June 2006

Environmental Technology Verification Report

LAB_BELL INC.
LUMINOTOX PECS TEST KIT

Prepared by
Battelle

Battelle
The Business of Innovation

Under a cooperative agreement with

EPA U.S. Environmental Protection Agency

ETV ETV ETV

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

ETV

EPA
U.S. Environmental Protection Agency

Battelle
The Business of Innovation

ETV Joint Verification Statement

TECHNOLOGY TYPE: **Rapid Toxicity Testing System**

APPLICATION: **Detecting Toxicity in Drinking Water**

TECHNOLOGY NAME: **LuminoTox PECs**

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The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permitters), and with individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The Advanced Monitoring Systems (AMS) Center, one of six technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center evaluated the performance of the Lab_Bell Inc. LuminoTox photosynthetic enzymatic complexes (PECs) Test Kit. This verification statement provides a summary of the test results.

VERIFICATION TEST DESCRIPTION

Rapid toxicity technologies use various biological organisms and chemical reactions to indicate the presence of toxic contaminants. The toxic contaminants are indicated by a change or appearance of color or a change in intensity. As part of this verification test, LuminoTox PECs Test Kit was subjected to various concentrations of contaminants such as industrial chemicals, pesticides, rodenticides, pharmaceuticals, nerve agents, and biological toxins. Each contaminant was added to separate drinking water samples and analyzed. In addition to determining whether LuminoTox PECs Test Kit could detect the toxicity caused by each contaminant, its response to interfering compounds, such as water treatment chemicals and by-products in clean drinking water, was evaluated.

LuminoTox PECs Test Kit was evaluated by

- Endpoints and precision—percent inhibition for all concentration levels of contaminants and potential interfering compounds and precision of replicate analyses

- Toxicity threshold for each contaminant—contaminant level at which higher concentrations generate inhibition significantly greater than the negative control and lower concentrations do not

- False positive responses—chlorination and chloramination by-product inhibition with respect to unspiked American Society for Testing and Materials Type II deionized water samples

- False negative responses—contaminants that were reported as producing inhibition similar to the negative control when present at lethal concentrations (the concentration at which 250 milliliters of water would probably cause the death of a 154-pound person) or a negative background inhibition that caused falsely low inhibition

- Other performance factors (sample throughput, ease of use, reliability).

The LuminoTox PECs Test Kit was verified by analyzing a dechlorinated drinking water sample from Columbus, Ohio (DDW), fortified with contaminants (at concentrations ranging from lethal levels to concentrations up to 1,000 times less than the lethal dose) and interferences (metals possibly present as a result of the water treatment processes). Dechlorinated water was used because free chlorine inhibits the photosynthetic process that the LuminoTox PECs Test Kit depends on to indicate toxicity and can degrade the contaminants during storage. Inhibition results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the LuminoTox PECs Test Kit to detect toxicity, as well as to measure the precision of the LuminoTox PECs Test Kit results. The response of the LuminoTox PECs Test Kit to possible interferents was evaluated by analyzing them at one-half of the concentration limit recommended by the EPA's National Secondary Drinking Water Regulations guidance. For analysis of by-products of the chlorination process, the unspiked DDW was analyzed because Columbus, Ohio, uses chlorination as its disinfectant procedure. For the analysis of by-products of the chloramination process, a separate drinking water sample was obtained from the Metropolitan Water District of Southern California (LaVerne, California), which uses chloramination as its disinfection process. The samples were analyzed after residual chlorine was removed using sodium thiosulfate. Sample throughput was measured based on the number of samples analyzed per hour. Ease of use and reliability were determined based on documented observations of the operators.

Quality control samples included method blank samples, which consisted of American Society for Testing and Materials Type II deionized water; positive control samples (fortified with atrazine); and negative control samples, which consisted of the unspiked DDW.

QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted a technical systems audit, a performance evaluation audit, and a data quality audit of 10% of the test data.

This verification statement, the full report on which it is based, and the test/QA plan for this verification test are all available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

The following description of the LuminoTox PECs Test Kit is based on information provided by the vendor. This technology description was not verified in this test.

The LuminoTox PECs Test Kit is a portable biosensor that indicates the presence of toxic chemicals in water. It uses PECs that have been stabilized through a method patented by Lab_Bell Inc. The PECs are membranes isolated from chloroplasts that are as simple to use as a chemical, but react more rapidly than a living organism because toxic compounds do not have to penetrate the cell wall of an organism. The photosynthetic electron chain is what is inhibited by contamination. When stimulated by light, the PECs emit fluorescence. The LuminoTox PECs Test Kit measures the fluorescence parameters produced both in background water and samples containing contaminants. Decreases in fluorescence parameters as a result of the presence of toxic contamination are expressed as percent inhibition.

The LuminoTox PECs Test Kit consists of the LuminoTox analyzer, a bottle of PECs for 50 tests, reaction buffer, a blank water control, and a positive control. Also provided are disposable syringes in which the test is performed and fabric syringe covers to protect the reaction from light. Aluminum foil can be used as a light protector.

The LuminoTox analyzer is 21.6 by 12.7 by 7.6 centimeters and weighs 1 kilogram. It is battery-operated and is portable. The analyzer has a built-in RS-232 serial port outlet, which can also be used for transferring data to a spreadsheet (which was not done during this test), and is compatible with a printer. A total of 100 measurements can be stored in the internal memory. The rechargeable battery operates for eight hours. Each kit costs $89, and the analyzer costs approximately $7,500.

VERIFICATION RESULTS

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Average Inhibition at Concentrations Relative to the LD Concentration (%)				Range of Standard Deviations (%)	Toxicity Thresh. (mg/L)
			LD	LD/10	LD/100	LD/1,000		
Contaminants in DDW	Aldicarb	260	26	2	0	-2	1-3	260
	Botulinum toxin complex B	0.3	0	-5	-8	-12	2-6	ND
	Colchicine	240	2	-3	-2	-6	1-5	ND
	Cyanide	250	47	31	-8	-7	1-7	25
	Dicrotophos	1,400	3	10	8	4	2-4	ND
	Nicotine	2,800	77	80	6	9	1-6	280
	Ricin	15	2	5	-7	-10	4-9	ND
	Soman	1.4	-5	-6	0	-1	4-6	ND
	Thallium sulfate	2,800	63	19	-3	-12	2-7	280
	VX	2	-5	-8	-2	3	3-5	ND

