} results, with a mean R value of 96%. The mean R values for $TEQ_{D/F}$ and total TEQ were

Table 6-1. Objective P1 Accuracy - Percent Recovery

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

NA = not applicable; insufficient data were reported to determine R or the sample was not spiked with those analytes.

125% and 94%, respectively. The mean and median R values for the TEQ_{PCB} and total TEQ were identical. The mean and median R values for $TEQ_{D/F}$ were not similar and were largely influenced by the $TEQ_{D/F}$ recovery for ERA Aroclor of 324%. The ERA Aroclor-certified $TEQ_{D/F}$ values were based on TCDD and TCDF only, whereas the reference method $TEQ_{D/F}$ values were based on contributions from all 2,3,7,8-substituted D/F analytes. The R values presented in Table 6-1 indicate that the reference method reported data that were on average between 94 and 125% of the certified values of the PE samples.

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

6.4 Evaluation of Primary Objective P2: Precision

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

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

Table 6-2. Evaluation of Interferences

PE Material with Known Interference	Mean TEQ (pg/g)
ERA PAH	0.195 (D/F + PCB)
ERA PCB 100	0.073 (D/F)
ERA PCB 10000	0.220 (D/F)
ERA TCDD 10	0.025 (PCB)
ERA TCDD 30	0.036 (PCB)

analytes) as might be expected due to the very low levels detected in these samples.

6.5 Comparability to Characterization Data

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

6.6 Performance Summary

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

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

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

Sample Type	Sample ID	RSD for TEQ_{PCB} (%)	RSD for $TEQ_{D/F}$ (%)	RSD for Total TEQ (%)
Environmental	Brunswick #1	8	6	6
	Brunswick #2	3	16	16
	Brunswick #3	5	8	8
	Midland #1	4	9	9
	Midland #2	10	6	6
	Midland #3	4	6	6
	Midland #4	77	9	10
	NC PCB Site #1	21	15	20
	NC PCB Site #2	21	2	21
	NC PCB Site #3	25	12	24
	Newark Bay #1	7	28	25
	Newark Bay #2	2	22	20
	Newark Bay #3	6	6	6
	Newark Bay #4	1	12	11
	Raritan Bay #1	6	5	4
	Raritan Bay #2	3	2	1
	Raritan Bay #3	3	5	4
	Saginaw River #1	8	25	23
	Saginaw River #2	7	19	18
	Saginaw River #3	60	19	19
	Solutia #1	36	13	13
	Solutia #2	4	7	7
	Solutia #3	11	5	5
	Titta. River Soil #1	7	6	5
	Titta. River Soil #2	9	10	10
	Titta. River Soil #3	12	26	26
	Titta. River Sed #1	19	27	26
	Titta. River Sed #2	14	37	37
	Titta. River Sed #3	13	9	8
	Winona Post #1	13	2	2
	Winona Post #2	4	9	9
	Winona Post #3	9	4	4
Extract	Envir Extract #1	71	50	50
	Envir Extract #2	83	2	2
	Spike #1	119	6	9
	Spike #2	1	5	3
	Spike #3	4	13	4
PE	Cambridge 5183	7	19	9
	Cambridge 5184	3	4	2
	ERA Aroclor	44	6	43
	ERA Blank	62	65	61
	ERA PAH	83	27	30
	ERA PCB 100	4	65 [a]	3
	ERA PCB 10000	7	91	7
	ERA TCDD 10	60	5	5
	ERA TCDD 30	39	6	6
	LCG CRM-529	14	2[a]	1
	NIST 1944	4	9	7
	Wellington WMS-01	5	3	3

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

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

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

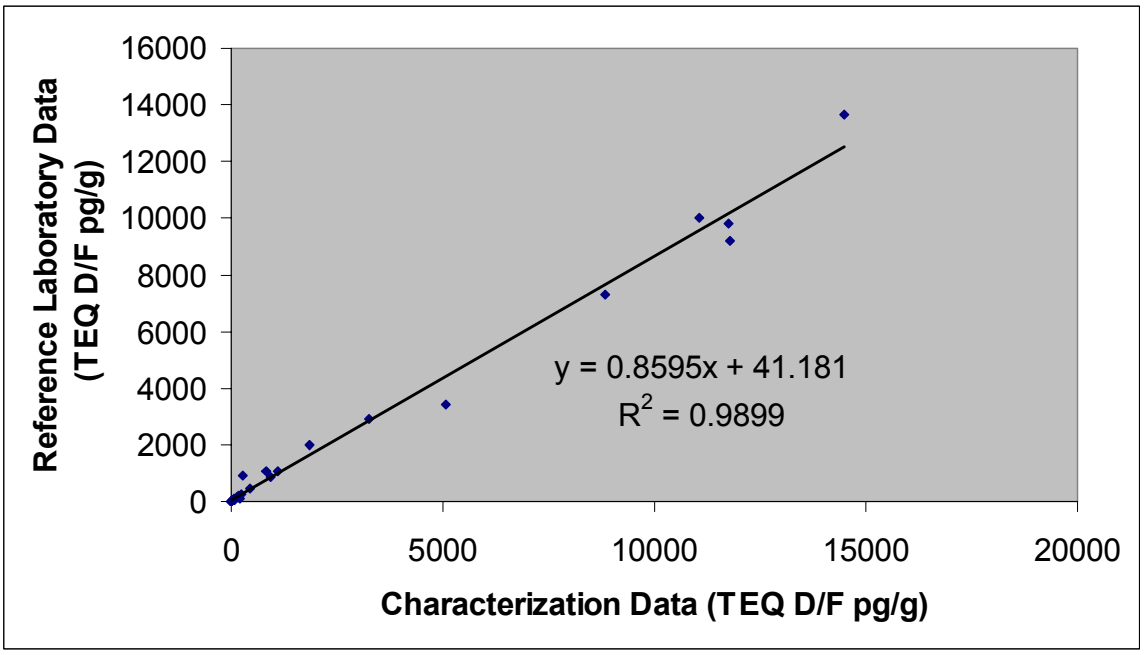


Figure 6-1. Comparison of reference laboratory and characterization D/F data for environmental samples.

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

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

Chapter 7
Performance of Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit

7.1 Evaluation of Dioxin ELISA Kit Performance

The Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit Wako (for environmental) is an immunoassay technology that reports total dioxin/furan concentration in a sample. It should be noted that the results generated by this technology may not directly correlate to HRMS $TEQ_{D/F}$ in all cases because it is known that the congener responses and cross-reactivity of the kit are not identical to the TEFs that are used to convert congener HRMS concentration values to $TEQ_{D/F}$. The effect of cross-reactivities may contribute to this technology's reporting results that are biased high or low compared to HRMS $TEQ_{D/F}$ results. Therefore, this technology should not be viewed as producing an equivalent measurement value to HRMS $TEQ_{D/F}$ but as a screening value to approximate HRMS $TEQ_{D/F}$ concentration. It has been suggested that correlation between the Wako and HRMS TEQ could be improved by first characterizing a site and calibrating the Wako results to HRMS results. Subsequent analysis using the Wako kit for samples obtained from this site may then show better correlation with the HRMS TEQ result. This approach was not evaluated during this demonstration.

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

7.1.1 Evaluation of Primary Objective P1: Accuracy

A summary of the Dioxin ELISA Kit Wako (for environmental) percent recovery (R) values is presented in Table 7-1. The description of how R values were calculated is presented in Section 4.7.1. The R values are calculated by comparing Wako's 2,3,7,8-TCDD EQ values with the certified $TEQ_{D/F}$ values. The minimum R value, the maximum R value, median R value, and the mean R value were 10%, 1,574%, 253%, and 443%, respectively. As presented in Table 7-1, only five R values could be calculated from the 12 PE samples because many of the PE samples were reported as nondetects by the Wako kit. Reporting these samples as nondetects in some cases was accurate. For example, ERA PCB 100, ERA PCB 10000, ERA Aroclor, and

Table 7-1. Objective P1 Accuracy - Percent Recovery

PE Sample ID	PE Sample Description	% Recovery	
1	Cambridge 5183 *	1574	
2	LCG CRM-529	10	
3	Wellington WMS-01	253	
4	Cambridge 5184	299	
5	NIST 1944	80	
6	ERA TCDD 10 *	NA	
7	ERA TCDD 30 *	NA	
8	ERA PAH *	NA	
9	ERA PCB 100 *	NA	
10	ERA PCB 10000 *	NA	
11	ERA Aroclor *	NA	
12	ERA Blank *	NA	
All Performance Samples		NUMBER	5
		MIN	10
		MAX	1574
		MEDIAN	253
		MEAN	443

NA = not applicable; insufficient data were reported to determine R or the sample was not spiked with those analytes

* No D/Fs were in the sample or the concentrations of D/Fs were outside of Wako's reporting range

ERA PAH contained only spiked PCBs or PAHs, so these samples should not have been detections for D/F. The lack of reported data for ERA TCDD 10 (certified $TEQ_{D/F}$ = 11 pg/g) and ERA TCDD 30 (certified $TEQ_{D/F}$ = 33 pg/g) indicates that these samples contained D/F concentrations that were below the detection capabilities of the kit. All four of the ERA TCDD 10 replicates were reported accurately by Wako as < reporting limits.

7.1.2 Evaluation of Primary Objective P2: Precision

A summary of the Dioxin ELISA Kit Wako (for environmental) RSD values is presented in Tables 7-2a and 7-2b. The description of how RSD values were calculated is presented in Section 4.7.2. The RSD values are presented in Table 7-2a for each sample where Wako reported values for three or more of the replicate samples. A summary by sample type is presented in Table 7-2b, along with the minimum RSD value, the maximum RSD value, the median RSD value, and the mean RSD value. Low RSD values (< 20 %) indicate high precision. In terms of sample type, the Dioxin ELISA Kit Wako (for environmental) values had the most precise data for the extract results, with a mean RSD value of 26%, but there were only two RSD values averaged in the extract value while the environmental and PE sample sets had considerably more values to average (16 and 6, respectively). Overall RSD values ranged from 11% to 145%, with a mean RSD of 62%.

7.1.3 Evaluation of Primary Objective P3: Comparability

The description of the statistical analyses used in the comparability evaluations are described in Section 4.7.3. The comparability of the Wako and reference laboratory data was assessed by calculating RPD for the $TEQ_{D/F}$ values, as presented in Table 7-3. The summary statistics presented in Table 7-3 provide an overall assessment of the RPD values. The Wako values agreed best with the reference laboratory D/F measurements for extract samples, with a median RPD value of -1%. The median RPD values for the environmental, PE, and overall $TEQ_{D/F}$ values were 52%, -37%, and 34%, respectively, with minimum and maximum overall values around minus 200% and positive 200%, respectively. RPD values between positive and negative 25% indicate good agreement between the reference laboratory and developer values. Of the $TEQ_{D/F}$ samples, 19 (18%) of the samples had RPD values between positive and negative 25%.

