NJCAT TECHNOLOGY VERIFICATION

Calux®

Xenobiotic Detection Systems (XDS)

July 2009

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1 Introduction

1.1 New Jersey Corporation for Advanced Technology (NJCAT) Program

NJCAT is a not-for-profit corporation to promote in New Jersey the retention and growth of technology based businesses in emerging fields such as environmental and energy technologies. NJCAT provides innovators with the regulatory, commercial, technological and financial assistance required to bring their ideas to market successfully. Specifically, NJCAT functions to:

Advance policy strategies and regulatory mechanisms to promote technology commercialization,

Identify, evaluate and recommend specific technologies, for which the regulatory and commercialization process should be facilitated,

Facilitate funding and commercial relationships/alliances to bring new technologies to market and new business to the state, and

Assist in the identification of markets and applications for commercialized technologies.

The technology verification program specifically encourages collaboration between vendors and users of technology. Through this program, teams of academic and business professionals are formed to implement a comprehensive evaluation of vendor specific performance claims. Thus, vendors have the competitive edge of an independent third party confirmation of claims.

NJCAT has developed and published Technical Guidance Documents containing a technology verification protocol that is consistent with the New Jersey Department of Environmental Protection (NJDEP) Technical Manual and the Interstate Technology and Regulatory Cooperation (ITRC) Program technical and regulatory documents. This technology verification review is consistent with the NJCAT general verification protocol contained in the guidance documents.

Pursuant to N.J.S.A 13:1D-134 *et seq* (Energy and Environmental Technology Verification Program) NJDEP and NJCAT have established a Performance Partnership Agreement (PPA) whereby NJCAT performs the technology verification review and NJDEP certifies the net beneficial environmental effect of the technology. In addition, NJDEP/NJCAT works in conjunction to develop expedited or more efficient timeframes for verified/certified technology.

The PPA also requires that:

- The NJDEP shall enter in reciprocal environmental technology agreements concerning the evaluation and verification protocols with the United States Environmental Protection Agency, other local required or national environmental agencies, entities or groups in other states and New Jersey for the purpose of encouraging and permitting the reciprocal acceptance of technology data and information concerning the evaluation and verification of energy and environmental technologies; and
- The NJDEP shall work closely with the State Treasurer to include in State bid specifications, as deemed appropriate by the State Treasurer, any technology verified under the energy and environmental technology verification program.

1.2 Technology Verification Report

On September 28, 2007, Xenobiotic Detection Systems (XDS), 1601 East Geer Street, Suite S, Durham, NC 27704, submitted a Limited Preliminary Application for participation in the NJCAT Technology Verification Program. The application was for the evaluation of their Calux® (Chemically-Activated Luciferase gene eXpression cell bioassay system) technology. XDS describes the technology as a unique, mechanistically based screening analysis for Dioxins, Furans and Polyhalogenated Biphenyls (PCBs). XDS has been involved in using their technology for a variety of different applications and types of samples.

After pre-screening by NJCAT staff (in accordance with the technology assessment guidelines), the application was accepted into the verification program. This verification report covers the evaluation of Calux® based on the performance claim of XDS (see Section 4.0) XDS provided information and data to NJCAT in support of the claim, including third party data. Numerous meetings, telephone discussions and e-mail exchanges were held with XDS to further understand the technology and to solicit additional review materials. Discussions and e-mail correspondence also took place with several others outside XDS to obtain expert opinions on the effectiveness and relevance of the technology.

1.3 Technology Description

Calux® by XDS is a patented, in-vitro, cell-based bioanalytic technology system that screens and quantifies polychlorinated diaromatic hydrocarbons, specifically the dioxin-like chemicals such as dioxins (PCDDs), furans (PCDFs), and PCBs in various media. The technology consists of two key patented components: (1) a genetically-engineered mouse cell line, which is designed to express the luciferase enzyme of the firefly as a measurable light signal in proportion to concentrations of dioxin-like chemicals in a sample and (2) an XCARB column separation and cleanup column that allows separation of dioxins/furans from PCBs in a sample, allowing separate reporting of each fraction. These two components become the basis for a complete bioanalytic measurement method when integrated with steps for sample preparation, extraction, sample cleanup and column separation, optical measurement of produced light, use of a 2, 3, 7, 8-TCDD comparison standard, and calculation from a standard curve based on a number of standard preparations. Results are generally reported in pg/g (ppt) Toxic Equivalent units (TEQs). The United States Environmental Protection Agency (EPA) has accepted the Calux ® technology to be the basis of one of their SW-846 Methods, designated as Method 4435. These methods are intended for various environmental regulatory applications. XDS provides the analysis of Method 4435 in their own laboratory for clients or provides SW-846 compatible kits, training and support to users who wish to run the analysis at their own sites.

XDS developed, maintains and provides engineered cells from their cell line to users. The line was developed by transfecting a Hepa 1c1c7 mouse cell line with a firefly luciferase gene to produce the recombinant cell line H1L6.1c3. This recombinant cell line is under the transactivational control of the aryl hydrocarbon receptor (AhR) which is a chemical-responsive DNA binding protein that is responsible for producing the toxic and biological effects of the dioxin-like chemicals. When these chemicals are present in samples, they bind with the AhR and go through a series of subsequent

bindings and translocation steps that provides an estimate of the relative potency of the sample, expressed as Toxic Equivalent Factors (TEQ's). Specifically, these steps are:

(1) AhR binds and forms a complex with any dioxin-like chemicals present.

(2) The complex migrates into the nucleus of the cell where it binds to ARNT protein resulting in the AhR:ARNT complex.

(3) This new complex then binds to a specific DNA sequence, the Dioxin Responsive Element (DRE), which is upstream from many genes including that of CYP1A1. This binding stimulates the expression of the adjacent gene.

(4) The gene expression causes light (luciferase from the firefly) to be emitted and measured.

1.3.1 Technology Status

The XDS technology has matured and has been adapted in a number of ways over the last ten years in food, feed and environmental applications, both in the US and abroad. In 1998, XDS was awarded a patent for its proprietary Calux® technology for the detection and measurement of dioxin-like chemicals. A second patent, for the separation of dioxin/furan and PCB fractions with XCARB, was awarded in 2004.