Parameter	Interference	Conc. (mg/L)	Average Inhibition (%)	Standard Deviation (%)
Potential interferences in DDW	Aluminum	0.5	0	4
	Copper	0.6	70	1
	Iron	0.15	7	6
	Manganese	0.25	5	6
	Zinc	2.5	12	5

False positive response	Both the chlorinated and chloraminated disinfection by-product samples produced an inhibition significantly greater than the negative control and, therefore, were considered false positive responses. However, the disinfectant by-product samples produced an inhibition of less than 15%, leaving enough fluorescence available for subsequent inhibition due to contamination.
False negative response	Botulinum toxin complex B, colchicine, dicrotophos, ricin, soman, and VX exhibited non-detectable responses at the lethal dose concentration.
Ease of use	The LuminoTox PECs Test Kit contained detailed instructions and clear illustrations. The contents of the LuminoTox PECs Test Kit were well identified with labels on the vials. Storage requirements were stated in the instructions and on the reagent vials. Preparation of the test samples for analysis was straightforward. However, the PECs had to be stored in ice between every sample analysis to keep them from coming to room temperature, which was somewhat inconvenient because the melting ice caused the lab bench and operators' hands to be wet most of the time. The necessity to record four numbers as raw data was somewhat burdensome; however, Lab_Bell has indicated that it is modifying this. No formal scientific education would be required to use the LuminoTox PECs Test Kit.
Field portability	The LuminoTox PECs Test Kit was transported from a laboratory setting to a storage room for the field portability evaluation. The LuminoTox PECs Test Kit was tested with one contaminant, cyanide, at the lethal dose concentration. The results of the test were very similar to the laboratory results. Inhibition in the laboratory was 47% ± 1%, and in the non-laboratory location, 51% ± 1%.
Throughput	Approximately 20 analyses were completed per hour, and approximately 50 samples could be analyzed with the supplies contained in one LuminoTox PECS Test Kit.

ND = Significant inhibition was not detected.

Original signed by Gregory A. Mack	6/22/06	Original signed by Andrew P. Avel	8/7/06
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Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

Lab_Bell Inc.
LuminoTox PECs Test Kit

by

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Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, has financially supported and collaborated in the extramural program described here. This document has been peer reviewed by the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use.

Foreword

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

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv/.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center1.html.

Acknowledgments

The authors wish to acknowledge the support of all those who helped plan and conduct the verification test, analyze the data, and prepare this report. We would also like to thank Karen Bradham, U.S. EPA National Exposure Research Laboratory; Steve Allgeier, U.S. EPA Office of Water; Ricardo DeLeon, Metropolitan Water District of Southern California; Yves Mikol, New York City Department of Environmental Protection; and Stanley States, Pittsburgh Water and Sewer Authority, for their careful review of the test/quality assurance plan and/or this verification report.

Contents

Figures

Tables

List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
ATEL	Aqua Tech Environmental Laboratories
DI	deionized water
DDW	dechlorinated drinking water from Columbus, Ohio
DPD	n,n-diethyl-p-phenylenediamine
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
HDPE	high-density polyethylene
LD	lethal dose
mM	millimolar
µL	microliter
mg/L	milligram per liter
mL	milliliter
mm	millimeter
NSDWR	National Secondary Drinking Water Regulations
%D	percent difference
PE	performance evaluation
PECs	photosynthetic enzymatic complexes
QA	quality assurance
QC	quality control
QMP	quality management plan
SOP	standard operating procedure
TSA	technical systems audit

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The EPA’s National Exposure Research Laboratory and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the Lab_Bell Inc. LuminoTox photosynthetic enzymatic complexes (PECs), hereafter referred to as the LuminoTox PECs Test Kit. Rapid toxicity technologies were identified as a priority verification category through the AMS Center stakeholder process.

Chapter 2
Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This verification report provides results for the verification testing of the LuminoTox PECs Test Kit. Following is a description of the LuminoTox PECs Test Kit, based on information provided by the vendor. The information provided below was not verified during this test.

The LuminoTox PECs Test Kit (Figure 2-1) is a portable biosensor that indicates the presence of toxic chemicals in water. It uses PECs that have been stabilized through a method patented by Lab_Bell Inc. The PECs are membranes isolated from chloroplasts that are as simple to use as a chemical, but react more rapidly than a living organism because toxic compounds do not have to penetrate the cell wall of an organism. The photosynthetic electron chain is what is inhibited by contamination. When stimulated by light, the PECs emit fluorescence. The LuminoTox PECs Test Kit measures the fluorescence parameters produced both in background water and samples containing contaminants. Decreases in fluorescence parameters as a result of the presence of toxic contamination are expressed as a percent inhibition.

Figure 2-1. Lab_Bell Inc. LuminoTox PECs Test Kit

The LuminoTox PECs Test Kit consists of the LuminoTox analyzer, a bottle of PECs for 50 tests, reaction buffer, a blank water control, and a positive control. Also provided are disposable syringes in which the test is performed and fabric syringe covers to protect the reaction from light. Aluminum foil can be used as a light protector.

The LuminoTox analyzer is 21.6 by 12.7 by 7.6 centimeters and weighs 1 kilogram. It is battery-operated and is portable. The analyzer has a built-in RS-232 serial port outlet, which can also be used for transferring data to a spreadsheet (which was not done during this test) and is compatible with a printer. A total of 100 measurements can be stored in the internal memory. The rechargeable battery operates for eight hours. Each kit costs $89, and the analyzer costs approximately $7,500.

Chapter 3
Test Design

The objective of this verification test of rapid toxicity technologies was to evaluate their ability to detect certain toxins and to determine their susceptibility to interfering chemicals in a controlled experimental matrix. Rapid toxicity technologies do not identify or determine the concentration of specific contaminants, but serve as a screening tool to quickly determine whether water is potentially toxic.

As part of this verification test, the LuminoTox PECs Test Kit was subjected to various concentrations of contaminants such as industrial chemicals, pesticides, rodenticides, pharmaceuticals, nerve agents, and biological toxins. Each contaminant was added to separate drinking water samples and analyzed. In addition to determining whether the LuminoTox PECs Test Kit can detect the toxicity caused by each contaminant, its response to interfering compounds such as water treatment chemicals and by-products in clean drinking water, was evaluated. Table 3-1 shows the contaminants and potential interferences that were evaluated during this verification test.