The agreement when sorting the developer and reference laboratory results for $TEQ_{D/F}$ into four intervals ($\leq$ 50 pg/g, 50 to 500 pg/g, 500 to 5,000 pg/g, and $\geq$ 5,000 pg/g) are described in Table 7-4. The agreement between the developer and reference laboratory data was 62%. Note that, as described in Section 4.7.3, results that were reported as semiquantitative results were counted as in agreement if the reference laboratory data was within that interval. For example, a reference laboratory result reported as 3,400 pg/g $TEQ_{D/F}$ was counted as in agreement with a Wako result reported as > 2,967 pg/g 2,3,7,8 TCDD EQ, even though the absolute quantitative values would be in different ranges.

Interval reporting addresses the question of whether a value reported by the technology would result in the same decision of what to do next with the sample if it was analyzed by the reference method. This interval assessment table indicates that 38% of the time, the Wako result would have indicated a different interval (and therefore a different decision to be made about the sample) than if it was analyzed by the reference laboratory, based on the concentration ranges chosen for these intervals.

The ERA blank samples contained levels of D/Fs that were below the reporting limits of the developer technologies (see Table 4-4 certified value: 0.046 pg/g $TEQ_{D/F}$). Wako reported concentrations were compared with the reference laboratory reported data for these samples in Table 7-5. Wako reported one of the eight values as a detection (114.00 pg/g) and seven results were reported as nondetects. As such, 88% agreed with the reference laboratory results. It should be noted that the reference laboratory data presented in Table 7-5 were calculated with nondetect values assigned a zero concentration. When applying the TEQ calculation method of assigning nondetects with a concentration of one-half the SDL, the reference data increased, but the conclusions regarding agreement with the developer data remain the same.

Table 7-2a. Objective P2 Precision - Relative Standard Deviation (All Samples)

Sample Type	Sample ID	Relative Standard Deviation (%)[a]
Environmental	Brunswick #1	NA
	Brunswick #2	NA
	Brunswick #3	74
	Midland #1	60
	Midland #2	35
	Midland #3	65
	Midland #4 *	NA
	NC PCB Site #1 *	NA
	NC PCB Site #2 *	NA
	NC PCB Site #3 *	NA
	Newark Bay #1 *	NA
	Newark Bay #2	NA
	Newark Bay #3	NA
	Newark Bay #4 *	NA
	Raritan Bay #1 *	NA
	Raritan Bay #2 *	NA
	Raritan Bay #3 *	NA
	Saginaw River #1	88
	Saginaw River #2	34
	Saginaw River #3	43
	Solutia #1	48
	Solutia #2	60
	Solutia #3	108
	Titta. River Soil #1	NA
	Titta. River Soil #2	16
	Titta. River Soil #3	74
	Titta. River Sed #1 *	NA
	Titta. River Sed #2	105
	Titta. River Sed #3 *	NA
	Winona Post #1	13
	Winona Post #2	60
	Winona Post #3	70
Extracts	Envir. Extract #1	41
	Envir. Extract #2	NA
	Spike #1 *	NA
	Spike #2	11
	Spike #3 *	NA

Sample Type	Sample ID	Relative Standard Deviation (%)[a]
PE	Cambridge 5183 *	47
	Cambridge 5184	145
	ERA Aroclor *	NA
	ERA Blank *	NA
	ERA PAH *	NA
	ERA PCB 100 *	NA
	ERA PCB 10000 *	82
	ERA TCDD 10 *	NA
	ERA TCDD 30 *	NA
	LCG CRM-529	64
	NIST 1944	54
	Wellington WMS-01	100

NA = not applicable (i.e., one or more of the replicates were reported as a nondetect value).

* No D/Fs were in the sample or the concentrations of D/Fs were outside of Wako's reporting range.

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

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

Sample Type	Relative Standard Deviation (%)				
	No.	MIN	MAX	MED	MEAN
Environ.	16	13	108	60	60
Extract	2	11	41	26	26
PE	6	47	145	73	82
Overall	24	11	145	60	62

Table 7-3. Objective P3 - Comparability Summary Statistics of RPD

Sample Type	$TEQ_{D/F}$ RPD (%)			
	N	MIN	MAX	MEDIAN
Environmental	76	-195	198	52
Extract	10	-198	98	-1
PE	20	-185	180	-37
Overall	106	-198	198	34

Table 7-4. Objective P3 - Comparability Using Interval Assessment

Agreement	$TEQ_{D/F}$
Number Agree	127
% Agree	62
Number Disagree	78
% Disagree	38

Table 7-5. Objective P3 - Comparability for Blank Samples

Replicate	Wako (pg/g 2,3,7,8-TCDD EQ)	Ref Lab[a] (pg/g $TEQ_{D/F}$)	Agree?
1	< 17.80	J0.0942 [b]	Yes
2	< 34.30	J0.0728	Yes
3	< 17.80	J0.237	Yes
4	< 34.30	J0.307	Yes
5	< 26.30	J0.113	Yes
6	114.00	J0.0524	No
7	< 45.30	J0.211	Yes
8	< 26.30	J0.0692	Yes
% agree	88% (7 of 8)		

[a] All nondetect and EMPC values were assigned a zero concentration for the reference laboratory TEQ calculation.
[b] J flag was applied to any reported value between the SDL and the lowest level calibration.

7.1.4 Evaluation of Primary Objective P4: Estimated Method Detection Limit

It should be noted that these detection limit calculations did not strictly follow the definition as presented in the *Code of Federal Regulations* (i.e., t-value with 6 degrees of freedom). PE samples in the precisely appropriate range for evaluation of this technology's detection limit were not available. Since detections were not reported for all seven replicate samples, the degrees of freedom and statistical power of the analysis were reduced accordingly. The only approach that led to the use of the definitional calculation with 6 degrees of freedom required special treatment of the nondetect values (i.e., assigning values that were one-half or equal to the nondetect value). However, these calculations are provided as estimated method detection limits (EMDLs) to give the reader a sense of the detection capabilities of the technology.

The EMDL of the Dioxin ELISA Kit Wako (for environmental) was determined by assessing the values that Wako reported for three PE samples: Wellington WMS-01, Cambridge 5183, and Extract Spike #1. The data from the Wellington sample were evaluated, but determined to have a D/F concentration (62 pg/g $TEQ_{D/F}$) that was higher than appropriate for evaluation of EMDL; they were not included. As shown in Table 7-6, because some of the results for the samples were nondetects, the $TEQ_{D/F}$ EMDL was calculated in three ways: by setting nondetect values to zero, by setting nondetect values to half of the reporting limit value, and by setting nondetect values to the reporting limit value itself. For the Extract Spike #1 samples, Wako reported only two samples as actual values (as opposed to nondetects); therefore, an EMDL could not be calculated for those samples by setting nondetect values to zero. One replicate for Cambridge 5183 (Wako 115) was reported as > 3,167 pg/g 2,3,7,8-TCDD EQ; that value was excluded as an outlier. The MDLs for the Cambridge 5183 and Extract Spike #1 samples ranged from 83 to 201 pg/g 2,3,7,8-TCDD EQ. The detection limit reported by Wako in the demonstration plan was 20 pg/g 2,3,7,8-TCDD EQ. PE samples with TEQ concentrations in the precisely appropriate range for evaluation of this technology's detection limit were not available, so these calculated values should be considered a rough estimate.

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

The description of false positive/false negative calculations is presented in Section 4.7.5. The summary of false positive/false negative results is presented in Table 7-7. In this evaluation, Wako results, reported in pg/g 2,3,7,8-TCDD EQ, were compared to the reference laboratory's results reported in pg/g $TEQ_{D/F}$. Wako reported 20 false positive (10%) and 26 false negative (13%) results, relative to the reference laboratory's reporting of samples above and below 20 pg/g $TEQ_{D/F}$. For samples around 50 pg/g $TEQ_{D/F}$, Wako's rate of false positives was the same (10%); but, there were fewer false negatives. As described in Section 7.1.4, the EMDLs were estimated to be 83 to 201 pg/g 2,3,7,8-TCDD EQ; but, because the concentration of PE samples

Table 7-6. Objective P4 - Estimated Method Detection Limit

Statistic	Cambridge 5183[a]			Extract Spike #1		
	Nondetect values set to zero	Nondetect values set to ½ value	Nondetect values set to reported value	Nondetect values set to zero	Nondetect values set to ½ value	Nondetect values set to reported value
Degrees of Freedom	2	5	5	NA	6	6
SD (pg/g 2,3,7,8-TCDD EQ)	29	30	25		32	26
EMDL (pg/g 2,3,7,8-TCDD EQ)	201	102	84		102	83

[a] Excludes Wako 115, which was reported as > 3,167 pg/g.
NA = not available (insufficient data to calculate).

Table 7-7. Objective P5 - False Positive/False Negative Results

Rate	$TEQ_{D/F}$	
	20 pg/g	50 pg/g
False Positive	10% (20 out of 207)[a]	10% (21 of 207)
False Negative	13% (26 out of 207)	8% (16 of 207)

[a] 207 sample results were evaluated instead of 209 due to two reference laboratory data points which were discarded due to sample preparation errors. See Section 6.4.

were not the most appropriate for evaluation of this technology's MDL, the calculated EMDLs should be considered rough estimates. Higher EMDLs are the result of less precise data at low concentration levels. The false positive and false negative results were calculated using the entire range of concentrations and demonstrate that Wako's results agreed with laboratory results, relative to the screening levels, for the majority (~80%) of the samples. As such, this evaluation suggests that the Wako kit could be an effective screening tool for determining sample results above and below 20 pg/g $TEQ_{D/F}$ and even more effective as a screen for samples above and below 50 pg/g $TEQ_{D/F}$.

7.1.6 Evaluation of Primary Objective P6: Matrix Effects

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

- Measurement location: not evaluated (all samples analyzed on-site)
- Matrix type: none
- Sample type: none
- PAH concentration: none
- Environmental site: none
- Known interferences: slight

In Table 7-8, precision summary values are presented by matrix type. A one-way ANOVA model was used to test the effect of soil vs. sediment vs. extract on RSD. These tests showed no significant effect on RSD. In Table 7-9, precision summary values are presented by PAH concentrations for environmental samples only. A one-way ANOVA model was used to test the effect of PAH concentration on RSD. These tests showed no effect. The summary of RSD values segregated by sample type is presented in Table 7-2b. A one-way ANOVA model was used to test the effect of sample type (PE vs. environmental vs. extract) on RSD. These tests showed no significant effect on RSD. The average RSD for the extracts (26%) was considerably less than that of the environmental samples (60%) and PE samples (82%), but there were only two RSD values averaged in the extract value while the environmental and PE sample sets had considerably more values to average (16 and 6, respectively). Based on the comparability results (RPD values), Wako's results were not more or less comparable for one particular environmental site, suggesting that matrix effects were not dependent on environmental site.