In Europe, Calux® has been used to support a number of directives and programs within the European Union. A screening program for food and feed as well as other sample types has been in place at the Scientific Institute of Public Health and the Food Safety and Security Agency of Belgium since 2001. A similar program has been undertaken in Poland since 2004.

The Hiyoshi Corporation of Japan has been licensed since 2000 to use the Calux® technology and is marketing it in Asia and Japan. Hiyoshi has successfully undergone the rigorous review of the Ministry of the Environment (MOE) and XDS Calux® as performed by Hiyoshi has been an accepted technology in Japan for the analysis of dioxin-like chemicals in a variety of matrices since 2005.

In August of 2001, the United States Food and Drug Administration (FDA) Center for Veterinary Medicine and the Office of Regulatory Affairs, Arkansas Regional Laboratory, signed a licensing agreement to use the XDS Calux® for investigation as a new technology for the detection of dioxin-like compounds.

The United States Environmental Protection Agency (EPA) Office of Water undertook a comparative study on residual materials (biosolids) from wastewater treatment plants in 2001. Comparison was made of the XDS Calux®, as well as another field technology, with high resolution GC/MS results which were generated in an EPA contract laboratory. This study will be discussed in Section 5 of this report.

The EPA Superfund Innovative Technology Evaluation (SITE) Program undertook the evaluation of the XDS Calux® in 2005. In this study, results were compared with those generated in an EPA

contract laboratory which used EPA high resolution GC/MS methods. A second comparative study was conducted to continue the evaluation of XDS Calux® on a site-specific basis. This study was reported in "Environmental Science and Technology," in 2007. These studies will also be discussed in Section 5 of this report.

XDS submitted an SW-846 method request, based on their technology, for approval by the EPA in 2004. Supporting data showed correlation between Calux® and GC/MS, These data were generated from the EPA pre-SITE study samples, Asian companies and from an international cross lab validation study. The submission was accepted and placed in SW-846 as Method 4435.

The Department of Health (DOH) of Hawaii evaluated the XDS Calux® for analyzing soil samples for contamination with dioxin-like chemicals. As a result, the Hawaii DOH accepts data from the XDS Calux® for use in making risk assessment and risk management decisions related to human habitation of land that was previously used for agriculture.

1.3.2 Specific Applicability

The XDS Calux® has a wide range of potential applications in various matrices. With appropriate extraction, cleanup, QA/QC, and method validation procedures, the technology could potentially be applied to screening of various medical, food, feed and environmental samples. The technology is only limited by its ability to properly extract, cleanup, remove interferences and place the extract in a suitable solvent for plating.

The most obvious application for Calux® is as an analysis tool where large numbers of samples need to be screened within reasonable budgets, fast turn-around is needed and, of course, where there is interest in using results against a TEQ-based action limit.

Screening hazardous and toxic waste sites generally is very expensive and time consuming. Screening tools are needed that can help move forward site investigations and remediation. Bio-analytical approaches as being offered by XDS are very much needed to be used in screening applications. Used in conjunction with high resolution GC/MS approaches, they could provide higher throughput and save time and cost. Also, unlike chemo-analysis, bioanalytic approaches yield an overall TEQ value in one single analysis without going through the steps of identifying each of the contributing TEQ congeners (sources of TEQ) and mathematically combining their individual TEQs, consistent with World Health Organization (WHO) and EPA protocols.

Because of the recent dioxin contamination events traced back to poisoned animal feed and food ingredients, enforcement authorities, especially in Europe and Japan, have placed limits on the levels of dioxins in food and feed and/or have implemented strict monitoring and control programs. The European Union (EU) countries have implemented action levels for milk, pork products, poultry products, animal food, fish products, vegetables, cereals and feeds. The action levels vary by product but are generally down in the low pg/g-fat levels for animal products and ug/kg-product levels for fruits, vegetables and feed materials. The EU strategy of analyzing feed and food substances requires the performance of numerous analytical determinations. Regulators there have looked for the most prudent way to safeguard human health from exposure of dioxin-like chemicals and have seen the XDS technology as effective because it can provide a reliable and rapid analysis

to large numbers of samples with rapid turnover rates. This could not be achieved by traditional chemical analysis methods. As discussed above, the technology was selected by the Belgian Government as a screening tool for protecting their food supply and has been certified by the European Union as a valid dioxin detection method. Additionally, XDS has licensed and provided training to the Hiyoshi Corporation of Japan to use the patented Calux® technology, mainly with a food-monitoring focus. US regulatory agencies have also undertaken surveys of potential contamination of food supply, but have not promulgated strict regulatory programs similar to those implemented in Europe or Japan. However, FDA has leased the XDS technology and has received training in its use.

This NJCAT evaluation focuses mainly on the potential application of XDS Calux® to the environmental protection field for monitoring and remediation of dioxin-like materials on land sites, in water bodies and in waste treatment processes. In these applications, there is a need for a screening procedure for soils, sediments and related site materials (vegetation, construction materials, debris, etc.) to monitor TEQ levels to provide data to support risk action levels of dioxin-like materials at contaminated sites. The Centers for Disease Control Agency (ATSDR) has established a decision framework for sites contaminated with dioxin-like compounds. The framework suggests: if samples are determined to have dioxin TEQ levels between 50 and 1000 pg/g, the site should be further evaluated; if samples are determined to have levels above 1000 pg/g, response action should be taken.

There had also been considerable regulatory interest in the United States in using TEQ approaches for dioxin-like materials in sewage sludge, also referred to as biosolids. In 1993, EPA promulgated a rule allowing for land application, surface disposal and incineration in sludge incinerators and established requirements for each of these uses. In 1999, EPA proposed a second rule for use and disposal of sewage sludge containing dioxin-like materials. This proposed rule included a numeric limit of 300 ppt TEQs for dioxins applied to the land, along with other monitoring, reporting and record keeping requirements. However, more recently, EPA has withdrawn their proposed rule to implement this, based on the fact that dioxins levels in sewage sludge were appearing to be of lower health risk and that levels were observed to be diminishing. Though not promulgating a bioanalytic testing approach at this time, EPA has recently proposed changing the Toxic Release Inventory (TRI) reporting to a TEQ based system for dioxin-like compounds. In proposing this, they are stating that a TEQ approach would offer a better understanding and more realistic description to the public of actual toxicity loadings.