This verification test was conducted from August to December 2005 according to procedures specified in the *Test/QA Plan for Verification of Rapid Toxicity Technologies* including Amendments 1 and 2.(1) The LuminoTox PECs Test Kit was verified by analyzing a dechlorinated drinking water sample from Columbus, Ohio (hereafter in this report referred to as DDW), fortified with various concentrations of the contaminants and interferences shown in Table 3-1. Where possible, the concentration of each contaminant or potential interference was confirmed independently by Aqua Tech Environmental Laboratories (ATEL), Marion, Ohio, or by Battelle, depending on the analyte.

The LuminoTox PECs Test Kit was evaluated by

- Endpoints and precision—percent inhibition for all concentration levels of contaminants and potential interfering compounds and precision of replicate analyses

- Toxicity threshold for each contaminant— contaminant level at which higher concentrations generate inhibition significantly greater than the negative control and lower concentrations do not

Table 3-1. Contaminants and Potential Interferences

Category	Contaminant
Biological toxins	Botulinum toxin complex B, ricin
Botanical pesticide	Nicotine
Carbamate pesticide	Aldicarb
Industrial chemical	Cyanide
Nerve agents	Soman, VX
Organophosphate pesticide	Dicrotophos
Pharmaceutical	Colchicine
Potential interferences	Aluminum, copper, iron, manganese, zinc, chloramination by-products, and chlorination by-products
Rodenticide	Thallium sulfate

- False positive responses—chlorination and chloramination by-product inhibition with respect to unspiked American Society for Testing and Materials (ASTM) Type II deionized (DI) water samples

- False negative responses—contaminants that were reported as producing inhibition similar to the negative control when present at lethal concentrations or negative inhibition that could cause falsely low percent inhibition

- Other performance factors (sample throughput, ease of use, reliability).

The LuminoTox PECs Test Kit was used to analyze the DDW samples fortified with contaminants at concentrations ranging from lethal levels to concentrations up to 1,000 times less than the lethal dose. The lethal dose of each contaminant was determined by calculating the concentration at which 250 milliliters (mL) of water would probably cause the death of a 154-pound person. These calculations were based on toxicological data available for each contaminant that are presented in Amendment 2 of the test/QA plan.(1) Inhibition (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the LuminoTox PECs Test Kit to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the LuminoTox PECs Test Kit results.

The response of the LuminoTox PECs Test Kit to compounds used during the water treatment process (identified as potential interferences in Table 3-1) was evaluated by analyzing separate aliquots of DDW fortified with each potential interference at one-half of the concentration limit recommended by the EPA's National Secondary Drinking Water Regulations (NSDWR)(2) guidance. For analysis of by-products of the chlorination process, the unspiked DDW was analyzed because Columbus, Ohio, uses chlorination as its disinfectant procedure. For the analysis of by-products of the chloramination process, a separate drinking water sample was obtained from the Metropolitan Water District of Southern California (LaVerne, California), which uses chloramination as its disinfection process. The samples were analyzed after residual chlorine was removed using sodium thiosulfate. Sample throughput was measured based on the number of samples analyzed per hour. Ease of use and reliability were determined based on documented observations of the operators.

3.1 Test Samples

Test samples used in the verification test included drinking water and quality control (QC) samples. Table 3-2 shows the number and type of samples analyzed. QC samples included method blanks and positive and negative control samples. The fortified drinking water samples were prepared from a single drinking water sample collected from the Columbus, Ohio, system. The water was dechlorinated using sodium thiosulfate and then fortified with various concentrations of contaminants and interferences. The DDW containing the potential interferences was analyzed at a single concentration level, while at least four dilutions were analyzed for each contaminant using the LuminoTox PECs Test Kit. Mixtures of contaminants and possible interfering compounds were not analyzed.

3.1.1 Quality Control Samples

QC samples included method blanks, positive controls, negative controls, and preservative blanks. The method blank samples consisted of ASTM Type II DI water and were used to ensure that no sources of contamination were introduced in the sample handling and analysis procedures. A positive control sample was included in the LuminoTox PECs Test Kit and was used as provided from the vendor. While performance limits were not placed on the results, inhibition significantly greater than the negative control for the positive control sample indicated to the operator that the LuminoTox PECs Test Kit was functioning properly. The negative control consisted of unspiked DDW and was used to set a background inhibition of the DDW, the matrix in which each test sample was prepared. To ensure that the preservatives in the contaminant solutions did not have an inhibitory effect, preservative blank samples were prepared. These preservative blanks consisted of DDW fortified with a concentration of preservative equivalent to that in the test solutions of botulinum toxin complex B, ricin, soman, and VX.

3.1.2 Drinking Water Fortified with Contaminants

Approximately 50 liters of Columbus, Ohio, tap water were collected in a low-density polyethylene container. The water was dechlorinated with sodium thiosulfate. Dechlorination was confirmed by adding an n,n-diethyl-p-phenylenediamine (DPD) tablet to a 10-mL aliquot of the water. Lack of color development in the presence of DPD indicated that the water was dechlorinated. All subsequent test samples were prepared from this DDW.

A stock solution of each contaminant was prepared in DDW at concentrations at or above the lethal dose level. The stock solution was further diluted to obtain one sample containing the lethal dose concentration for each contaminant and three additional samples with concentrations 10, 100, and 1,000 times less than the lethal dose. Additional concentrations of thallium sulfate were prepared and analyzed because of the large difference in response between two concentration levels. One dilution level was almost completely inhibitory and the next dilution level was non-inhibitory, so several intermediate concentrations were analyzed to better determine the toxicity threshold of that contaminant. Table 3-2 lists each concentration level and the number of samples analyzed at each level.