Table 7-8. Objective P6 - Matrix Effects Using RSD as a Description of Precision by Matrix Type

Matrix Type	RSD for $TEQ_{D/F}$ (%)				
	N	MIN	MAX	MED	MEAN
Soil	15	13	145	63	60
Sediment	7	34	105	71	74
Extract	2	11	41	26	26
Overall	24	11	145	62	60

Table 7-9. Objective P6 - Matrix Effects Using RSD as a Description of Precision by PAH Concentration Levels (Environmental Samples Only)

PAH Concentration Level (ng/g)	RSD for $TEQ_{D/F}$ (%)				
	N	MIN	MAX	MED	MEAN
> 100,000	1	74	74	74	74
10,000–100,000	3	13	70	48	60
1,000–10,000	7	34	108	64	60
< 1,000	5	16	105	57	48
Overall (Environmental Samples Only)	16	13	108	60	60

The effect of known interferences was also assessed by evaluating the results of PE materials that contained one type of contaminant (PCBs or PAHs) but was not spiked with D/Fs. Table 7-10 summarizes the detections for D/F reported by Wako for these PE samples. For the ERA PAH sample that contained only spiked PAHs, Wako reported three values as nondetects and one as 38.5 pg/g 2,3,7,8-TCDD EQ. The PCB-only spiked samples were reported with two detections (mean 132 pg/g 2,3,7,8-TCDD EQ) for the lower PCB spike and all four replicates reported as D/F detects (mean 76 pg/g 2,3,7,8-TCDD EQ) for the higher PCB spike.

7.1.7 Evaluation of Primary Objective P7: Technology Costs

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

7.2 Observer Report: Evaluation of Secondary Objectives

Wako's dioxin ELISA screening kit is an anti-dioxin monoclonal antibody in an ELISA format. The following procedural steps were observed: ASE of a 10-gram sample aliquot (this procedure was omitted for samples received as extracts), evaporation by nitrogen to dryness, multilayer silica cleanup, concentration by large TurboVap, phthalocyanine immobilized silica cleanup, solvent exchange to 50 microliters of dimethyl sulfoxide (DMSO) using nitrogen evaporation, addition of buffer and antibody, incubation, addition of conjugate, addition of reaction mixture to microplate wells, incubation, plate washing, addition of color-developing solution and stop solution, and analysis with a microplate reader at 450 nanometers. Each plate contained a seven-point curve and four QC samples prepared in the same manner as the sample extracts. Each sample extract was processed in duplicate during the ELISA process. A total of 40 samples could be analyzed with a 96-well plate. Analysis was accomplished in a few seconds with the operating software generating the curve and sample results almost simultaneously. The software marked samples that were out of range and these samples were reanalyzed with a dilution of another 10-µL aliquot.

The demonstration plan only stated the procedural steps for the ELISA kit and did not address the extraction and sample extract cleanup necessary for this technology. Also, the operating procedure listed in the demonstration plan did not match the operating procedure that was observed. The kit's instructions did match the procedures observed including an extraction

Table 7-10. Objective P6 - Matrix Effects Using PE Materials

PE Sample	% Recovery for Spiked Analytes [a]	Mean TEQ (pg/g 2,3,7,8-TCDD EQ) Reported by Wako for Analytes that were not Spiked in the PE Sample
ERA PAH	NA	38.5[b]
ERA PCB 100	NA	132 [c]
ERA PCB 10,000	NA	76

[a] NA = not applicable; percent recovery value could not be calculated.

[b] Three replicates were reported as nondetects.

[c] Two replicates were reported as nondetects.

and cleanup flow diagram, as well as detailed instructions for kit operation.

7.2.1 Evaluation of Secondary Objective S1: Skill Level of Operator

From observation, a fully trained environmental chemistry technician would be the minimum qualifications of a person capable of meeting the kit's extraction and cleanup needs. Wako's ELISA kit only would require a user skilled in pipetting and computing to generate accurate data. Wako prefers only experienced personnel use the kit. The operators of the technology at the demonstration were highly trained laboratory staff. Each process in the system was operated by personnel highly experienced with that process. For example, all ASE extractions observed were conducted by an ASE specialist. The ELISA operation was also completed by someone with significant ELISA experience. If one person was going to perform all steps of the kit from sample extraction to analysis, that person would have to be highly trained in all areas of the sample process system. A person would also have to be very conscious of contamination reduction in such areas as glassware washing and equipment cleaning, considering several components of the system are non-disposable and must be reused. The personnel in the following tabulation were observed processing demonstration samples for Wako:

Operator Name	Job Title	Education Level	Years of Experience
Tomohiro Itoh	Researcher	Bachelor of Engineering	1 year
Sheldon Henderson	ASE Product Marketing Specialist	Master of Business Administration	15 years
Kimihiko Sano	Experimental Assistant	Master of Pharmacy	half year
Nobukazu Miyamoto	Researcher	Doctor of Agriculture	3 years
Minoru Imokawa	Researcher	Bachelor of Agriculture	3 years
Masako Hayakawa	Experimental Assistant	Bachelor of Pharmacy	half year

The provided instructions were in Japanese but were translated for the observer by Wako. Once translated, the instructions were very easy to follow. One step, which might be better explained in the instructions, was the time intervals between each sample receiving some component of the reaction solutions. For example, the analysts very carefully added a solution to a sample, waited 10 seconds, and then added the same solution to the next sample in line. A better explanation of this practice and why it is necessary would be beneficial. The sample process could be stopped, well sealed, and stored at room temperature after extraction, after any concentration, or after any column cleanup without any adverse effects. In addition, the ELISA process contains an 18- to 20-hour refrigerated incubation period. According to the developers, once the extracts are in DMSO, it is stable for 3 weeks. The recommended extraction and extract cleanup requires large amounts of hazardous solvents, large laboratory equipment, significant amounts of dedicated bench-top space, and fume hoods. Although the actual kit is simplistic, the observed sample processing prior to kit use is very complex. Wako only supplies the kit and does not process samples for users.

7.2.2 Evaluation of Secondary Objective S2: Health and Safety Aspects

The sample process generated a large amount of hazardous waste including: flammable solvents, spent reactants (corrosive impregnated silica, copper, sodium

sulfate), spent samples, and glass (pipettes, disposable cleanup columns, filters). The process also generated small amounts of hazardous aqueous waste. A considerable amount of lab trash was also generated which included used personal protective equipment, absorbent paper, and aluminum foil. A complete inventory of the waste generated was performed after the demonstration for the processing of 209 samples by Wako and the following was recorded. None of the containers was verified as full. Also, some items left as waste could have been reused, but were not because of the cost shipping them back to Japan.

(1) Three boxes of used silica cleanup columns
(2) One 5-gallon container with broken glass and used phthalocyanine immobilized silica columns
(3) One large box with almost empty solvent bottles (some bottles contained approximately 100 mL of solvent)
(4) Four large boxes of used ASE collection vials
(5) Three gallons of spent organic solvent contained in a large container
(6) Eleven 5-gallon containers with spent organic solvents
(7) One 5-gallon container with used ASE filters
(8) Nine filled broken glass boxes that contained glass waste

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

7.2.3 Evaluation of Secondary Objective S3: Portability

The observed Dioxin ELISA Kit Wako (for environmental) required an exacting sample extraction and cleanup, similar to what is required for traditional HRMS dioxin analysis. The extraction and sample clean-up required one mobile lab fully outfitted with fume hoods, water, nitrogen and/or purified compressed air, and significant electrical power (~ 15 kVA power for the mobile laboratory). The ELISA procedure required less infrastructure, requiring only a refrigerator, watch, pipettes, small bench space, microplate reader with computer, and a controlled environment. Wako decided to segregate the ELISA operations in another trailer, which also accommodated material storage. This set up took six Wako personnel approximately one and a half days to become operational. Although the developer used both a mobile lab and a trailer, the observer felt that one mobile lab would be sufficient to house the sample processing system if adequate storage cabinets were provided. To accommodate the guidance given in the kit instructions, one would have to bring a fully functional environmental lab to the field. Once these accommodations are met, the turnaround time of samples would possibly be faster in the field than a remote lab simply because no sample shipment is needed. During the demonstration, Wako used four nitrogen cylinders during sample preparation.

7.2.4 Evaluation of Secondary Objective S4: Throughput

All 209 samples were processed by Wako in the field by the eighth day. To accomplish this, two Dionex ASE extractors were employed along with three various nitrogen evaporators, fume hood, sink, refrigerator, computer, microplate reader, at least 15 feet of bench space for column cleanups, compressed filtered air and/or compressed nitrogen, and hazardous and nonhazardous material storage areas. This was all distributed between one mobile lab and one trailer. Five personnel were needed to operate the process at this speed. Wako lost 6 to 8 hours of sample processing due to meetings, Visitor's Day, and nitrogen supply line problems (leaks).

The developer stated it could process 40 samples in three days using the process that was observed in the field. This process involved 3.5 personnel for sample extraction and preparation and 1.5 personnel for sample analysis. The process is not amenable to an inexperienced user. One person had multiple roles, including performing the phthalocyanine immobilized silica cleanup, solvent exchange into DMSO, and software operator of the microplate reader. As part of the observation, 23 samples received as extracts were processed in just over 24 hours. It was common during the week of observation to see simultaneously 40 samples being extracted on an ASE; 40 samples being put through a multilayer silica column; and another 24 samples being put through phthalocyanine immobilized silica columns. The process did seem to bottleneck at the DMSO solvent exchange step early in the demonstration, but near the end of the first week the

DMSO solvent exchange operator was able to keep up with the sample flow. Based on observation, regardless of how small a sample batch size is, no data could be generated in less than two days (two 12-hours shifts) mainly because of the minimal 18-hour incubation period. The developer's estimate that 40 samples could be completed in three days seems reasonable. In fact, with the system that was set up in the field, 40 samples went through extraction one day, sample preparation the second day and analysis the third day. Meaning, if the flow of samples was maintained, eventually 40 samples could be finished every day.

7.2.5 Miscellaneous Observer Notes

According to the developer, the kit is usually sold to experienced clients who require very little support or training. Wako provides training classes and phone support that easily meets the needs of their experienced clients. The kit is primarily sold in Japan, but Wako would provide a training video if kits are sold in the United States. Wako currently has several sales offices in the United States.