The main measurement approach presently favored by regulators for dioxin analysis is high resolution GC/MS, preceded by extensive sample processing. This is very expensive and has a long turn-around time. Because of this, there has been a strong interest in developing field kits for sample screening as well as quantitative approaches for dioxin-like chemicals. Several field kit products, in addition to XDS Calux®, are available on the market.

One of them is supplied by Cape Technologies. The company provides various kits and analytical services which can enable users to follow SW-846 Method 4425. These kits use an immunoassay to measure dioxins and furans in TEQs.

Another vendor, Eichrom, provides a field kit technology for dioxin-like chemicals which is called "Procept." This company also supplies kits and services, which measure TEQs from dioxins and furans and have a method, SW-846 Method 4430. The technology is based on the use of binding Ah receptors and it goes through a series of steps, making an indirect measurement of the DNA Response Element (DRE), using real time polymerase chain reaction (PCR) which correlates to TEQ.

1.3.3 Range of Contaminants and Concentrations Measured

Calux® is designed to either screen or quantify the combined toxic effects (TEQs) of polychlorinated diaromatic hydrocarbons, specifically the dioxin-like chemicals such as dioxins, furans and PCBs, together or in a mixture. The technology can well operate in the low ppt range which generally meets the regulatory decision needs for most hazardous waste samples. Sample sizes of 1 - 10g will typically give a detection limit of less than 1pg/g. Samples can be brought into the proper analytical range by both adjusting sample size and dilution. Detection limits can be adjusted to meet the needs of the project. As the sample size increases, the detection limit will decrease.

1.4 Project Description

The objective of this NJCAT evaluation is to verify the XDS Calux® claim about the performance of their technology as stated in Section 4 of this report. The project entailed a number of meetings and discussions with the key contacts, review of performance information and data identified by the vendor, review of several EPA and other technical reports, review of several analytical methodologies, and web site searches for background information.

1.5 Key Contacts

The key individuals involved with this evaluation are:

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2 Evaluation of Applicant

2.1 Corporate History

Xenobiotic Detection Systems (XDS) has been heavily involved with the development and commercialization of the technology for a number of years. The company was started in 1995 by Dr. George C. Clark and Dr.Michael S. Denison. They have offered services to various industrial, consulting and governmental clients and aim at providing data with the highest quality, responsive service and short turn-around time. Their expertise lies in the analysis of dioxins/furans (PCDDs/Fs), polychlorinated biphenyls (PCBs), and Endocrine Disruptor Compounds with the developing LUMI-CELL®ER bioassay.

In 1997, XDS received major research and development funding from the National Institutes of Health. In 1998, they were awarded a patent for their proprietary Calux® technology for measurement of dioxin-like chemicals. XDS has been licensing the use of this technology since 2000. In 2004, XDS was awarded a patent for its proprietary sample processing procedures which enable the separation of polychlorinated biphenyls from chlorinated dioxins/dibenzofurans, making it possible to determine what portion of the total TEQ in a sample is due to each of these classes of compounds.

XDS has actively participated with Calux® in inter-laboratory round-robin studies and certification programs.

2.2 Organization and Management

The three key individuals who operate XDS are:

- Dr, George C. Clark is President and CEO of the company. He has been with the company since its inception and operates out of the home office and laboratory in Durham, North Carolina.

- Dr. Michael S. Denison is a stockholder and is a professor at the University of California Davis.

- Dr. Jun Liu is the Laboratory Director of the laboratory facility in Durham, North Carolina.

2.3 Operating Experience Related to the Technology

XDS is a very hands-on company and they have worked with the technology through its inception. This includes the development, publishing about it in the scientific literature, field testing and evaluation, packaging, technical support and as a supplier. They also perform the analysis, hands-on, for fee at the Durham laboratory. XDS has experience in analyzing a wide variety of sample types and matrices for dioxins/furans and PCBs. Below is a list of typical sample sources and types which XDS has claimed to have analyzed:

- Biological samples (fish, tissue)
- Environmental samples such as soils, sediments and water
- Foodstuffs and food packaging
- Fuel Oils
- Industrial waste samples (biosolids, effluents, fly ash, still bottoms)
- Human blood and tissue
- Pulp and paper industry products, byproducts, filtrates, and wastes

Stack source emissions (MM5 train, M23) from the following sources:

- Cement kilns
- Diesel fired boilers
- Hazardous waste incinerators
- Medical waste incinerators
- Municipal waste incinerators
- Secondary aluminum smelters
- Oil fired boilers
- Waste-to-energy plants
- Wood-fired boilers
- Ambient air

In addition to the development of the SW-846 method for soils/sediments/solids, XDS has developed a method for animal feed validated in Belgium.

2.4 Patents

The Calux ® technology is proprietary and is based on two patents:

- “Bioassay for detecting 2,3,7,8-Tetrachlorodibenzo-para-dioxin and TCDD-like compounds and novel recombinant cell line useful therefore” - U.S. Patent Number 5,854,010, December 29, 1998.

- "Methods and Apparatus for separating and detecting specific polyhalogenated diaromatic hydrocarbons" - U.S. Patent Number 6,720,431, B2, April 13, 2004

2.5 Technical Resources, Staff and Capital Equipment

XDS has been in business since 1995 and has been developing licensing agreements for transfer of the technology since 2000. XDS's operations are housed in a 3000 square foot custom designed laboratory located in Durham, North Carolina. XDS's laboratory was initially completed in February 1995. The facility features:

- Sample login and sample storage areas including refrigerators and freezers.
- Sample preparation laboratory designed to maximize throughput and minimize sample cross contamination.
- Separate instrument operation rooms to accommodate two luminometer and sterile cell culture rooms. This design minimizes cross-contamination within the cell culture and sample preparation rooms.
- Data review adjacent to the instrumentation room allows analysts to access and review instrument generated data in quiet, spacious preparation areas.
- Secure data archive room.
- Complete instrumentation and equipment to support the dioxin, furan, PCB and endocrine disruptor analyses.

3 Design and Operation of the Technology

When used in conformance with SW-846 Method 4435 in the screening mode, typical analysis of soil or sediments includes these steps:

- Taking 1 or 2-g samples and placing them in solvent-cleaned vials with polytetrafluoroethylene caps.

- Extracting the samples with a 20% solution of methanol in toluene using sonication in a water bath.

- Extracting two additional times with toluene using sonication in a water bath.

- Combining and filtering the three extracts and concentrating with vacuum centrifugation.