Table 3-2. Summary of Quality Control and Contaminant Test Samples

Type of Sample	Sample Characteristics	Concentration Levels	No. of Sample Analyses
Quality control	Method blank (ASTM Type II water)	NA	15
	Positive control	Used as provided in kit, 0.2 mg/L atrazine	15
	Negative control (unspiked DDW)	NA	60
	Preservative blank: botulinum toxin complex B	0.015 millimolar (mM) sodium citrate	4
	Preservative blank: VX and soman	0.21% isopropyl alcohol	4 with VX, 4 with soman
	Preservative blank: ricin	0.00024% NaN_3, 0.45 mM NaCl, 0.03 mM phosphate	4
DDW fortified with contaminants	Aldicarb	260; 26; 2.6; 0.26 milligrams/liter (mg/L)	4 per concentration level
	Botulinum toxin complex B	0.3; 0.03; 0.003; 0.0003 mg/L	4 per concentration level
	Colchicine	240; 24; 2.4; 0.24 mg/L	4 per concentration level
	Cyanide	250; 25; 2.5; 0.25 mg/L	4 per concentration level
	Dicrotophos	1,400; 140; 14; 1.4; mg/L	4 per concentration level
	Nicotine	2,800; 280; 28; 2.8 mg/L	4 per concentration level
	Ricin	15; 1.5; 0.15; 0.015 mg/L	4 per concentration level
	Soman	1.4; 0.14; 0.014; 0.0014 mg/L	4 per concentration level
	Thallium sulfate	2,800; 2,100; 1,400; 700; 280; 28; 2.8 mg/L	4 per concentration level
	VX	2.0; 0.2; 0.02; 0.002 mg/L	4 per concentration level
DDW fortified with potential interferences	Aluminum	0.5 mg/L	4
	Copper	0.6 mg/L	4
	Iron	0.15 mg/L	4
	Manganese	0.25 mg/L	4
	Zinc	2.5 mg/L	4
Disinfectant by-products	Chloramination by-products	NA	4
	Chlorination by-products	NA	60

NA = not applicable, samples not fortified with any preservative, contaminant, or potential interference.

3.1.3 Drinking Water Fortified with Potential Interferences

Individual aliquots of the DDW were fortified with one-half the concentration specified by the EPA's NSDWR for each potential interference. Table 3-2 lists the interferences, along with the concentrations at which they were tested. Four replicates of each of these samples were analyzed. To test the sensitivity of the LuminoTox PECs Test Kit to by-products of the chlorination process as potential interferences, the unspiked DDW (same as the negative control) was used since the water sample originated from a utility that uses chlorination as its disinfectant procedure. In a similar manner, by-products of the chloramination process were evaluated using a water sample from the Metropolitan Water District of Southern California. The residual chlorine in both of these samples was removed using sodium thiosulfate, and then the samples were analyzed in replicate with no additional fortification of contaminants.

3.2 Test Procedure

The procedures for preparing, storing, and analyzing test samples and confirming stock solutions are provided below.

3.2.1 Test Sample Preparation and Storage

A drinking water sample was collected as described in Section 3.1.2 and, because free chlorine inhibits the photosynthetic process that the LuminoTox PECs Test Kit depends on to indicate toxicity and can degrade the contaminants during storage, was immediately dechlorinated with sodium thiosulfate. Dechlorination of the water sample was qualitatively confirmed by adding a DPD tablet to a 10-mL aliquot of the DDW. All the contaminant samples, potential interference samples, preservative blanks, and negative control QC samples were made from this water sample, while the method blank sample was prepared from ASTM Type II DI water. The positive control sample, 0.2 mg/L atrazine, was provided by the vendor. All QC samples were prepared prior to the start of the testing and stored at room temperature. The stability of each contaminant for which analytical methods are available was confirmed by analyzing it three times over a two-week period. Throughout this time, each contaminant maintained its original concentration to within approximately 25%. Therefore, the aliquots of DDW containing the contaminants were prepared within two weeks of testing and were stored at room temperature without chemical preservation. The contaminants without analytical methods were analyzed within 48 hours of their preparation. To maintain the integrity of the test, test samples provided to the operators were labeled only with sample identification numbers so that the operators did not know their content.

3.2.2 Test Sample Analysis Procedure

The first step of sample analysis was to add the reaction buffer to the bottle of dried PECs, swirl, and then wait for 15 minutes before shaking well to finalize the dissolution of the PECs. Next, 2 mL of each control and test sample were each taken up into 3-mL syringes that were then covered with an opaque cloth provided by the vendor or with aluminum foil. A sample set typically included one method blank, one positive control sample, four replicates of the negative control, and four replicates each of four or five concentrations of contaminant. After adding 100 µL of the PECs solution to each control and water sample, each syringe was mixed by inverting five times. The solutions were allowed to react for 10 minutes, and the content of the syringe was added to a cuvette residing within the LuminoTox analyzer. The cover was closed for one minute, and the sample readings were taken from the LuminoTox analyzer. The analyzer generated four readings, two absolute fluorescence readings, and an efficiency and an inhibition reading. If the proper control sample (one very similar to the test sample) was entered into the analyzer, the percent inhibition could be obtained directly. Two operators performed all the analyses using the LuminoTox analyzer. One operator performed testing with contaminants that did not require special chemical and biological agent training and one performed testing with those that did. Both held bachelor's degrees in the sciences and were trained by the vendor to operate the LuminoTox analyzer.

3.2.3 Stock Solution Confirmation Analysis

The concentrations of the contaminant and interfering compound stock solutions were verified with standard analytical methods, with the exception of colchicine, ricin, and botulinum toxin complex B—contaminants without standard analytical methods. Aliquots to be analyzed by standard methods were preserved as prescribed by the method. In addition, the same standard methods were used to measure the concentration of each contaminant/potential interference in the unspiked DDW so that background concentrations of contaminants or potential interferences were accounted for within the displayed concentration of each contaminant/potential interference sample. Table 3-3 lists the standard methods used to measure each analyte; the results from the stock solution confirmation analyses (obtained by analyzing the lethal dose concentration for the contaminants and the single concentration that was analyzed for the potential interferences); and the background levels of the contaminants and potential interferences measured in the DDW sample, which were all non-detect or negligible.

Standard methods were also used to characterize several water quality parameters such as alkalinity; dissolved organic carbon content; specific conductivity; hardness; pH; concentration of haloacetic acids, total organic carbon, total organic halides, and trihalomethanes; and turbidity. Table 3-4 lists these measured water quality parameters for both the water sample collected in Columbus, Ohio, representing a water system using chlorination as the disinfecting process, and the water sample collected at the Metropolitan Water District of Southern California, representing a water system using chloramination for disinfection.