The developer stated that the kit is readily available and is supplied with the following materials:

(1) One 96-well plate with the wells coated with secondary antibody
(2) Positive Control tube
(3) PC solutions
(4) Buffer B solution
(5) Primary antibody solution
(6) Purified water
(7) Buffer A
(8) POD-conjugate solution
(9) Parafilm
(10) Reaction mixture
(11) Wash solution
(12) Color developing solution
(13) Citrate buffer
(14) Stop solution
(15) Dilution tubes
(16) Instructions

All materials needed for the suggested extraction and cleanup would have to be supplied by the user. The extraction and cleanup materials are, however, listed in the kit instructions. Also, the following materials for the ELISA process would have to be supplied by the user and, unless noted, are listed in the "materials needed" section of the kit instructions:

(1) Refrigerator
(2) Sample tubes and rack
(3) Micropipettes
(4) Methanol
(5) Acetone
(6) Stirring rods
(7) Paper towels*
(8) Scotch tape*
(9) Aluminum foil*
(10) Microplate reader
(11) Watch*

* not listed on the materials needed in the instructions

Each microplate submitted for analysis contains a seven-point curve and three QC samples (samples with known amount of dioxin). Each sample, including the three QC samples, is run in duplicate. These are considered requirements and are outlined in the instructions. The observer felt that it would be beneficial to confirm results by traditional HRMS analysis at or near any action levels required by a program using this technology, but this was not required by Wako for operation of the technology. No extractions and/or cleanup QC (such as method blanks, sample extraction duplicates, etc.) was mentioned by the developers or the kit's instructions.

Chapter 8
Economic Analysis

During the demonstration, the Wako kit and the reference laboratory analytical methods were each used to perform more than 200 sample analyses, including samples with a variety of distinguishing characteristics such as high levels of polychlorinated biphenyls and PAHs. Collectively, the samples provided different levels and types of contamination necessary to properly evaluate the technologies and to perform a comprehensive economic analysis of each technology. The purpose of the economic analysis was to estimate the total cost of generating results by using the Wako kit and then comparing this cost to the reference method. This cost estimate also is provided so that potential users can understand the costs involved with using this technology.

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

8.1 Issues and Assumptions

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

8.1.1 Capital Equipment Cost

The capital equipment cost was the cost associated with the purchase of the Dioxin ELISA Kit Wako (for environmental). Components of the kit are presented in detail in Chapter 2 and 7. The cost information was obtained from a standard price list provided by Wako.

8.1.2 Cost of Supplies

The cost of supplies was estimated based on the supplies required to analyze all demonstration samples using the Dioxin ELISA Kit Wako (for environmental) that were not included in the capital equipment cost category. Examples of such supplies include filters, cleanup columns, gas cylinders, solvents, and distilled water. The supplies that Wako used during the demonstration fall into two general categories: consumable (or expendable) and reusable. Examples of expendable supplies utilized by Wako during the demonstration include hexane, acetone, fluorobenzene, toluene, distilled water, nitrogen cylinders, and Eppendorf pipettes. Examples of reusable supplies include a microplate reader, accelerated solvent extractor, and concentrators. It should be noted that this type of equipment may or may not be already owned by a potential Dioxin ELISA Kit Wako (for environmental) user; however, for this economic analysis, an assumption was made that the user does not possess these items.

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

8.1.3 Support Equipment Cost

This section details the equipment used at the demonstration such as the mobile laboratory, construction trailer, fume hood, and laptop computer required by the technology. Costs for these items will be reported per actual costs for the demonstration.

8.1.4 Labor Cost

The labor cost was estimated based on the time required for work space setup, sample preparation, sample analysis, and reporting. For the demonstration, developers reported results by submitting a chain-of-custody (COC)/results form. The measurement of the time required for Wako to complete all 209 sample analyses during the field demonstration (522 labor-hours) was estimated by the sign-in log sheets that recorded the time the Wako operators were on-site. Time was removed for site-specific training activities and Visitors Day. Time estimates were rounded to the nearest hour.

During the demonstration, the skill level required for the operators to complete analyses and report results was evaluated. As stated in Section 7.2.1, based on the field observations, a fully trained environmental chemistry technician would be the minimum qualifications of a person capable of meeting the kit's extraction and cleanup needs. Wako's ELISA kit only would require a user skilled in pipetting and computing to generate accurate data. Four technicians are needed for pretreatment, while two technicians are needed for ELISA. Furthermore, specific technical training would be necessary to use the accelerated solvent extractor. This information was corroborated by Wako.

Education levels of the actual field operators are included in Section 7.2.1. For the economic analysis, costs were estimated using both actual and projected necessary skill levels for operators.

8.1.5 Investigation-Derived Waste Disposal Cost

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

8.1.6 Costs Not Included

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

Electricity. During the demonstration, some of the capital equipment was operated using AC power. The costs associated with providing the power supply were not included in the economic analysis as it is difficult to estimate the electricity used solely by the Wako technology. The total cost for electricity usage over the 10-day demonstration was $288. With seven mobile labs/trailers and miscellaneous equipment being operated continuously during the course of the demonstration, the cost of Wako electricity usage would be no more than $82. There was significantly more cost (approximately $13,000) to install an electrical board and additional power at the demonstration site, but this was a function of the demonstration site and not the responsibility of the individual developers, so this cost was not included in the economic analysis.

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

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

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

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

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

Items Costing Less Than $10. The cost of inexpensive items was not included in the economic analysis when the estimated cost was less than $10. Items where it is estimated that the cost was less than $10 included:
- Distilled water
- Personal protective equipment
- Waste containers
- Lab stools.

8.2 Dioxin ELISA Kit Costs

This section presents information on the individual costs of capital equipment, supplies, support equipment, labor, and IDW disposal for the Dioxin ELISA Kit Wako (for environmental), as well as a summary of these costs. Additionally, Table 8-1 summarizes the kit costs. As described in Section 4.6, Wako analyzed all 209 samples during the field demonstration and zero samples in its laboratory (total 209 demonstration samples). It is important to note that costs estimated in this section are based on actual costs to analyze the samples during the field demonstration. Cost estimates for analyzing the entire set of 209 demonstration samples were then determined based on the field demonstration costs, even though a subset of 24 samples was reanalyzed in Wako's laboratories. Because the number of samples analyzed in the field is equal to the number of samples in the entire demonstration set for Wako, itemized costs for the field demonstration samples and the entire set of demonstration samples will be identical.

8.2.1 Capital Equipment Cost

The capital equipment cost was the cost associated with the purchase of the technology in order to perform sample preparation and analysis. The Dioxin ELISA Kit Wako (for environmental) can be purchased for $898. One kit contains enough supplies for 42 samples (duplicate analyses per sample). In the demonstration, eight wells were used per sample, so only 10 samples were analyzed per plate. Because the kit is consumable, Wako does not rent the kit. During the field demonstration, Wako utilized 21 ELISA kits for approximately nine days to analyze 209 samples.

8.2.2 Cost of Supplies

The supplies that Wako used during the demonstration fall into two general categories: expendable or reusable. Table 8-1 lists all the expendable and reusable supplies that Wako used during the demonstration and the corresponding costs. Expendable supplies are ones that are consumed during the preparation or analysis. Reusable costs are items that must be used during the analysis but ones that can be repeatedly reused. The estimated life of reusable supplies could not be assessed during this economic analysis.

The total cost of the supplies employed by Wako during the demonstration was $81,958. Supplies have to be purchased from a retail vendor of laboratory supplies. Reusable items listed in Table 8-1 can be substituted with other models that operate under the same specifications, thereby modifying the cost of supplies to the potential kit user. Costs to rent or lease rather than purchase resusable items were not provided by Wako.

8.2.3 Support Equipment Cost

Wako analyzed demonstration samples in a 32-foot mobile lab equipped with two fume hoods and one 32-foot construction trailer equipment with a refrigerator. The rental cost for the mobile lab for use during sample extraction and sample analysis was $3,500, while the rental cost for the construction trailer was $1,919. The minimum rental rate for both the mobile lab and construction trailer was one month. Wako only used the mobile laboratory and construction trailer for two weeks. Since weekly or daily rental rates for the mobile lab were not an option, the entire cost is reported. A laptop computer is a necessity for the efficient operation of this technology. This is a one-time purchase that is reusable.

Table 8-1. Cost Summary

Item	Quantity Used During Field Demo		Unit Cost ($)[a]	Cost ($) 209 samples
Capital Equipment				
Purchase of Dioxin ELISA Kit	21	Kits	898	18,858 [b]
Supplies				
Expendable				
Nitrogen Cylinder	5	unit	31	155
Cylinder Regulator	2	unit	182	364
Presep® Multilayer Silica Gel (5 pcs.)	1	unit	165	165
Presep® Phthalocyanine Immobilized Silica Gel (10 pcs.)	10	unit	330	330
Copper, Reduced, Granular (100 g)	1	unit	91	91
Toluene (3-liter)	1	unit	76	76
Acetone (3-liter)	1	unit	63	63
Hexane (3-liter)	1	unit	61	61
Fluorobenzene (10 g)	1	unit	26	26
DMSO (500 mL)	1	unit	27	27
Eppendorf Pipette and Tips	1	unit	1,000	1,000
Reusable				
Accelerated Solvent Extractor	1	unit	44,000	44,000
TurboVap-LV Concentrator	1	unit	8,000	8,000
TurboVap-500 Concentrator	1	unit	8,000	8,000
Pressurized Gas Blowing Concentrator	1	unit	100	100
Low Temperature Circulator	1	unit	5,000	5,000
Compressor	2	unit	5,000	5,000
Microplate Reader	1	unit	9,000	9,000
Refrigerator	1	unit	500	500
Support Equipment				
Mobile Laboratory	1	unit	3,500	3,500
Construction Trailer	1	unit	1,919	1,919
Laptop Computer	1	unit	1,000	1,000
Labor				
Operator	522	labor hours	80[c]	41,891
IDW Disposal[d]	1	unit	1,168	1,168
Total Cost				**$150,294**

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

[b] Wako used 21 kits during the demonstration because they analyzed more sample replicates than are normally performed. One kit can be used for 42 samples when duplicate sample analyses are performed, so the cost of the kits to perform 209 sample analyses would have been greatly reduced ($4,490) if duplicates were performed.

[c] Labor rate for field technicians to operate technology rather than research scientists was $50.75 an hour, $26,492 for 209 samples.

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

8.2.4 Labor Cost

As described in Section 8.1.4, 522 labor-hours were spent in the field to analyze 209 samples. An hourly rate of $32.10 was used for a research scientist performing sample extractions and sample analysis, and a multiplication factor of 2.5 was applied to labor costs in order to account for overhead costs.[9] Based on this hourly rate and multiplication factor, a labor rate of $41,891 was determined for the analysis of the 209 samples during the field demonstration.