- Suspending the concentrate in hexane and rapidly passing it through the patented XCARB column procedure in two separate elution steps. These steps separate the PCDDs/PCDF's from the PCBs so that separate estimates of TEQ can be made for each chemical fraction.

- Exchanging the eluate solvents for dimethyl sulfoxide solvent (DMSO).

- Preparing a series of dilutions and pipeting aliquots from them into 96 well dosing plates which contain the genetically engineered cells and media.

- Incubating the plates in a humidified incubator to produce optimal light expression of the luciferase.

- Removing the plate's media and examining them microscopically for viability.

- Quantifying the luciferase expression (light) with a luminometer.

- Comparing responses to a standard calibration curve prepared from a set of standards of 2,3,7,8 TCDD.

- Calculating the TEQs.

These steps are described in complete detail in XDS Standard Operating Procedures (SOPs) and conform to SW-846 Method 4435. The technology can be employed in several ways. It can be used as a screening tool with single sample analysis as described above. It can also be used to provide a semi-quantitative analysis, also conforming to SW-846 Method 4435, with three aliquots taken from each of the dilutions in the series. Results are reported as a mean of all three aliquots. It can also be used on a site specific basis, providing quantitative results. The site specific approach utilizes a combination of replicate samples, a model calibrated to high resolution GC/MS responses to the site samples and full quality control.

XDS provides the Calux® technology to users in three different ways. The first way is that XDS provides analyses on a fee for service basis with the range of fees being $300 to $600 per sample depending upon single screen or quantitative mode of analysis and the time for reporting of results. Almost all of their business in the United States has been on a fee for service basis.

The second way is by XDS supplying everything to a user to run the bioassay method themselves. This service is provided by licensing, training, outfitting a laboratory, and validation. They have done this in various laboratories including the USFDA, and authorities in Belgium, Poland, and Japan. XDS believes that it makes sense to invest in the technology in this way when there is a need for greater than 500 samples to be analyzed.

The third way would be for XDS to help the user run the method by themselves in their own laboratory. This is done with a minimum field kit of material and supplies not available to be obtained elsewhere. This includes a shipment of the Calux® cells seeded in 96 well micro-titer plates and XCARB columns to perform the affinity chromatography step for isolating the PCDDs/PCDFs and the PCB fractions from a sample. In all three cases, all SOPs are consistent with the published EPA method 4435 and are supplied to the field user along with training being required for field analysis.

4 Technology Performance Claim

Claim: XDS- Calux® is an effective tool for high throughput, low cost screening of sediment, soil and site material samples for toxicity from dioxin-like materials to determine if they exceed action limits starting as low as 50ppt.

5 Technology Performance

5.1 Case Studies

Three case studies were identified by XDS as relevant in providing supporting information and data to substantiate their claim. All three cases were aimed at evaluating the Calux® as an environmental screening, decision-making tool. All the studies were carried out by independent parties. They are as follows:

(1) "Report on the Comparison of Cell-Based Assays with Mass Spectrometry Methods for the Analysis of PCDDs/PCDFs and PCBs in Biosolids", June 2002

This study, which we will refer to as the Biosolids Study, was conducted by the EPA Office of Water, Office of Science and Technology and Engineering and Analysis Division in June 2002. At that time, EPA was in the process of proposing regulations for the land disposal of sewage sludge. The regulations were to address 29 specific congeners of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and coplanar PCBs. The proposed standard was to be 300ng/kg (ppt) TEQs. All of the background data for the proposed regulation was generated using high resolution GC/MS procedures - Method 1613B for PCBs and Method 1668A for dioxins/furans. Because of the high cost of these methods, lack of available laboratories to perform the analysis and inability of state labs to certify them, EPA was advised to consider other types of procedures than high resolution GC/MS.

As a result of this, EPA conducted the study to investigate the ability of the Calux® to determine TEQs of the dioxin-like chemicals found in sewage sludge (biosolids). The aim was to treat the Calux® as a "screening method," in that there was no expectation that the technology would produce results for the biosolids samples that would exactly match the GC/MS methods. Rather, Calux® was evaluated to determine whether a biosolids sample would contain more or less than the proposed action limit of 300ppt on a dried-weight basis, as compared to the GC/MS results.

DynaCorp Systems & Solutions was tasked by EPA to carry out the study. Archived samples from a previous investigation of 94 biosolids facilities were used for the study. These were shown to be held in a stable condition, which is expected for dioxin-like chemicals. Ten percent of the samples existed as duplicates, which were introduced into the study to help determine precision of the technology's analysis. The design was to include as many samples in the study as possible that were over 300ppt in order judge how well the technology could predict whether or not the sample TEQ concentration was over or under the action limit. The final design of the study included 22 samples, ranging from 8ppt to 718ppt total TEQ.

The data evaluation compared the Calux® with the GC/MS study results. This was done by plotting a log-transformed linear regression of their respective results and evaluating the slope and intercept using a Student's t-Test. Also, calculations of predictive index were made, using an approach patterned for epidemiological studies which is described in the literature. Predictive index is a statistical measure that shows the ability of a method to predict a certain outcome.

In addition to using the linear regression analysis and predictive index calculations, the study conducted a limited evaluation of the precision of Calux® using field duplicate samples. Results for

9 sets of duplicate samples were used to compare the internal relative percent difference (RPD) between the two methods as well as applying the F-test to examine the pooled variances of the Calux® method relative to the GC/MS method.

The EPA report concluded that the study demonstrated that, as expected, the Calux® technology did not produce results that were identical to the TEQ GC/MS results for dioxins/furans and PCBs in the biosolid samples. However, when employed as a screening method associated with a specific action limit, the Calux® could predict when the GC/MS concentration of a sample would not exceed an action level, (i.e. negative predictive values.) In other words, if a sample result is less than 300ppt by Calux®, then it is likely to be less than 300ppt by GC/MS.

(2) "Innovative Technology Verification Report – Xeniobiotic Detection Systems. Inc., Calux® by XDS," March 2005

This study, which we refer to as EPA SITE Study 1, was conducted by the Superfund Innovative Technology Evaluation (SITE) Program and managed by the Environmental Sciences Division, National Exposure Laboratory of the Office of Research and Development, EPA. The SITE Program was created to provide reliable cost and performance data in order to speed the acceptance of innovative remediation, characterization and monitoring technologies by the regulatory and user community. Recognizing the time consuming and high cost of traditional analytical approaches for determining concentrations of dioxin-like chemicals for hazardous waste site remediation decisions, the EPA wanted to explore the feasibility of using simple, rapid, cost-effective analytical methods that would allow field personnel to quickly assess the contamination at a site and could be used to direct or monitor remediation or risk assessment activities. This required that a thorough performance assessment of these alternative methods be carried out.