Table 3-3. Stock Solution Confirmation Results

	Method	Average Concentration ± Standard Deviation N = 4 (mg/L)(b)	Background in DDW (mg/L)
Contaminant			
Aldicarb	Battelle method	260 ± 7	<0.005
Botulinum toxin complex B	(a)	NA	NA
Colchicine	(a)	NA	NA
Cyanide	EPA 335.3(3)	249 ± 4 296 ± 26 (field portability)	0.006
Dicrotophos	Battelle method	1,168 ± 18	<3.0
Nicotine	Battelle method	2,837 ± 27	<0.01
Ricin	(a)	NA	NA
Soman	Battelle method	1.3 ± 0.1 (10/18/05) 1.16 ± 0.06 (10/21/05)	<0.025
Thallium sulfate	EPA 200.8(4)	2,469 ± 31	<0.001
VX	Battelle method	1.89 ± 0.08 (10/17/05) 1.77 ± 0.03 (10/20/05)	<0.0005
Potential Interference			
Aluminum	EPA 200.7(5)	0.50 ± 0.02	<0.2
Copper	EPA 200.7(5)	0.60 ± 0.03	<0.02
Iron	EPA 200.7(5)	0.155 ± 0.006	<0.04
Manganese	EPA 200.7(5)	0.281 ± 0.008	<0.01
Zinc	EPA 200.7(5)	2.63 ± 0.05	0.27

NA = Not applicable.

(a) No standard method available. QA audits and balance calibration assured accurately prepared solutions.

(b) Target concentration was highest concentration for each contaminant or interference on Table 3-2.

Table 3-4. Water Quality Parameters

Parameter	Method	Dechlorinated Columbus, Ohio, Tap Water (disinfected by chlorination)	Dechlorinated Southern California Tap Water (disinfected by chloramination)
Alkalinity (mg/L)	SM 2320 B(6)	40	71
Specific conductivity (µmho)	SM 2510 B(6)	572	807
Hardness (mg/L)	EPA 130.2(7)	118	192
pH	EPA 150.1(7)	7.6	8.0
Total haloacetic acids (µg/L)	EPA 552.2(8)	32.8	17.4
Dissolved organic carbon (mg/L)	SM 5310 B(6)	2.1	2.9
Total organic carbon (mg/L)	SM 5310 B(6)	2.1	2.5
Total organic halides (µg/L)	SM 5320B(6)	220	170
Total trihalomethanes (µg/L)	EPA 524.2(9)	74.9	39.2
Turbidity (NTU)	SM 2130(10)	0.1	0.1

NTU = nephelometric turbidity unit.

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center(11) and the test/QA plan for this verification test.(1)

4.1 Quality Control of Stock Solution Confirmation Methods

The stock solutions for the contaminants cyanide and thallium sulfate and for the potential interferences aluminum, magnesium, zinc, iron, and copper were analyzed at ATEL using standard reference methods. As part of ATEL's standard operating procedures (SOPs), various QC samples were analyzed with each sample set. These included matrix spike, laboratory control spike, and method blank samples. According to the standard methods used for the analyses, recoveries of the QC spike samples analyzed with samples from this verification test were within acceptable limits of 75% to 125%, and the method blank samples were below the detectable levels for each analyte. For VX, soman, aldicarb, nicotine, and dicrotophos, the confirmation analyses were performed at Battelle using a Battelle SOP or method. Calibration standard recoveries of VX and soman were always between 62% and 141%, and most of the time were between 90% and 120%. Dicrotophos standard recoveries ranged from 89% to 122%. Aldicarb standard recoveries ranged from 95% to 120%. Nicotine standard recoveries ranged from 96% to 99%. Standard analytical methods for colchicine, ricin, and botulinum toxin complex B were not available and, therefore, not performed. QA audits and balance calibrations assured that solutions for these compounds were accurately prepared.

4.2 Quality Control of Drinking Water Samples

A method blank sample consisting of ASTM Type II DI water was analyzed once by the LuminoTox PECs Test Kit for approximately every 20 drinking water samples that were analyzed. Because inhibition has to be calculated with respect to a control sample, none were calculated for the method blank samples. The method blanks were used as the control for calculating the inhibition of the DDW for the disinfecting by-product evaluation. A positive control sample of 0.2 mg/L atrazine also was analyzed once for approximately every 20 drinking water samples. While performance limits were not placed on the results of the positive control sample, the vendor informed Battelle that, if the positive control samples did not cause significant inhibition, it would indicate to the operator that the LuminoTox PECs Test Kit was not functioning properly. For 15 positive control samples, an inhibition of 69% ± 6% was measured. These inhibition values seemed to indicate the proper functioning of the LuminoTox PECs Test Kit. A negative control sample (unspiked DDW) was analyzed with approximately every four samples. The percent inhibition calculation for each sample incorporated the average inhibition of the negative control samples analyzed with that particular sample set; therefore, by definition, the average inhibition of four negative control samples was 0%.

4.3 Audits

A performance evaluation (PE) audit, a technical systems audit (TSA), and an audit of data quality were performed for this verification test.

4.3.1 Performance Evaluation Audit

The accuracy of the reference method used to confirm the concentrations of the stock solutions of the contaminants and potential interferences was confirmed by analyzing solutions of each analyte from two separate commercial vendors. The standards from one source were used to prepare the stock solutions during the verification test, while the standards from a second source were analyzed as the PE sample. The percent difference (%D) between the measured concentration of the PE sample, and the nominal concentration of that sample was calculated using the following equation:

$$\%D = \frac{M}{A} \times 100\% \qquad (1)$$

where M is the absolute value of the difference between the measured and the nominal concentration, and A is the nominal concentration. The %D between the measured concentration of the PE standard and the nominal concentration had to be less than 25% for the measurements to be considered acceptable. Table 4-1 shows the results of the PE audit for each compound. All %D values were less than 25.

PE audits were performed when more than one source of the contaminant or potential interference was commercially available and when methods were available to perform the confirmation; therefore, PE audits were not performed for all of the contaminants. To assure the purity of the other standards, documentation, such as certificates of analysis, was obtained for colchicine, botulinum toxin complex B, and ricin. In the cases of VX and soman, which were obtained from the U.S. Army, the reputation of the source, combined with the confirmation analysis data, provided assurance of the concentration analyzed.

4.3.2 Technical Systems Audit

The Battelle Quality Manager conducted a TSA to ensure that the verification test was performed in accordance with the test/QA plan(1) and the AMS Center QMP.(11) As part of the audit, the Battelle Quality Manager reviewed the contaminant standard and stock solution confirmation methods, compared actual test procedures with those specified in the test/QA plan, and reviewed data acquisition and handling procedures. Observations and findings from this audit were documented and submitted to the Battelle Verification Test Coordinator for response. No findings were documented that required any significant action. The records concerning the TSA are permanently stored with the Battelle Quality Manager.