Based on observation, it is anticipated that lower-cost field technicians, with proper training and skill levels, could have analyzed the samples in a similar amount of time. As such, the labor rate for the analysis of 209 samples during the field demonstration could have been as low as $26,492 (hourly rate of $20.30 with 2.5 multiplication factor for 522 labor-hours).

8.2.5 Investigation-Derived Waste Disposal Cost

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

During the demonstration, Wako analyzed 209 samples. The total cost to dispose of the waste generated for these samples was $1,168.

8.2.6 Summary of Dioxin ELISA Kit Costs

The total cost for performing dioxin analyses using the Dioxin ELISA Kit Wako (for environmental) during the field demonstration was $150,294. The dioxin analyses were performed for 58 soil and sediment PE samples, 128 soil and sediment environmental samples, and 23 extracts. When Wako performed multiple dilutions or reanalyses for a sample, these were not included in the number of samples analyzed.

The total cost of $150,294 for analyzing the demonstration samples under the Wako kit included $18,858 for capital equipment; $81,958 for supplies; $6,419 for support equipment; $41,891 for labor; and $1,168 for IDW disposal. Of these five costs, the largest cost was for the supplies (55 percent of the total cost).

8.3 Reference Method Costs

This section presents the costs associated with the reference method used to analyze the 209 demonstration samples for dioxin. Typical costs of these analyses can range from $800 to $1,100 per sample, depending on the method selected, the level of quality assurance/quality control incorporated into the analyses, and reporting requirements. The reference laboratory utilized EPA Method 1613B for all soil and sediment samples for comparison with the Dioxin ELISA Kit Wako (for environmental). The reference method costs were calculated using information from the reference laboratory invoices.

To allow an accurate comparison of the Dioxin ELISA Kit Wako (for environmental) and reference method costs, the reference method costs were estimated for the same number and type of samples as was analyzed by Wako. For example, although the reference laboratory analyzed soil and sediment samples for coplanar PCBs, the associated sample analytical costs were not included in the reference method costs because Wako did not analyze samples for PCBs during the demonstration.

Table 8-2 summarizes the projected and actual reference method costs. At the start of the demonstration, the reference laboratory's projected cost per sample was $785 for dioxin/furan analysis. This cost covered the preparation and analysis of the demonstration samples, required method QC samples, electronic data deliverable, and the data package for each. The actual cost for the 209 demonstration analyses was $213,580 for D/F. This was higher than the projected ($162,915) due to reanalysis, re-extractions, dilutions, and additional cleanups that were above the 30% repeats allowable by the original quote. The turnaround time by the reference laboratory for reporting all 209 samples was approximately eight months (171 business days). The quoted turnaround time was three months.

8.4 Comparison of Economic Analysis Results

The total costs for the Dioxin ELISA kit Wako (for environmental) ($150,294) and the reference method ($213,580) are listed in Tables 8-1 and 8-2, respectively. The total cost for the use of the Wako kit was $63,286 less than the reference method. Furthermore, the Wako

Table 8-2. Reference Method Cost Summary

Analyses Performed	Number of Samples Analyzed	Cost per sample Quotation ($)	Itemized Cost ($)	
			Quotation[a]	Actual
Dioxin/Furans, EPA Method 1613B, GC/HRMS	23 extracts	735	16,905	213,580
	186 soil/sediment	785	146,010	
Total Cost	209 samples		162,915	

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

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

The Dioxin ELISA Kit Wako (for environmental) is a screening method only that reports 2,3,7,8-TCDD EQ (total D/F concentration in the sample), unlike the reference method which reports concentrations for individual congeners. Although the kit's analytical results did not have the same level of detail as the reference method analytical results (or comparable QA/QC data), the kit provided dioxin/furan analytical results on site in a nine-day at significant cost and time savings compared to the reference laboratory.

Chapter 9
Technology Performance Summary

The purpose of this chapter is to provide a performance summary of the Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit Wako (for environmental) by summarizing the evaluation of the primary and secondary objectives of this demonstration in Tables 9-1 and 9-2, respectively. Detailed information about these evaluations, including a complete evaluation of the reference laboratory data, can be found in previous sections of this report.

The data generated and evaluated during this demonstration showed that the Wako kit in many cases did not directly correlate with HRMS $TEQ_{D/F}$ values. It did show that the kit could be an effective screening tool for determining sample results above and below 20 pg/g $TEQ_{D/F}$ and even more effective as a screen for samples above and below 50 pg/g $TEQ_{D/F}$, particularly considering that both the cost ($150,294 vs. $213,580) and the time (nine days on-site vs. eight months) to analyze the 209 demonstration samples were significantly less than the reference laboratory. Because the Wako kit is not expected to directly correlate to HRMS $TEQ_{D/F}$ in all cases, the technology should not be viewed as producing an equivalent measurement value to HRMS $TEQ_{D/F}$ values. It has been suggested that correlation between the Wako and HRMS TEQ could be improved by first characterizing a site and calibrating the Wako results to HRMS results. Subsequent analysis using the Wako kit for samples obtained from this site may then show better correlation with the HRMS TEQ result. This approach was not evaluated during this demonstration.

Table 9-1. Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit Wako (for environmental) Performance Summary - Primary Objectives

Objective	Statistic	Performance
P1: Accuracy	Number of data points	5
	Median Recovery (%)	253
	Mean Recovery (%)	443
P2: Precision	Number of data points	24
	Median RSD (%)	60
	Mean RSD (%)	62
P3: Comparability	Number of data points	106
	Median RPD (%)	34
	Interval agreement (%)	62
	Blank agreement (%)	88
P4: Estimated method detection limit	EMDL (pg/g 2,3,7,8-TCDD EQ)	83–201
P5: False Positive/False Negative Rate	False positive rate at 20 pg/g TEQ (%)	10
	False positive rate at 50 pg/g TEQ (%)	10
	False negative rate at 20 pg/g TEQ (%)	13
	False negative rate at 50 pg/g TEQ (%)	8
P6: Matrix Effects	• Measurement location: not evaluated (all samples analyzed on-site) • Matrix type: none • Sample type: none • PAH concentration: none • Environmental site: none • Known interferences: slight	
P7: Cost	Cost for Wako to deploy at the field demonstration site and analyze all 209 samples: $150,294.	

Table 9-2. Wako Pure Chemical Industries, Ltd. Dioxin ELISA Kit Wako (for environmental) Performance Summary - Secondary Objectives

Objective	Performance
S1: Skill level of Operator	Based on observation during the field demonstration, a fully trained environmental chemistry technician would be the minimum qualifications of a person capable of meeting the kit's extraction and cleanup needs. The Dioxin ELISA Kit Wako (for environmental) only would require a user skilled in pipetting and computing to generate accurate data. Wako prefers only experienced personnel use the kit.
S2: Health and Safety Aspects	The sample process generated a large amount of hazardous waste including: flammable solvents, spent reactants (corrosive impregnated silica, copper, sodium sulfate), spent samples, and glass (pipettes, disposable cleanup columns, filters). The process also generated small amounts of hazardous aqueous waste. A considerable amount of lab trash was also generated that included used personal protective equipment, absorbent paper, and aluminum foil. A fume hood is necessary for the operation of this technology.
S3: Portability	The observed Dioxin ELISA kit provided by Wako required an exacting sample extraction and cleanup, similar to what is required for traditional HRMS dioxin analysis. The extraction and sample cleanup required one mobile lab fully outfitted with fume hoods, water, nitrogen and/or purified compressed air, and significant electrical power (~ 15 kVA power for the mobile laboratory). The ELISA procedure required less infrastructure, requiring only a refrigerator, watch, pipettes, small bench space, microplate reader with computer, and a controlled environment.
S4: Sample Throughput	During the field demonstration, all 209 demonstration samples were processed by Wako, equating to a sample throughput rate of 23 samples per day. This was accomplished in about 9 full working days (522 labor-hours), with six people performing various aspects of the sample preparation and analytical procedure.

Chapter 10
References

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Appendix A
SITE Monitoring and Measurement Technology Program
Verification Statement

United States Environmental Protection Agency

Office of Research and Development
Washington, DC 20460

SITE Monitoring and Measurement Technology Program Verification Statement

TECHNOLOGY TYPE:	**Enzyme-Linked Immunosorbent Assay**
APPLICATION:	**MEASUREMENT OF DIOXIN AND DIOXIN-LIKE COMPOUNDS**
TECHNOLOGY NAME:	**Dioxin ELISA Kit Wako (for environmental)**
COMPANY:	**Wako Pure Chemical Industries, Ltd.**
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VERIFICATION PROGRAM DESCRIPTION

The U.S. Environmental Protection Agency (EPA) created the Superfund Innovative Technology Evaluation (SITE) Monitoring and Measurement Technology (MMT) Program to facilitate deployment of innovative technologies through performance verification and information dissemination. The goal of this program is to further environmental protection by substantially accelerating the acceptance and use of improved and cost-effective technologies. The program assists and informs those involved in designing, distributing, permitting, and purchasing environmental technologies. This document summarizes results of a demonstration of the Wako Pure Chemical Industries, Ltd. Dioxin Enzyme-Linked Immunosorbent Assay (ELISA) Kit Wako (for environmental).

PROGRAM OPERATION

Under the SITE MMT Program, with the full participation of the technology developers, the EPA evaluates and documents the performance of innovative technologies by developing demonstration plans, conducting field tests, collecting and analyzing demonstration data, and preparing reports. The technologies are evaluated under rigorous quality assurance protocols to produce well-documented data of known quality. The EPA's National Exposure

Research Laboratory, which demonstrates field sampling, monitoring, and measurement technologies, selected Battelle as the verification organization to assist in field testing technologies for measuring dioxin and dioxin-like compounds in soil and sediment.

DEMONSTRATION DESCRIPTION

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

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

The secondary objectives for the demonstration were as follows:

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

A total of 209 samples was analyzed by each technology, including a mix of performance evaluation (PE) samples, environmentally contaminated samples, and extracts. Wako analyzed all 209 samples on-site during the field demonstration. The PE samples were used primarily to determine the accuracy of the technology and consisted of purchased reference materials with certified concentrations. The PE samples also were used to evaluate precision, comparability, EMDL, false positive/negative results, and matrix effects. Dioxin-contaminated samples from Warren County, North Carolina; the Saginaw River, Michigan; the Tittabawassee River, Michigan; Midland, Michigan; Winona Post, Missouri; Nitro, West Virginia; Newark Bay, New Jersey; Raritan Bay, New Jersey; and Brunswick, Georgia were used to evaluate precision, comparability, false positive/negative results, and matrix effects. Extracts prepared in toluene were used to evaluate precision, EMDL, and matrix effects. All samples were used to evaluate qualitative performance objectives such as technology cost, the required skill level of the operator, health and safety aspects, portability, and sample throughput. AXYS Analytical Services (Sidney, British Columbia) was contracted to perform the reference analyses by high-resolution mass spectrometry (HRMS) (EPA Method 1613B). The purpose of the verification statement is to provide a summary of the demonstration and its results; detailed information is available in *Technologies for Monitoring and Measurement of Dioxin and Dioxin-like Compounds in Soil and Sediment— Wako Pure Chemical Industries, Dioxin ELISA Kit Wako (for environmental)* (EPA/540/R-05/002).