A demonstration was conducted by EPA, with cooperation from the Michigan Department of Environmental Quality, at the Green Point Environmental Learning Center in Saginaw, Michigan from April 26, to May 5, 2004. XDS participated in the demonstration with their Calux® technology along with four other technology developers. Technical aspects of the project were tasked to Battelle. The technologies were operated by the developers with oversight from the EPA evaluation team. Calux® results for dioxin/furan TEQs and PCB TEQs and total TEQs were compared to TEQ results generated by a reference laboratory, AXYS Analytical Services. The laboratory used EPA high resolution GC/MS Methods 1613B and 1668A.

The demonstration objectives were to be accomplished by evaluating the results generated by the GC/MS reference laboratory and Calux® technology on a total of 209 sample analyses. Performance of the Calux® was measured in terms of accuracy, precision, comparability, estimated method detection limit, false positive / false negative results, matrix effects, costs, skill level of operator, health and safety aspects, portability and sample throughput. 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 of soils and sediments collected from 10 different dioxin contaminated sites. These sites provided samples with a variety of distinguishing characteristics including high levels of PCBs and PAHs. Samples came from around the country including Warren County, North Carolina; Tittabawassee River Flood Plain; Midland, Michigan;

Winoma Post; Solutia; New York/ New Jersey harbors; Newark Bay; Raritan Bay; Tittabawasee River; Saginaw River and Brunswick Wood Preserving Site.

The report concluded that, based on the data, the XDS Calux® could be an effective tool for screening samples above or below 1pg/g TEQ for $TEQ_{D/F}$ and total TEQ and that it could be effective for all 3 types of TEQ values to determine results above or below 50pg/g TEQ, particularly considering that both the cost ($89,564 vs. $398,029) and time (six weeks vs. eight months) to analyze the 209 demonstration samples.

(3) "Application of Site-Specific Calibration Data Using the CALUX by XDS for Dioxin-like Chemicals in Soil and Sediment Samples" Environmental Science and Technology, 2007, 41, 8376-8382

This paper, written by Amy Dindal, Elizabeth Thompson, and Stephen Billets, describes a follow up study to EPA SITE Study 1, discussed above. This study further compared the Calux® to the conventional GC/MS approach on hazardous waste site samples, but went further to explore using a site-specific approach to improve Calux® precision and comparability to the high resolution GC/MS method. We refer to this study as EPA SITE Study 2.

The study used a total of 112 soil and sediment samples which were mostly archived from the previous study but also included some unique samples that were not used as part of that study. The samples came from five of the original ten sites. XDS implemented its "comprehensive" analytical protocol (i.e., the sample extract was analyzed three times) to provide a more precise estimate of the TEQ. The data were evaluated in four ways: (1) uncalibrated to GC/MS (2) calibrated to GC/MS using an overall statistical model, (3) calibrated to GC/MS using site-specific models generated on a site-specific basis and (4) calibrated to GC/MS using site specific calibration factors.

The results showed that TEQ data produced by the Calux® were more precise than the first study. They also showed that site-specific statistical models were better tools for understanding the relationship between Calux® and GC/MS than a single overall model generated from multiple sites. When a site-specific calibration approach was used, it was found to be a simple and accurate way of correcting Calux® data and improving comparability to GC/MS. The paper concluded that Calux®, when used in conjunction with GC/MS, can be a useful tool for risk assessment and risk management decisions on remediation of hazardous waste and contaminated sites.

5.2 Technology Evaluation Analysis

In order to evaluate the soundness of the XDS performance claim (Section 4), it is useful to break the claim down into three major questions:
(1) Can Calux® be used to screen sediment, soil, and site material sample matrices (i.e., operate in a screening mode; appropriate for these matrices)?

(2) Is Calux® effective - able to provide date of sufficient quality to screen sediments, soils and site materials down to 50ppt levels?

(3) Can Calux® be performed at a relatively low cost / and high throughput (compared to high resolution GC/MS)?

The three case studies, discussed above, provide data to help address these questions. The EPA Biosolids Study and the EPA SITE Study 1 were intended mainly to evaluate the Calux® as a tool for making screening decisions and provide information and data to help evaluate this application. They did not evaluate the technology as a tool to be used for stand-alone quantitative analysis. The EPA SITE Study 2 provides data to help evaluate the technology as a screening tool, but also shows that Calux® can be used as a quantitative tool for site specific applications, when used with increased replicate analysis, in conjunction with GC/MS and aided with site specific data modeling tools.

In all three of the case studies, a comparison is made with the high resolution GC/MS - TEQ analysis. By convention, GC/MS becomes the reference method and the burden of proof is on the candidate technology to prove comparability. However, a strict comparison based on TEQ cannot really be made between a chemical-specific analytical method such as GC/MS and a cell based bioassay system such as Calux® for two reasons:

- First, it is possible that the Ah-receptor binding compounds that are being measured in the Calux® analysis are not all accounted for in the GC/MS result which uses the 1998 World Health Organization (WHO) Toxicity Equivalent Factors (TEF) protocol to generate the TEQ results. For example, brominated dioxins, furans and biphenyls and to some degree PAHs will respond to the bioassay but are not measured by GC/MS and counted in the WHO calculations. It may, in fact, be possible that the Calux® analysis gives a more complete response for toxicity from a risk assessment point of view, because it includes these other toxic compounds.
- Second, the TEF developed by EPA and WHO have round number multiples of 1 or 5, e.g., 0.5, 0.1, 0.001 and are calculated as estimates of the relative potency of the individual compounds. Bioanalytic systems, such as the Calux® cell-based system, are unlikely to respond to a dioxin-like chemical with exactly the same multiples as the GC/MS TEF values. The GC/MS method will give a calculated sum of TEF values, while the Calux® will give a single, combined response to toxicity.

Despite this inability to make a strict comparison, an evaluation of the Calux® against the stated claim can still be made from the existing data base from these three studies. This evaluation of the claim is done below, responding to the three questions.