Table 4-1. Summary of Performance Evaluation Audit

		Measured Concentration (mg/L)	Nominal Concentration (mg/L)	%D
Contaminant	Aldicarb	0.057	0.050	14
	Cyanide	1,025	1,000	3
	Dicrotophos	1.10	1.00	10
	Nicotine	0.120	0.100	20
	Thallium	1,010	1,000	1
Potential interference	Aluminum	960	1,000	4
	Copper	1,000	1,000	0
	Iron	960	1,000	4
	Manganese	922	1,000	8
	Zinc	1,100	1,000	10

4.3.3 Audit of Data Quality

At least 10% of the data acquired during the verification test were audited. Battelle's Quality Manager traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting, to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.4 QA/QC Reporting

Each internal assessment and audit was documented in accordance with Sections 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center.(11) Once the assessment report was prepared, the Battelle Verification Test Coordinator ensured that a response was provided for each adverse finding or potential problem and implemented any necessary follow-up corrective action. The Battelle Quality Manager ensured that follow-up corrective action was taken. The results of the TSA were sent to the EPA.

4.5 Data Review

Records generated in the verification test were reviewed before they were used to calculate, evaluate, or report verification results. Table 4-2 summarizes the types of data recorded. The review was performed by a technical staff member involved in the verification test, but not the staff member who originally generated the record. The person performing the review added his/her signature or initials and the date to a hard copy of the record being reviewed.

Table 4-2. Summary of Data Recording Process

Data to be Recorded	Responsible Party	Where Recorded	How Often Recorded	Disposition of Data[a]
Dates, times of test events	Battelle	Laboratory record books	Start/end of test, and at each change of a test parameter	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Sample preparation (dates, procedures, concentrations)	Battelle	Laboratory record books	When each sample was prepared	Used to confirm the concentration and integrity of the samples analyzed; procedures entered into laboratory record books
Test parameters (contaminant concentrations, location, etc.)	Battelle	Laboratory record books	When set or changed	Used to organize/check test results, manually incorporated in data spreadsheets as necessary
Stock solution confirmation analysis, sample analysis, chain of custody, and results	Battelle or contracted laboratory	Laboratory record books, data sheets, or data acquisition system, as appropriate	Throughout sample handling and analysis process	Transferred to spreadsheets/agreed upon report

[a] All activities subsequent to data recording were carried out by Battelle.

Chapter 5
Statistical Methods and Reported Parameters

The statistical methods presented in this chapter were used to verify the performance parameters listed in Section 3.

5.1 Endpoints and Precision

The fluorescence analyzer provided with the LuminoTox PECs Test Kit reported two values of fluorescence units for each sample analyzed. One of the values (F_1) represented a lower intensity fluorescence measurement and the other value (F_2) represented a higher intensity fluorescence measurement. These two measurements were used to calculate a percent inhibition with respect to the negative control. This was done using the following equations, which were provided by the vendor:

$$efficiency = \frac{F_{2\,sample} - F_{1\,sample}}{\overline{F_2}_{\;negative\ control}} \qquad (2)$$

$$\%\ inhibition = \left(1 - \frac{E_{sample}}{\overline{E}_{negative\ control}}\right) \times 100\% \qquad (3)$$

where efficiency (E) is a measure of the fluorescence produced by the PECs with respect to the average high-intensity fluorescence measurement values produced by the replicate negative control samples ($\overline{F_2}_{\;negative\ control}$) and the percent inhibition is the relative decrease in fluorescence production with respect to the average efficiency for four negative control samples ($\overline{E}_{negative\ control}$). As shown in the above equations, efficiency is calculated directly from the raw data, while the percent inhibition is calculated from the efficiency. The response of the negative control samples is accounted for in the calculation of percent inhibition of each sample. Therefore, the percent inhibition of the four negative control samples within each sample set always averaged zero percent. The negative control sample was always DDW, except when the inhibition of the disinfection by-products was being determined; in that case, ASTM Type II DI water served as the control sample.

The standard deviation (SD) of the results for the replicate samples was calculated, as follows, and used as a measure of the LuminoTox PECs Test Kit precision at each concentration. The standard deviation around the average negative control results represented the variability of the inhibition caused by the negative control water. Similarly, the standard deviation of the rest of the contaminant concentrations represented the precision of the inhibition caused by the background water combined with the contaminant.

$$SD = \left[\frac{1}{n-1} \sum_{k=1}^{n} \left(I_k - \overline{I} \right)^2 \right]^{1/2} \qquad (4)$$

where n is the number of replicate samples, I_k is the percent inhibition measured for the k^{th} sample, and $\overline{I}$ is the average percent inhibition of the replicate samples. Because the average inhibition was frequently near zero for this data set, relative standard deviations often would have greatly exceeded 100%, making the results difficult to interpret. Therefore, the precision results were left in the form of standard deviations of the percent inhibition so the reader could easily view the uncertainty around the average percent inhibition for results that were both near zero and significantly larger than zero.

5.2 Toxicity Threshold

The toxicity threshold was defined as the lowest concentration of contaminant to exhibit a percent inhibition significantly greater than the negative control. Also, each concentration level higher than the toxicity threshold had to be significantly greater than the negative control, and the inhibition produced by each lower concentration analyzed had to be significantly less than that produced by the toxicity threshold concentration. Since the inhibition of the test samples was calculated with respect to the inhibition of each negative control sample, the percent inhibition of the negative control was always zero. A significant difference in the inhibition at two concentration levels required that the average inhibition at each concentration level, plus or minus its respective standard deviation, did not overlap.

5.3 False Positive/Negative Responses

A response was considered false positive if an unspiked drinking water sample produced an inhibition significantly greater than zero when determined with respect to DI water. Depending on the degree of inhibition in the sample, toxicity from subsequent contamination of that sample may not be detectable or could be exaggerated as a result of the baseline inhibition. Drinking water samples collected from water systems using chlorination and chloramination as the disinfecting process were analyzed in this manner. An inhibition was considered significantly different from zero if the average inhibition, plus or minus the standard deviation, did not overlap with the zero inhibition plus or minus the standard deviation.

A response was considered false negative when the LuminoTox PECs Test Kit, subjected to a lethal concentration of some contaminant in the DDW, did not indicate inhibition significantly greater than the negative control (zero inhibition) and the other concentration levels analyzed (for lethal dose inhibition less than 100%). The inhibition of the lethal dose sample was required to be significantly greater than the other concentration levels because it more thoroughly incorporated the uncertainty of all the measurements made by the LuminoTox PECs Test Kit in determining a false negative result. A difference was considered significant if the average inhibition plus or minus the standard deviation did not encompass the value or range of values that were being compared. In addition, background water samples that increased the light production of the LuminoTox PECs Test Kit organisms (i.e., negative inhibition) were considered false negative because such samples could cancel out the effect of a contaminant that inhibits light production, making it seem that the contaminant had no toxic effect.