TECHNOLOGY DESCRIPTION

The technology description and operating procedure below are based on information provided by Wako Pure Chemical Industries, Ltd. A monoclonal antibody specific to dioxin is mixed with a sample solution or the positive control (PC) provided with the Dioxin ELISA Kit Wako (for environmental). 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 Wako (for environmental) contains the secondary antibody microplate, the PC, buffers A and B, the primary antibody, peroxidase conjugate (lyophilized), wash solution concentrate, substrate, citrate buffer, stop solution, and a plate seal. The monoclonal antibodies used for the Dioxin ELISA Kit Wako (for environmental) 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-tetrachlorodibenzo-*p*-dioxin (TCDD). The technology results are reported in picogram/gram (pg/g) 2,3,7,8-TCDD equivalents (EQ).

VERIFICATION OF PERFORMANCE

The Wako kit is an immunoassay technology that reports total dioxin/furan concentration in the sample. It should be noted that the results generated by this technology may not directly correlate to HRMS total toxicity equivalents of dioxins/furans ($TEQ_{D/F}$) in all cases because it is known that the congener responses and cross-reactivity of the kit are not identical to the toxicity equivalency factors that are used to convert congener HRMS concentration values to $TEQ_{D/F}$. Therefore, this technology should not be viewed as producing an equivalent measurement value to HRMS $TEQ_{D/F}$ but as a screening value to approximate HRMS $TEQ_{D/F}$ concentration. It has been suggested that correlation between the Wako and HRMS TEQ could be improved by first characterizing a site and calibrating the Wako results to HRMS results. Subsequent analysis using the Wako kit for samples obtained from this site may then show better correlation with the HRMS TEQ result. This approach was not evaluated during this demonstration.

Accuracy: The determination of accuracy was based on the agreement of the Wako results with the certified or spiked levels of the PE samples that were obtained from commercial sources. Accuracy was assessed by percent recovery (R), which is the average of the replicate results from the Dioxin ELISA Kit Wako (for environmental) divided by the certified or spiked value of the PE sample, multiplied by 100%. Ideal R values are near 100%. The overall R values were 443% (mean), 253% (median), 10% (minimum), and 1,574% (maximum).

Precision: Replicates were incorporated for all samples (PE, environmental, and extracts) included in the 209 samples analyzed in the demonstration. Replicates were incorporated for all samples (PE, environmental, and extracts) included in the 209 samples analyzed in the demonstration. Three samples had seven replicates in the experimental design, one sample had eight replicates, and all other samples had four replicates. Precision was determined by calculating the standard deviation of the replicates, dividing by the average concentration of the replicates, and multiplying by 100%. Ideal RSD values are less than 20%. The overall RSD values were 62% (mean), 60% (median), 11% (minimum), and 145% (maximum).

Comparability: The Dioxin ELISA Kit Wako (for environmental) results were compared to EPA Method 1613B results for total $TEQ_{D/F}$. The results were compared by determining the relative percent difference (RPD) by dividing the difference of the two numbers by the average of the two numbers and multiplying by 100%. Ideal RPD values are between positive and negative 25%. The overall RPD values were 34% (median), -198% (minimum), and 198% (maximum). The Wako results were also compared to the reference laboratory results using an interval approach to determine if the Wako results and the reference laboratory results would place the samples in the same action-level interval, thereby resulting in the same action-oriented decision. The developer and reference data were grouped into four toxicity equivalent (TEQ) concentration ranges. The ranges were $\leq$ 50 pg/g, 50 to 500 pg/g, 500 to 5,000 pg/g, and $\geq$ 5,000 pg/g. The intervals were determined based on current guidance for cleanup levels. The percentage of developer results that agreed with reference laboratory results for $TEQ_{D/F}$ was 62%.

Estimated method detection limit: EMDL was calculated for the technology generally according to the procedure described in 40 CFR Part 136, Appendix B, Revision 1.11. Lower EMDL values indicate better sensitivity. The calculated EMDLs ranged from 83 to 201 pg/g 2,3,7,8-TCDD EQ values, depending on whether nondetect values were assigned values of zero, one-half the reporting limit value, or the reporting limit value itself. The detection limit reported by Wako in the demonstration plan was 20 pg/g 2,3,7,8-TCDD EQ. PE samples with TEQ concentrations in the precisely appropriate range for evaluation of this technology's detection limit were not available, so these calculated values should be considered a rough estimate.

False positive/negative results: Samples that were reported as less than a specified level by the reference laboratory but greater than the specified level by Wako were considered false positive. Conversely, those samples that were reported as less than the specified level by Wako but reported as greater than the specified level by the reference laboratory were considered false negatives. Note that results that were reported as semiquantitative results were counted as in agreement if the reference laboratory data was within that interval. For example, a reference laboratory result reported as 3,400 pg/g $TEQ_{D/F}$ was counted as in agreement with a Wako result reported as > 2,967 pg/g 2,3,7,8-TCDD EQ, even though the absolute quantitative values would be in different ranges. Ideal false positive and false negative rates were zero. The kit had a false positive rate of 10% and a false negative rate of 13% around 20 pg/g TEQ. Comparison of values around 50 pg/g indicated the same false positive rate (10%), but less false negatives (8%).

These data suggest the Wako kit could be an effective screening tool for determining sample results above and below 20 pg/g TEQ and even more effective as a screen for samples above and below 50 pg/g TEQ.

Matrix effects: The likelihood of matrix-dependent effects on performance was investigated by evaluating results in a variety of ways. No significant effect was observed for the reproducibility of Wako results by matrix type (soil, sediment, and extract), sample type (PE vs. environmental vs. extract), or by polynuclear aromatic hydrocarbon concentration. The Wako results were not more or less comparable to the reference laboratory results based on environmental site.

Cost: The full cost of the technology was documented and compared to the cost of the reference analyses. The total cost for the Dioxin ELISA kit Wako (for environmental) to analyze all 209 samples was $150,294. The total cost for the reference laboratory to analyze all 209 samples by EPA Method 1613B was $213,580. The total cost for the use of the Wako kit was $63,286 less than the reference method.

Skills and training required: Based on observation during the field demonstration, a fully trained environmental chemistry technician would be the minimum qualifications of a person capable of meeting the kit's extraction and cleanup needs. Wako prefers only experienced personnel use the kit and to be skilled in pipetting and computing skills to generate accurate data.

Health and safety aspects: The sample process generated a large amount of hazardous waste, including flammable solvents, spent reactants (corrosive impregnated silica, copper, sodium sulfate), spent samples, and glass (pipettes, disposable cleanup columns, and filters). The process also generated small amounts of hazardous aqueous waste. A considerable amount of lab trash was also generated, which included used personal protective equipment, absorbent paper, and aluminum foil. A fume hood is necessary for the operation of this technology.

Portability: The observed Dioxin ELISA kit provided by Wako required an exacting sample extraction and cleanup, similar to what is required for traditional HRMS dioxin analysis. The extraction and sample cleanup required one mobile lab fully outfitted with fume hoods, water, nitrogen and/or purified compressed air, and significant electrical power (~ 15 kilowatt/ampere (kVA) power for the mobile laboratory). The ELISA procedure required less infrastructure, requiring only a refrigerator, watch, pipettes, small bench space, microplate reader with computer, and a controlled environment.

Sample throughput: During the field demonstration, all 209 demonstration samples were processed by Wako, equating to a sample throughput rate of 23 samples per day. This was accomplished in about 9 full working days (522 labor-hours), with six people performing various aspects of the sample preparation and analytical procedure. The reference analyses took 8 months to complete the same 209 sample analyses.

Appendix B
Supplemental Information Supplied by the Developer

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

Wako's comment toward EPA report

1. Goal of Demonstration

Wako Pure Chemical Ind., Ltd. should design a project of field determination of dioxins as the goal. Wako designed its measurement program as a screening method for this demonstration.

1.1.Reporting results

1.1.1 The concentration of dioxins:

(1) If the sample concentration was 50 pg/g or less of dioxins, we decided to report semiquantitative results under the demonstration coordinator's advice.

(2) If the sample concentration was between 50 to 1,000 pg/g of dioxins, we decided to determine the exact concentration of dioxins in the sample.

(3) If the sample concentration was 1,000 pg/g or more of dioxins, we decided to report semiquantitative results under the demonstration coordinator's advice.

1.1.2 The term of determination

We planned the time frame for determination to be just in the demonstration period from April 26 to May 5 in Saginaw, Michigan.

1.1.3 The working hour in the demonstration period

We made a timetable for each day and it was the working hours from 7:30 am to 7:30 pm

1.1.4 Determination of the number of sample

We believed that we should analyze all samples (209 samples) under the term of demonstration. And, we did not have enough time to re-analyze on site for the demonstration period.

1.1.5 Demonstration site

We came from Japan to participate in the demonstration at Saginaw, Michigan

1.1.6 The properties of samples

The samples distributed by EPA contained various concentrations of dioxins, furans and PCBs from extremely high to spiked. The samples included a variety of matrices such

as the polyaromatic compounds. The samples were distributed blindly and randomly.

2. Experimental design

We made the experimental design to meet these requirements from the demonstration as follows.

2.1 The amount of sample

We designed the method to take 10g of each sample from distributed materials on site.

2.2 The frequency of dilution

Our consideration was samples needed to divide with dilution at four stages.

2.3 The range of fixed quantity

Our kit has the range of fixed quantity is from about 20 to 2,000 pg/g.

We did the same preprocessing "full clean up" as HRGC/HRMS because we had to treat samples including various matrices. However we used the multilayer silica-gel column for the preprocessing to simplify the process. Due to "full clean up", waste (glassware and solvent, etc.) had increased and sample throughput decreased more than originally projected. One of our main targets was to measure 40 to 50 samples a day.