QUESTION 1: Can Calux® be used to screen sediment, soil and site material sample matrices (i.e., operate in a screening mode; appropriate for these matrices)?

The first question is about the applicability of the Calux® technology for samples of sediments, soils and related materials that might be encountered at a hazardous waste site. EPA SITE Studies 1 and 2 show successful screening demonstrations of screening samples collected from dioxin-like chemicals from a wide range of soils and sediments. These came from well known waste sites and river basins. The EPA biosolids study uses sewage sludge as a matrix for demonstrating the effectiveness of the Calux® as a screening tool. Although the biosolids matrix technically falls outside the claim stated above, biosolids, nevertheless, have similarities in matrix characteristics to

many soils and sediments and the successful demonstration of Calux® at 300ppt as a screening tool on biosolids can also help support the claim for its appropriateness for soil and sediment samples.

The claim also includes site materials that might be associated with soils or sediments, should the need arise to analyze them at hazardous waste sites. This could include a range of materials that could include rocks, vegetation, concrete, old structural materials or anything else that might be of a remediation concern. Although we found no evaluation data specifically on these materials, the extraction and two column cleanup system of the Calux® technology should be able to accommodate these materials and provide screening information, providing the samples can be adequately prepared for analysis.

QUESTION 2 Is Calux® effective - able to provide date of sufficient quality to screen sediments, soils and site materials down to 50ppt levels?

The second question deals directly with effectiveness – being able to screen samples to determine with a high degree of probability if they are higher or lower in TEQ concentration than a stated action limit which might be as low as 50ppt. There are a number of different evaluation measurements that can be gleaned out of the three case studies that are relevant.

(a) Predictability index calculations in the EPA Biosolids Study

The Predictability index tested in the Biosolids Study helps demonstrate the value of Calux® as a screening tool for action level decision-making in biosolids at 300ppt. Predictability is an important quality for a screening method in order to correctly classify a sample concentration as being over or under an action limit. This was tested in the Study when 22 samples of biosolids were analyzed by a reference laboratory using EPA GC/MS Methods 1613B and 1668A and results were compared to results on the same samples by the Calux® technology. The biosolids samples ranged in total TEQ concentrations over and under a proposed action limit of 300ppt. The statistical evaluation used was patterned after an epidemiological approach described in Hennekens et al., 1987. First, a predictive index was calculated comparing the results of GC/MS and Calux®. This consisted of four outcomes, based on the compared results, using the 300ppt action limit. The outcomes were:

- Number of results over action level by both GC/MS and Calux® (A)
- Number of results under action limit by GC/MS, but over action limit by Calux® (B)
- Number of results over action limit by GC/MS, but under action limit by Calux® (C)
- Number of results under action limit by both GC/MS and Calux® (D)

According to these outcomes, four measures of screening method performance were then calculated from the data. These were:

(1) Sensitivity - This is the probability that a sample will have a result greater than the action limit (a positive result) with the screening test when the GC/MS results for the sample are greater than the action limit. On this, the Calux® scored agreement on 4/4 outcomes which was 100%

(2) Specificity - This is the probability that a sample will have a screening result less than the action limit (a negative result) when the GC/MS results are also less than the action limit. On this, Calux® agreed on 16/18 outcomes which was 88.9%

(3) Positive Predictive Value - This is the probability that a sample with the GC/MS result greater than the action limit will have a positive result with the screening test. On this, Calux® scored 4/6 outcomes or 66.7%

(4) Negative Predictive Value - This is the probability that a sample with GC/MS results less than the action limit will have a negative result with the screening test. On this, Calux® scored 16/16 outcome or 100%

The EPA report concluded that Calux® as a screening tool can successfully predict when the GC/MS concentration of a sample would not exceed the action level (i.e., negative predictive value), based on 300ppt. Also, it concluded that Calux® performed reasonably well with a positive predictive value of 67%, despite its potential to respond to brominated compounds that are not measured by the GC/MS method.

(b) False positive / false negative results in EPA SITE Study 1

The results of the EPA SITE Study 1 false positive / false negative evaluation support the ability of Calux® technology as a screening tool for soils and sediments, at as low as 50ppt. This evaluation measures the tendency of a method to improperly characterize a sample as either a false positive or a false negative. In this Study, a comparison was again made to high resolution GC/MS results which were generated in the reference laboratory. Samples were arranged by concentration in two TEQ testing intervals - above and below 1 pg/g and above and below 50 pg/g for TEQ_{PCB}, $TEQ_{D/F}$ and total TEQ, respectively. As such, samples that were reported as $\leq$ 1 (or 50) pg/g TEQ by the reference method but > 1 (or 50) pg/g by Calux® were considered false positives. Conversely, those that were reported as $\leq$ 1 (or 50) pg/g TEQ by Calux® but $\geq$ 1 (or 50) pg/g by the reference method, were considered false negatives. The Calux® results are shown below:

Table 1 False Positive / False Negative Results

	TEQ_{PCB} at 1pg/g	*TEQ_{PCB} at 50pg/g*
False positives	15% (29 of 194)	9% (18 of 194)
False negatives	23% (45 of 194)	6% (11 of 194)
	$TEQ_{D/F}$ at 1pg/g	*$TEQ_{D/F}$ at 50pg/g*
False positives	6% (12 of 207)	10% (20 of 207)
False negatives	0% (0 of 207)	0.5% (1 of 207)
	Total TEQ at 1pg/g	*Total TEQ at 50pg/g*
False positives	4% (8 of 207)	6% (12 of 207)
False negatives	1% (2 of 207)	0% (0 of 207)

Calux® had a fairly high rate of false positives and false negatives around 1pg/g TEQ_{PCB} (15% and 23% respectively) but had significantly fewer false positives and false negatives for total TEQ (4 and 1% respectively) and at $TEQ_{D/F}$ (6% and 0% respectively). When the data were compared to the reference method values around 50pg/g TEQ, the false positive and false negative rates were well controlled at 10% or below. The EPA report concluded that the Calux® data suggested that it could be an effective tool to screen samples at or below a 1pg/g action limit for dioxins/furans and total TEQ, and that it could be effective for all three types of polychlorinated diaromatic hydrocarbons at or below an action limit of 50pg/g.