5.4 Other Performance Factors

Ease of use (including clarity of the instruction manual, user-friendliness of software, and overall convenience) was qualitatively assessed throughout the verification test through documented observations of the operators and Verification Test Coordinator. Sample throughput was evaluated quantitatively based on the number of samples that could be analyzed per hour.

Chapter 6
Test Results

6.1 Endpoints and Precision

Tables 6-1a-k present the percent inhibition data for 10 contaminants; and Table 6-2 gives the percent inhibition data for preservatives with concentrations similar to what would be contained in a lethal dose of botulinum toxin complex B, ricin, soman, and VX. Given in each table are the concentrations analyzed, the percent inhibition of each replicate at each concentration, and the average and standard deviation of the inhibition of the four replicates at each concentration. Contaminant test samples that produced negative percent inhibition values indicated an increase in light production by the LuminoTox PECs Test Kit and were considered non-toxic.

6.1.1 Contaminants

Aldicarb, cyanide, nicotine, and thallium sulfate generated an inhibition significantly larger than the negative control. Cyanide and nicotine generated detectable inhibition at the two highest concentration levels analyzed; and aldicarb and thallium sulfate (upon the initial analysis), produced detectable inhibition only at the lethal dose concentration level. Because of the rather large inhibition (63%) for thallium sulfate at only the lethal dose concentration, additional dilutions were performed to more closely determine the toxicity threshold. Those results are shown in Table 6-1j. During these additional dilutions, toxicity due to thallium sulfate was detectable down to 280 mg/L. Interestingly, inhibition at that concentration was not detectable during the initial analyses. Colchicine and dicrotophos did not generate a detectable inhibition.

It is important to note that the botulinum toxin complex B, ricin, soman, and VX stock solutions used to prepare the test samples were stored in various preservatives that included sodium azide, sodium chloride, and sodium phosphate for ricin; sodium citrate only for botulinum toxin complex B, and isopropyl alcohol for soman and VX. During the previous ETV test of this technology category, the preservatives were not accounted for in the negative control; therefore, the results from each test should be interpreted accordingly. The results for this test are more thorough because they show the sensitivity (or lack thereof) to both the preservative and the contaminant. In the in the earlier verification test, toxicity could have been the result of either. Table 3-2 details the concentrations of preservatives in the lethal dose samples of each

Table 6-1a. Aldicarb Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-2	0	2
	-1		
	2		
	0		
0.26	-1	-2	2
	-2		
	0		
	-4		
2.6	1	0	1
	0		
	-2		
	0		
26	0	2	3
	2		
	7		
	1		
260 (Lethal Dose)	25	26	3
	26		
	23		
	30		

Table 6-1b. Botulinum Toxin Complex B Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-3	0	2
	0		
	3		
	-1		
0.0003	-11	-12	2
	-14		
	-10		
	-11		
0.003	-11	-8	4
	-9		
	-10		
	-1		
0.03	-4	-5	3
	-8		
	-5		
	-2		
0.3 (Lethal Dose)	-3	0	6
	3		
	7		
	-7		
Lethal Dose Preservative Blank	-8	-10	3
	-9		
	-13		
	-12		

Table 6-1c. Colchicine Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-5	0	4
	-1		
	1		
	5		
0.24	-8	-6	2
	-5		
	-4		
	-7		
2.4	-3	-2	1
	-2		
	-2		
	-2		
24	-1	-3	5
	-9		
	-4		
	1		
240 (Lethal Dose)	3	2	3
	-3		
	5		
	1		

Table 6-1d. Cyanide Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	6	0	5
	3		
	-3		
	-5		
0.25	-8	-7	5
	-13		
	-6		
	-1		
2.5	-7	-8	7
	-17		
	-10		
	-1		
25	37	31	6
	27		
	37		
	25		
250 (Lethal Dose)	47	47	1
	49		
	47		
	46		
Field Portability Negative Control	3	0	3
	1		
	0		
	-4		
Field Portability 250	50	51	1
	52		
	50		
	53		

Table 6-1e. Dicrotophos Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	16	0	11
	-9		
	-6		
	-2		
1.4	2	4	4
	-1		
	4		
	10		
14	10	8	2
	5		
	8		
	9		
140	9	10	2
	12		
	11		
	9		
1,400 (Lethal Dose)	5	3	2
	0		
	5		
	3		

Table 6-1f. Nicotine Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-4	0	6
	-1		
	-3		
	8		
2.8	1	9	6
	15		
	12		
	9		
28	3	6	5
	0		
	8		
	12		
280	81	80	2
	77		
	79		
	82		
2,800 (Lethal Dose)	77	77	1
	76		
	76		
	77		

Table 6-1g. Ricin Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-2	0	2
	-1		
	2		
	1		
0.015	-15	-10	5
	-12		
	-3		
	-8		
0.15	-3	-7	4
	-9		
	-4		
	-11		
1.5	1	5	4
	4		
	7		
	9		
15 (Lethal Dose)	4	2	9
	4		
	-10		
	11		
Lethal Dose Preservative Blank	-16	-10	6
	-14		
	-6		
	-5		

Table 6-1h. Soman Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	2	0	4
	-5		
	4		
	-1		
0.0014	-7	-1	5
	-2		
	3		
	2		
0.014	6	0	6
	-7		
	2		
	-3		
0.14	-7	-6	4
	-9		
	-8		
	0		
1.4 (Lethal Dose)	-11	-5	6
	-10		
	1		
	-2		
Lethal Dose Preservative Blank	-6	-3	6
	6		
	-6		
	-6		

Table 6-1i. Thallium Sulfate Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-3	0	7
	-7		
	-1		
	10		
2.8	-7	-12	6
	-20		
	-14		
	-8		
28	-12	-3	7
	3		
	-6		
	2		
280	8	1	5
	-2		
	-2		
	-1		
2,800 (Lethal Dose)	60	63	2
	65		
	63		
	62		

Table 6-1j. Thallium Sulfate Percent Inhibition Results—Additional Dilutions

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-1	0	1
	-1		
	1		
	1		
280	16	19	4
	23		
	18		
	15		
700	27	31	5
	31		
	35		
	37		
1,400	49	49	1
	50		
	49		
	49		
2,100	64	65	1
	66		
	64		
	64		
2,800	73	73	1
	72		
	73		
	74		