3. About the measurement result

Our first priority of this demonstration was "In the period, all samples (209 samples) are measured on the environmental site". Therefore, neither the minimum limit of determination nor the upper limit was measured again. We reported results as below the fixed quantity lower bound (<20pg/g) or above the fixed quantity upper bound (>2,000pg/g). We considered that it was enough as the report of this demonstration whatever we were able to measure 20pg/g, because it was a prevailing opinion that 50pg/g or less of dioxins value was an unnecessary concentration for the treatment. And a high concentration sample (more than >) could be measured if diluted. However, the percent recovery (R) and the relative percent difference (RPD) values were not calculated for the samples which our reported as "< (value)" and "> (value)". They are reported as "NA" (see table 7-1, 7-2a and 7-3) because the equations that are used to calculate those values (see section 4.7 of the report) require that an actual number be used. These values were used the results as reported (in < and >) in other evaluations, such as the interval assessment. For these evaluations, our data could use as reported because of the nature of the assessment.

of various characteristics in the period in the environmental site. (conventional alternative: Standard pretreatment procedure in Japan, “full clean up”)
However, we would like to say it is not likely that various matrices like these samples exist at one contaminated site. It seems that the extraction and preprocessing would be able to be simplified according to the situation if the sample characteristics are definable.
We want to examine further better extraction and pretreatment procedure, and to find a more effective method.

Appendix C
Reference Laboratory Method Blank and Duplicate Results Summary

Table C-1. Summary of Method Blank Performance

Sample Batch Number	Criteria Met	Method Blank TEQ[a] (pg/g)	Sample TEQ Range[a] (pg/g)	Comments
D/F WG12107	Y	0.000812	26.1–74.1 (Newark Bay) 9.93–13.3 (Raritan Bay)	
D/F WG12148	N	0.133	13.5–50.4 (Newark Bay) 49.5–15,200 (Brunswick)	Many samples had concentrations >20x blank. Few that didn't were not significantly affected on a total TEQ basis.
D/F WG12264	N	0.0437	1.0–94.1 (Titta. River sediment) 0.237–6900 (PE)	Most samples had concentrations >20x blank. Low level Tittabawassee River sediment samples L6749-2 (Ref 48[b]), -9 (Ref 130), -10 (Ref 183), and -12 (Ref 207) were evaluated based on their replication within the demonstration analyses and comparison to characterization results and considered unaffected by method blank exceedances. Low level PE samples L6760-1 (Ref 25), -3 (Ref 28), and -4 (Ref 29) were D/F blanks with resulting TEQs sufficiently low enough to still be distinguished as blank samples.
D/F WG12534	N	0.610	25.3–7100 (PE)	Sample concentrations > 20x blank.
D/F WG12641	N	0.0475	31–269 (Midland) 72.8 (Brunswick) 123 (Titta. River sediment) 0.159–7690 (PE)	All but PE sample Ref 177 (0.159 TEQ) had significantly higher total TEQ than blank. Ref 177 was confirmed by running in another batch and results, which agreed within 18%. Additionally, Ref 177 was compared to its replicates within the program and considered acceptable.
D/F WG12737	N	0.348	25.7–192 (Midland) 35.2– 1300 (Titta. River soil)	Sample concentrations >20x blank.
D/F WG12804	N	0.0153	3.89–188 (PE)	A few analytes higher than criteria but no significant contribution to total TEQ.
D/F WG13547	N	0.0553	57.5– 3,000 (Nitro) 37.9 (North Carolina) 122 (Saginaw River) 26.4– 222 (Midland)	Several analytes exceeded criteria, but blank total TEQ contribution to sample is relatively small.

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

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

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

Table C-2. Sample Batch Duplicate Summary

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

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

[a] Nondetects were assigned a concentration of zero unless otherwise noted and are referred to as U=0 DL values.
[b] U=1/2 DL indicates that non-detects were assigned a concentration equal to one-half the SDL and EMPC concentrations were assigned a value equal to the EMPC.

Appendix D
Summary of Developer and Reference Laboratory Data

Appendix D. Wako and Reference Laboratory One-to-One Matching

Sample Type	Sample Number	Measurement Location	Sample Description	REP	Developer[a] pg/g 2,3,7,8-TCDD EQ	Reference Laboratory[b] $TEQ_{D/F}$ (pg/g)
Environmental	WAKO 176	Field	Brunswick #1	1	<17.8	67.2
Environmental	WAKO 97	Laboratory	Brunswick #1	2	415	71.6
Environmental	WAKO 141	Laboratory	Brunswick #1	3	159	61.7
Environmental	WAKO 53	Field	Brunswick #1	4	<27.2	67.8
Environmental	WAKO 208	Field	Brunswick #2	1	<34.3	49.5
Environmental	WAKO 151	Field	Brunswick #2	2	<22.8	72.8
Environmental	WAKO 33	Field	Brunswick #2	3	90.2	56
Environmental	WAKO 37	Field	Brunswick #2	4	<45.3	60.4
Environmental	WAKO 194	Laboratory	Brunswick #3	1	99	12600
Environmental	WAKO 78	Laboratory	Brunswick #3	2	250	15200
Environmental	WAKO 185	Laboratory	Brunswick #3	3	64.5	13100
Environmental	WAKO 56	Field	Brunswick #3	4	65.2	13600
Environmental	WAKO 207	Laboratory	Midland #1	1	238	222
Environmental	WAKO 162	Field	Midland #1	2	64.5	241
Environmental	WAKO 82	Laboratory	Midland #1	3	110	269
Environmental	WAKO 81	Laboratory	Midland #1	4	290	268
Environmental	WAKO 147	Field	Midland #2	1	132	208
Environmental	WAKO 202	Field	Midland #2	2	59.3	179
Environmental	WAKO 26	Field	Midland #2	3	117	197
Environmental	WAKO 84	Laboratory	Midland #2	4	155	192
Environmental	WAKO 72	Laboratory	Midland #3	1	78.8	185
Environmental	WAKO 32	Field	Midland #3	2	323	174
Environmental	WAKO 187	Laboratory	Midland #3	3	126	176
Environmental	WAKO 191	Laboratory	Midland #3	4	133	161
Environmental	WAKO 159	Field	Midland #4	1	<21.8	25.7
Environmental	WAKO 60	Field	Midland #4	2	<28.5	26.4
Environmental	WAKO 132	Field	Midland #4	3	<28.5	31
Environmental	WAKO 86	Field	Midland #4	4	<51.0	25.8
Environmental	WAKO 51	Field	NC PCB Site #1	1	>2800	788
Environmental	WAKO 127	Field	NC PCB Site #1	2	1817	1100
Environmental	WAKO 117	Field	NC PCB Site #1	3	>3167	852
Environmental	WAKO 29	Field	NC PCB Site #1	4	>4400	906
Environmental	WAKO 160	Field	NC PCB Site #2	1	>2967	3400
Environmental	WAKO 106	Field	NC PCB Site #2	2	>3783	3300
Environmental	WAKO 199	Field	NC PCB Site #2	3	>3733	3430
Environmental	WAKO 76	Field	NC PCB Site #2	4	>4517	3490
Environmental	WAKO 130	Field	NC PCB Site #3	1	>3217	8320
Environmental	WAKO 134	Field	NC PCB Site #3	2	>4517	8410
Environmental	WAKO 41	Field	NC PCB Site #3	3	>3783	9360
Environmental	WAKO 58	Field	NC PCB Site #3	4	>2800	10800
Environmental	WAKO 182	Field	Newark Bay #1	1	<28.2	23
Environmental	WAKO 100	Field	Newark Bay #1	2	<25.2	14
Environmental	WAKO 183	Field	Newark Bay #1	3	<28.2	14.5
Environmental	WAKO 66	Field	Newark Bay #1	4	28.5	13.5
Environmental	WAKO 68	Field	Newark Bay #2	1	72.7	50.6
Environmental	WAKO 90	Field	Newark Bay #2	2	<33.3	47.4
Environmental	WAKO 124	Field	Newark Bay #2	3	63.2	74.1
Environmental	WAKO 135	Field	Newark Bay #2	4	<28.5	50.4
Environmental	WAKO 171	Field	Newark Bay #3	1	<24.8	38.9
Environmental	WAKO 104	Field	Newark Bay #3	2	<25.2	44.9
Environmental	WAKO 116	Field	Newark Bay #3	3	>3167	40.2
Environmental	WAKO 178	Field	Newark Bay #3	4	<17.8	41.9

Sample Type	Sample Number	Measurement Location	Sample Description	REP	Developer[a] pg/g 2,3,7,8-TCDD EQ	Reference Laboratory[b] $TEQ_{D/F}$ (pg/g)
Environmental	WAKO 131	Field	Newark Bay #4	1	36.5	33.6
Environmental	WAKO 44	Field	Newark Bay #4	2	59.7	26.1
Environmental	WAKO 52	Field	Newark Bay #4	3	<37.5	27.6
Environmental	WAKO 152	Field	Newark Bay #4	4	<22.8	26.8
Environmental	WAKO 156	Field	Raritan Bay #1	1	<21.8	10.2
Environmental	WAKO 70	Field	Raritan Bay #1	2	<25.7	10.3
Environmental	WAKO 149	Field	Raritan Bay #1	3	<22.8	10.4
Environmental	WAKO 89	Field	Raritan Bay #1	4	<33.3	11.4
Environmental	WAKO 95	Field	Raritan Bay #2	1	<33.3	13.3
Environmental	WAKO 189	Field	Raritan Bay #2	2	<47.0	13.1
Environmental	WAKO 105	Field	Raritan Bay #2	3	<25.2	12.8
Environmental	WAKO 181	Field	Raritan Bay #2	4	<28.2	13
Environmental	WAKO 73	Field	Raritan Bay #3	1	<25.7	10.4
Environmental	WAKO 28	Field	Raritan Bay #3	2	<32.0	11.1
Environmental	WAKO 125	Field	Raritan Bay #3	3	<24.8	10.6
Environmental	WAKO 177	Field	Raritan Bay #3	4	<17.8	9.93
Environmental	WAKO 54	Field	Saginaw River #1	1	197	1050
Environmental	WAKO 164	Laboratory	Saginaw River #1	2	692	683
Environmental	WAKO 197	Field	Saginaw River #1	3	217	1070
Environmental	WAKO 59	Field	Saginaw River #1	4	97.5	694
Environmental	WAKO 80	Field	Saginaw River #2	1	258	1110
Environmental	WAKO 121	Field	Saginaw River #2	2	358	953
Environmental	WAKO 205	Field	Saginaw River #2	3	228	1320
Environmental	WAKO 42	Field	Saginaw River #2	4	477	864
Environmental	WAKO 123	Field	Saginaw River #3	1	105.0	99.7
Environmental	WAKO 158	Field	Saginaw River #3	2	118	146
Environmental	WAKO 113	Field	Saginaw River #3	3	106.7	122
Environmental	WAKO 62	Field	Saginaw River #3	4	32.5	99.6
Environmental	WAKO 128	Field	Solutia #1	1	60	57.5
Environmental	WAKO 67	Field	Solutia #1	2	154	76.9
Environmental	WAKO 118	Field	Solutia #1	3	>3167	62
Environmental	WAKO 112	Field	Solutia #1	4	86.2	61.6
Environmental	WAKO 34	Laboratory	Solutia #2	1	1252	2090
Environmental	WAKO 129	Field	Solutia #2	2	410	1950
Environmental	WAKO 150	Field	Solutia #2	3	432	1860
Environmental	WAKO 101	Laboratory	Solutia #2	4	1372	2160
Environmental	WAKO 98	Field	Solutia #3	1	435	2810
Environmental	WAKO 143	Laboratory	Solutia #3	2	>3817	2800
Environmental	WAKO 55	Field	Solutia #3	3	2367	3000
Environmental	WAKO 203	Field	Solutia #3	4	362	3080
Environmental	WAKO 27	Field	Titta. River Soil #1	1	1330	35
Environmental	WAKO 155	Field	Titta. River Soil #1	2	<21.8	35.2
Environmental	WAKO 36	Field	Titta. River Soil #1	3	<45.3	40
Environmental	WAKO 198	Field	Titta. River Soil #1	4	38.5	35.8
Environmental	WAKO 200	Field	Titta. River Soil #2	1	270	420
Environmental	WAKO 209	Field	Titta. River Soil #2	2	217	450
Environmental	WAKO 50	Laboratory	Titta. River Soil #2	3	303	523
Environmental	WAKO 103	Field	Titta. River Soil #2	4	227	506
Environmental	WAKO 85	Field	Titta. River Soil #3	1	355	1050
Environmental	WAKO 92	Field	Titta. River Soil #3	2	597	676
Environmental	WAKO 161	Field	Titta. River Soil #3	3	72.3	1220
Environmental	WAKO 144	Field	Titta. River Soil #3	4	918	1300
Environmental	WAKO 24	Field	Titta.. River Sed #1	1	<32.0	1.05
Environmental	WAKO 46	Field	Titta. River Sed #1	2	86.5	1.11