(c) Comparability using the interval approach in EPA SITE Study 1

The claim for effectiveness is reasonably well supported by the interval approach evaluation conducted as part of EPA SITE Study 1. This looked at how well the Calux® technology could compare with the GC/MS reference laboratory in screening samples and placing the results in discrete quantitative intervals. Agreement between the reference laboratory and Calux® results was assessed for TEQ_{PCB}, $TEQ_{D/F}$ and total TEQ at 4 intervals: <50pg/g; 50 - 500pg/g; 500 - 5000pg/g and $\geq$ 5000pg/g. The agreement between the Calux® and the Reference Laboratory was 82% for TEQ_{PCB}, 69% for $TEQ_{D/F}$ and 72% for total TEQs. Interval reporting addresses the question on whether a value reported by Calux® would result in the same action decision as if it were analyzed by the Reference Laboratory. The interval assessment indicated that 18 to 31% of the time, the Calux® analysis would have resulted in a different decision than if it was analyzed in the Reference Laboratory, based on TEQs determined for the study and the concentrations chosen for the interval.

(d) Estimated method detection limit in EPA SITE Study 1

Of the three studies, only the EPA SITE Study 1 evaluated the detection capability of the Calux® technology. Although this detection limit study was quite limited, it showed the ability of Calux® to detect at well below the claimed screening level of 50pg/g. In this study, an estimated method detection limit (EMDL) was calculated generally according to the procedure in 40CFR Part 136, Appendix B, Revision 1.11. Seven samples of an extract were prepared in toluene spiked with 0.5pg/mL of 2,3,7,8 TCDD. Because only the 2,3,7,8 TCDD congener was used, the EMDL could only be calculated for $TEQ_{D/F}$. Since several non-detects were reported, they had to be treated so as to give them some numerical value. Three different ways were tried to treat these: by setting non-detect values equal to zero; by setting non-detects equal to one-half of the reporting value and by setting non-detects equal to the reporting value itself. The calculated EMDLs were very close, regardless of the method of data treatment: 0.62pg/g TEQ, 0.63pg/g TEQ and 0.53pg/g TEQ.

(e) Comparability using RPD in EPA SITE Study 1

The Calux® technology was used in EPA SITE Study 1 in the screening mode and was not expected to provide quantitative results. Nevertheless, it is useful to see the quantitative performance which was assessed in the RPD comparison with the reference laboratory. The comparability of the Calux® to the GC/MS methods was assessed by measuring the relative percent difference (RPD) between the reference laboratory which used high resolution GC/MS and the Calux® results for TEQ_{PCB}, $TEQ_{D/F}$ and total TEQ. Differences between the reference laboratory and the Calux® results were calculated and this difference was divided by the average of the

reference laboratory and the Calux® results, and multiplied by 100. Signed rather than absolute differences were used to provide an idea of whether Calux® was biased either high or low with respect to the reference laboratory. If the result was negative, Calux® would be biased high and vice- versa. The values were:

TEQ_{PCB} -17% (median) -200% (minimum) 200% (maximum)

$TEQ_{D/F}$ -102% (median) -198% (minimum) 196% (maximum)

Total TEQ -92% (median) -191% (minimum) 186% (maximum)

The evaluation indicates that Calux® values were generally higher than the reference laboratory as evidenced by all median values being negative and that TEQ_{PCB} results were reported most consistently with the reference laboratory results. The EPA report considered an achievement of RPD values between positive and negative 25% to be in good agreement. Of the TEQ_{PCB}, $TEQ_{D/F}$ and total TEQ values, 5%, 9%, and 11% fell within this range. It should be noted, that although 75% of the values did not meet this objective, the bias tended to be generally high. This is usually more desirable for a screening procedure which generally aims to accept results more conservatively on the high side (false positives) rather than the low side (false negatives).

(f) Accuracy data in EPA SITE Study 1

All three studies were designed to give more attention to demonstrating Calux®'s comparability to the GC/MS reference method rather than to determine its true accuracy in measuring TEQs. Only the EPA SITE Study 1 tried to make an assessment of accuracy. This assessment was based on agreement of the Calux® technology results with the certified or spiked levels of Performance Evaluation (PE) Samples that were obtained from commercial sources. Accuracy was assessed by percent recovery, which is the average of the replicate results from Calux® divided by the certified or spiked value of the PE sample, multiplied by 100%. An ideal percent recovery value would be near 100%. A statistical summary of the PE sample results is below:

Table 2 EPA Study Accuracy Results

	$\%TEQ_{PCB}$	$\%TEQ_{D/F}$	%Total TEQ
mean	548	514	217
median	25	307	141
minimum	3	120	15
maximum	1736	1842	868

Examination of the Calux® means, medians and individual data show a fairly high bias for $TEQ_{D/F}$ and total TEQ with the best agreement with the certified values for total TEQ as shown with a mean of 217% and a median of 141%. The results for the TEQ_{PCB} were generally biased lower as noted with a median recovery of 25%.

It should be noted again that evaluation was designed to evaluate the use of Calux® as a screening procedure, not to really assess accuracy as if it were being used in a quantitative way. Calux® was being used in a screening mode whereby only one extraction of the sample was taken and processed with a single determination at a variety of dilutions to obtain an estimate.

(g) Precision data in EPA SITE Studies 1 and 2

Precision is the measure of being able to consistently produce the same result over repeated measurements. It is another important quality of a screening tool but the requirements for precision are somewhat less stringent for a screening tool than when a method is used as a quantitative measurement. In EPA SITE Study 1, precision of the Calux® was based on all samples (PE, environmental and extracts) included in the 209 samples analyzed in the determination. Three of the samples had seven replicates in the experimental design, one sample had eight replicates, and all of the others had four replicates. Relative Standard Deviations (RSD) were calculated. The overall RSD values were as follows:

$TEQ_{PCB:}$ 105% (mean), 97% (median), 26% (minimum) and 199% (maximum)

$TEQ_{D/F:}$ 41% (mean), 32% (median), 2% (minimum) and 124% (maximum)

Total TEQ 53% (mean), 42% (median), 3% (minimum) and 165% (maximum)

(h) Use of Calux® in a quantitative mode

The main objective of EPA SITE Study 2 was to demonstrate improved Calux® performance over EPA SITE Study 1 by trying a number of enhancements over and above running the Calux® in the single analysis screening mode. The first step was to improve the precision by analyzing triplicate extracts rather than single extract analysis. This approach demonstrated a significant improvement in precision and the ability of Calux® to become more of a quantitative tool when advanced out of the screening mode. Averages of the total TEQ values and RSDs achieved by Calux® are below:

Table 3 Improved precision with replicate analysis (%RSD)

SAMPLE	AVERAGE TEQ (pg/g)	%RSD
Winona cell 10	57238	14
Winona cell 12	51597	22
Winona cell 2	56021	10
Winona cell 4	55599	18
Winona cell 8	59542	22
Tittabawassee River DNR1	1613	9
Tittabawassee River DNR2	127	23
Tittabawassee River FFP1	8828	23
Tittabawassee River FFP2	2511	17
Tittabawassee River IMP2	2101	28
Newark Bay NB1	61	13
Newark Bay NB2	53	13
Newark Bay NB3	39	19
Newark Bay NB5	22	15
Newark Bay NB6	73	9
Solutia SS1	840	38
Solutia SS2	109	25
Solutia SS3	3946	24
Solutia SS4	2177	47
Solutia SS5	1234	28
Solutia SS6	3913	27
Raritan Bay RB1	51	18
Raritan Bay RB2	39	10
Raritan Bay RB3	43	10
Raritan Bay RB4	43	14
Raritan Bay RB5	42	17

The other enhancements that were demonstrated in the EPA SITE Study 2 provided more information on the value of Calux® when used in conjunction with high resolution GC/MS in a site-specific application. An effort was made to evaluate Calux® when used in conjunction with GC/MS in site specific applications, using various models and site factors. This included calibrating the Calux® with an overall statistical model, calibrating it using a site-specific model and calibrating it using site-specific calibration factors. Ultimately, site-specific calibration was shown to be the best approach because it was a simple and accurate way of correcting the Calux® data and improving comparability with GC/MS. This work highlighted the value of Calux® when used as a companion tool to GC/MS for expedited and TRIAD-type field applications. These applications are of considerable interest to EPA and NJDEP.

QUESTION 3 Can Calux® be performed at a relatively low cost / and high throughput (compared to high resolution GC/MS)?

The third question deals with two elements of the claim statement: cost and throughput.

Cost: The June 2002 Biosolids Study did not actually measure and compare cost differences between high resolution GC/MS analysis and the Calux®. However, the report did conclude that based on a demonstrated 83% positive prediction value, there would be economic advantages to using the Calux® as a screening technique. The report referred to costs that at time were estimated to be $200 - $400 per sample, while the GC/MS analysis ranged from $1600 - $2000 per sample.

EPA SITE Study 1 measured the cost differences between the two methods in a very methodical way. A complete comparison was made of the total costs for the demonstration including capital equipment, supplies, support equipment, labor and investigation derived disposal. The report concluded that the total costs for the Calux® to conduct a screening analysis of 209 samples was $89,564 compared to the reference laboratory which was $398,029. The XDS cost was based on XDS analyzing 43 samples in five days on-site during the demonstration and completing the remaining 166 samples in its laboratory within six weeks of the demonstration. The report also concluded that Calux® in the field will produce additional cost savings because the results will be available within a few hours of sample collection. This will allow critical decisions regarding sampling and analysis to be made in the field, resulting in a more complete data set. Additional possible advantages to using field technologies such as Calux® include reduction of multiple re-mobilization-demobilization cycles to a single cycle, dramatically increased spatial resolution mapping for higher statistical confidence, reduced insurance costs and disposal costs, and compression of time to reduce administrative overhead. However, the cost comparison did not include GC/MS confirmation costs that would be incurred in a real world use of the Calux® in the screening mode.

Throughput: EPA SITE Study 1 also measured throughput in a methodical way, again comparing the measured Calux® throughput to that of the GC/MS method approach used by the reference laboratory. When operated in the screening mode of one analysis per sample, XDS demonstrated significantly increased throughput over conventional GC/MS. The EPA report stated that during the field demonstration, 43 samples were processed by XDS, equating to a sample throughput rate of 9 samples per day. This work was accomplished in about 5 full working days (42 labor-hours), with one person exclusively performing the work. XDS completed the remaining 166 samples in their laboratory within 6 weeks of the demonstration. (EPA Report Note, that typical non-expedited turnaround times are 21 to 30 days in the XDS laboratory). For comparison, the reference laboratory took 8 months to report all 209 samples.

6.0 Verification of Performance Claim

Based on a review of performance data and information from the above studies and several interviews, the questions below are sufficiently answered in the affirmative:

(1) Can it be used to screen sediment, soil, and site material sample matrices?

(2) Is it effective - able to provide date of sufficient quality to screen sediments, soils and site materials down to 50ppt levels?

(3) Can it be performed at a relatively low cost/ and high throughput?

Therefore there are sufficient data and information to support the XDS Calux® claim: X**DS- Calux® is an effective tool for high throughput, low cost screening of sediment, soil and site material samples for toxicity from dioxin-like materials to determine if they exceed action limits starting as low as 50ppt.**

7.0 Limitations

There are several limitations to the Calux® technology:

(1) It is not a completely stand-alone technology. Although very useful as a screening tool, the technology needs to be guided and understood in terms of the GC/MS analysis. When used, especially in the screening mode, results will have to be carefully evaluated and routinely confirmed by GC/MS.

(2) Test results will tend to be biased somewhat high, especially when high levels of PAHs are present, unless used in the site-specific mode with replicate analysis and site modeling to GC/MS results.

8.0 Net Environmental Benefits

The XDS CALUX® technology saves time and money in analysis, allowing for lower cost site characterization and remediation of PCDDs/PCDFs and PCBs. This saved money and time can be redirected to other environmental restoration efforts.

9.0 References

1. "Application of Site-Specific Calibration Data Using the CALUX by XDS Bioassay for Dioxin-like Chemicals in Soil and Sediment Samples", Amy Dindal, Elizabeth Thompson, Laura Aume, Stephen Billets, Environmental Science and Technology, 2007, 41, 8376 - 8382

2. Private telephone communication, Amy Dindal, Battelle, March 11, 2009

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6. Private telephone communication, Jon Josephs, Region 2 EPA-ORD coordinator, April 3, 2009

7. Private telephone communication, Jon Josephs, Region 2 EPA-ORD Coordinator, April 8, 2009