Sample Type	Sample Number	Measurement Location	Sample Description	REP	Developer[a] pg/g 2,3,7,8-TCDD EQ	Reference Laboratory[b] $TEQ_{D/F}$ (pg/g)
Environmental	WAKO 188	Field	Titta. River Sed #1	3	<47.0	1
Environmental	WAKO 169	Field	Titta. River Sed #1	4	50.2	1.7
Environmental	WAKO 102	Field	Titta. River Sed #2	1	55.2	52.8
Environmental	WAKO 195	Field	Titta. River Sed #2	2	302	123
Environmental	WAKO 153	Field	Titta. River Sed #2	3	<22.8	66.1
Environmental	WAKO 61	Field	Titta. River Sed #2	4	52.2	94.1
Environmental	WAKO 154	Field	Titta. River Sed #3	1	<21.8	13
Environmental	WAKO 126	Field	Titta. River Sed #3	2	39.8	11.2
Environmental	WAKO 65	Field	Titta. River Sed #3	3	34.7	12.7
Environmental	WAKO 74	Field	Titta. River Sed #3	4	<25.7	13.8
Environmental	WAKO 30	Field	Winona Post #1	1	64.3	7290
Environmental	WAKO 99	Field	Winona Post #1	2	77.2	7370
Environmental	WAKO 140	Field	Winona Post #1	3	68.3	7450
Environmental	WAKO 167	Laboratory	Winona Post #1	4	57	7160
Environmental	WAKO 71	Field	Winona Post #2	1	68.2	9720
Environmental	WAKO 47	Field	Winona Post #2	2	190	9770
Environmental	WAKO 122	Field	Winona Post #2	3	65.3	9200
Environmental	WAKO 186	Field	Winona Post #2	4	227	11300
Environmental	WAKO 91	Laboratory	Winona Post #3	1	293	10300
Environmental	WAKO 108	Field	Winona Post #3	2	91.2	9770
Environmental	WAKO 136	Field	Winona Post #3	3	63.7	9320
Environmental	WAKO 145	Laboratory	Winona Post #3	4	138	9870
Extract	WAKO 19	Field	Envir Extract #1	1	405	175
Extract	WAKO 21	Field	Envir Extract #1	2	243	444
Extract	WAKO 12	Field	Envir Extract #1	3	383	176
Extract	WAKO 11	Field	Envir Extract #1	4	150	439
Extract	WAKO 5	Field	Envir Extract #2	1	68.3	55.3
Extract	WAKO 15	Field	Envir Extract #2	2	<24.5	53.3
Extract	WAKO 22	Field	Envir Extract #2	3	<34.3	53.1
Extract	WAKO 23	Field	Envir Extract #2	4	<32.0	53.6
Extract	WAKO 16	Field	Spike #1	1	<24.5	0.504
Extract	WAKO 8	Field	Spike #1	2	<24.5	0.509
Extract	WAKO 9	Field	Spike #1	3	<24.5	0.537
Extract	WAKO 6	Field	Spike #1	4	87.5	0.524
Extract	WAKO 2	Field	Spike #1	5	71.0	0.585
Extract	WAKO 13	Field	Spike #1	6	<24.5	0.576
Extract	WAKO 1	Field	Spike #1	7	<37.5	0.52
Extract	WAKO 3	Field	Spike #2	1	69.2	91.6
Extract	WAKO 17	Field	Spike #2	2	76.2	91.8
Extract	WAKO 14	Field	Spike #2	3	<24.5	89.1
Extract	WAKO 20	Field	Spike #2	4	60.5	100
Extract	WAKO 4	Field	Spike #3	1	61.5	0.324
Extract	WAKO 10	Field	Spike #3	2	<24.5	0.348
Extract	WAKO 18	Laboratory	Spike #3	3	<29.7	0.363
Extract	WAKO 7	Field	Spike #3	4	<34.3	0.268
Performance	WAKO 31	Field	Cambridge 5183	1	<46.2	4.78
Performance	WAKO 175	Field	Cambridge 5183	2	<17.8	4.08
Performance	WAKO 166	Field	Cambridge 5183	3	31.3	4.06
Performance	WAKO 87	Laboratory	Cambridge 5183	4	88.7	3.56
Performance	WAKO 192	Field	Cambridge 5183	5	<47.0	3.89
Performance	WAKO 115	Field	Cambridge 5183	6	>3167	5.93
Performance	WAKO 110	Field	Cambridge 5183	7	64.3	3.89
Performance	WAKO 49	Field	Cambridge 5184	1	1620	187
Performance	WAKO 138	Field	Cambridge 5184	2	85.5	188

Sample Type	Sample Number	Measurement Location	Sample Description	REP	Developer[a] pg/g 2,3,7,8-TCDD EQ	Reference Laboratory[b] $TEQ_{D/F}$ (pg/g)
Performance	WAKO 63	Field	Cambridge 5184	3	217	173
Performance	WAKO 163	Field	Cambridge 5184	4	122	180
Performance	WAKO 179	Field	ERA Aroclor	1	<17.8	36.4
Performance	WAKO 114	Field	ERA Aroclor	2	658	32.9
Performance	WAKO 109	Field	ERA Aroclor	3	60.5	37.9
Performance	WAKO 119	Field	ERA Aroclor	4	>3900	35.5
Performance	WAKO 173	Field	ERA Blank	1	<17.8	0.0942
Performance	WAKO 196	Field	ERA Blank	2	<34.3	0.0728
Performance	WAKO 172	Field	ERA Blank	3	<17.8	0.237
Performance	WAKO 206	Field	ERA Blank	4	<34.3	0.307
Performance	WAKO 35	Field	ERA Blank	5	<26.3	0.113
Performance	WAKO 69	Field	ERA Blank	6	114	0.0524
Performance	WAKO 43	Field	ERA Blank	7	<45.3	0.211
Performance	WAKO 57	Field	ERA Blank	8	<26.3	0.0692
Performance	WAKO 96	Field	ERA PAH	1	<33.3	0.159
Performance	WAKO 165	Field	ERA PAH	2	38.5	0.141
Performance	WAKO 88	Field	ERA PAH	3	<33.3	0.161
Performance	WAKO 157	Field	ERA PAH	4	<21.8	0.248
Performance	WAKO 204	Field	ERA PCB 100	1	57.8	0.0386
Performance	WAKO 83	Field	ERA PCB 100	2	<35.2	NA[c]
Performance	WAKO 48	Field	ERA PCB 100	3	207	0.053
Performance	WAKO 201	Field	ERA PCB 100	4	<34.3	0.127
Performance	WAKO 107	Laboratory	ERA PCB 10000	1	50.2	0.204
Performance	WAKO 148	Field	ERA PCB 10000	2	25.7	0.507
Performance	WAKO 39	Field	ERA PCB 10000	3	168	0.105
Performance	WAKO 139	Field	ERA PCB 10000	4	61.5	0.0628
Performance	WAKO 93	Field	ERA TCDD 10	1	<33.3	8.69
Performance	WAKO 174	Field	ERA TCDD 10	2	<17.8	9.28
Performance	WAKO 94	Field	ERA TCDD 10	3	<33.3	8.44
Performance	WAKO 193	Field	ERA TCDD 10	4	<47.0	8.2
Performance	WAKO 40	Field	ERA TCDD 30	1	63.8	27.4
Performance	WAKO 168	Field	ERA TCDD 30	2	<24.8	25.3
Performance	WAKO 75	Field	ERA TCDD 30	3	<25.7	24.8
Performance	WAKO 180	Field	ERA TCDD 30	4	<17.8	23.9
Performance	WAKO 133	Field	LCG CRM-529	1	457	NA(c)
Performance	WAKO 64	Field	LCG CRM-529	2	367	6930
Performance	WAKO 170	Field	LCG CRM-529	3	1127	6900
Performance	WAKO 25	Field	LCG CRM-529	4	<32.0	7190
Performance	WAKO 38	Field	NIST 1944	1	332	237
Performance	WAKO 137	Field	NIST 1944	2	75.8	206
Performance	WAKO 45	Field	NIST 1944	3	223	252
Performance	WAKO 142	Field	NIST 1944	4	168	219
Performance	WAKO 111	Field	Wellington WMS-01	1	109	68
Performance	WAKO 190	Field	Wellington WMS-01	2	<47.0	65.7
Performance	WAKO 120	Laboratory	Wellington WMS-01	3	93.2	61.9
Performance	WAKO 146	Field	Wellington WMS-01	4	387	66.1
Performance	WAKO 77	Field	Wellington WMS-01	5	37	68
Performance	WAKO 184	Field	Wellington WMS-01	6	<28.2	65.7
Performance	WAKO 79	Field	Wellington WMS-01	7	<35.2	65.4

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

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

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