Table 6-1k. VX Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-2	0	2
	0		
	1		
	1		
0.002	8	3	5
	-5		
	5		
	3		
0.02	-2	-2	5
	-9		
	4		
	-1		
0.2	-11	-8	4
	-12		
	-5		
	-4		
2 (Lethal Dose)	0	-5	3
	-5		
	-7		
	-7		
Lethal Dose Preservative Blank	5	-1	4
	-2		
	-4		
	-2		

contaminant. These data could be evaluated in two ways to determine the sensitivity of the LuminoTox PECS Test Kit to contaminants stored in preservatives. The first approach would be to determine the inhibition of the test samples containing preservatives with respect to the background negative control, as was the case for the contaminants not stored in preservatives. This technique, however, could indicate that the LuminoTox PECs Test Kit was sensitive to the contaminant when, in fact, it was sensitive to one of the preservatives. Since these contaminants are only available (either commercially or from the government) in aqueous formulations with the preservatives, this may be appropriate. The second approach would be to fortify negative control samples with the same concentrations of preservative contained in all the samples so that the inhibition resulting from the preservatives could be subtracted from the inhibition caused by the contaminant. This approach would greatly increase the number of samples required for analysis. Therefore, for this test, aspects of both approaches were incorporated without substantially increasing the number of samples. Negative control samples fortified with a concentration of each preservative equivalent to the concentration in the lethal dose test samples (preservative blanks) were analyzed prior to and with every set of test samples. For those sets of test samples for which it was especially difficult to determine whether inhibitory effects were from the contaminant or the preservative, the preservative blank was diluted identically to all the contaminant samples and analyzed with them so a background subtraction could take place if necessary.

During the initial analysis of the preservative blanks (Table 6-2), none of the preservative blank samples generated an inhibition significantly greater than the DDW negative control. Because the preservatives apparently did not have toxic effects at the lethal dose concentration, no additional dilutions of preservative blanks were required to determine whether there were toxic effects from each individual concentration level. Each contaminant concentration level was evaluated and compared with the negative control to determine any toxic effects. The inhibition of the lethal dose preservative blank was determined with each contaminant sample set and is shown with the results from each of the contaminants regardless of the result of the initial preservative blank analysis.

Table 6-2. Lethal Dose Level Preservative Blank Percent Inhibition Results

Preservative Blank	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-3	0	7
	6		
	5		
	-8		
Ricin	22	12	12
	21		
	6		
	-3		
Soman/VX	-2	-5	3
	-8		
	[a]		
	-4		
Botulinum Toxin Complex B	9	0	7
	3		
	-6		
	-6		

[a] Removed because it was an obvious outlier.

For all four of these contaminants, neither the contaminant sample nor the lethal dose preservative blank generated an inhibition that was significantly greater than the negative control. This indicated that none of these contaminants produced a toxic effect on the LuminoTox PECs Test Kit.

6.1.2 Potential Interferences

All of the potential interference samples were prepared in DDW and compared with the negative control to determine the level of inhibition. This determination is crucial because the ability of the LuminoTox PECs Test Kit to detect toxicity is dependent on the background light production in whatever drinking water matrix is being used. If the background drinking water sample completely inhibits the background light, inhibition caused by contaminants could not be detected. Table 6-3 presents the results from the samples analyzed to test the effect of potential interferences on the LuminoTox PECs Test Kit. Of the five metal solutions that were evaluated as possible interferences, two, zinc (12% ± 5%) and copper (70% ± 1%), exhibited inhibition that was significantly different from the DDW negative control (0% ± 2%). Because zinc exhibited a very small inhibition, therefore leaving plenty of light for inhibition due to contamination, it

Table 6-3. Potential Interferences Results

Potential Interferences	Concen-tration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative control (Metals)	NA	0	0	2
		-2		
		0		
		2		
Aluminum	0.5	-1	0	4
		2		
		-4		
		5		
Copper	0.6	69	70	1
		69		
		71		
		70		
Iron	0.15	1	7	6
		6		
		15		
		6		
Manganese	0.25	6	5	6
		14		
		2		
		-1		
Zinc	2.5	20	12	5
		10		
		8		
		11		
Negative control (By-products)		3	0	3
		2		
		-2		
		-3		
Chlorination by-products	NA	[a]	14	11
Chloramination by-products	NA	5	7	2
		7		
		7		
		10		

NA = Not applicable.
[a] = Average inhibition across all negative control samples (N=60).

should be considered only a slight interference. Therefore, water samples containing similar concentration of zinc could be used as a representative negative control sample because enough background light for inhibition by contaminants remains even though in it is somewhat inhibited by zinc. Copper, on the other hand, should be considered a possible interference because the majority of background light would be inhibited by the matrix if a similar copper concentration was present, leaving less residual light to be inhibited by contamination.

To investigate whether the LuminoTox PECs Test Kit is sensitive to by-products of disinfecting processes, DDW samples from water systems that use chlorination and chloramination were analyzed and compared with ASTM Type II DI water as the control sample. In the absence of a background water sample, it seems likely that DI water may be used as a “clean water” control; therefore, it would be helpful to know what the results would be if this is done. The sample from the water supply disinfected with chlorination (N=60) exhibited an average inhibition of 14% ± 11%, while the sample from the water supply disinfected by chloramination exhibited an inhibition of 7% ± 2% on four replicates. The difference in number of replicates is because the dechlorinated water was used as the negative control with each sample set; therefore, much more data were collected on that particular water. These inhibition data suggest that samples disinfected by either process are not likely to interfere with the LuminoTox PECs Test Kit results because the inhibition caused by the “clean” drinking water matrices left most of the background fluorescence to potentially be inhibited by contamination. Even for copper, which produced a greater inhibition, the inhibition was not complete; and it can be accounted for by using negative control samples that are very similar to the water being analyzed. For both scenarios, if samples are analyzed daily, a good practice would be to archive a negative control sample each day in case of contamination the next day.

6.1.3 Precision

Across all the contaminants and potential interferences, the standard deviation (not relative standard deviation) was measured and reported for each set of four replicates to evaluate the precision of the LuminoTox PECs Test Kit. Out of 80 opportunities, the standard deviation of the four replicate inhibition measurements was less than 5% inhibition 51 times (63% of the time), between 5 and 10% inhibition 26 times (33% of the time), and greater than 10% inhibition 3 times (4%). As described in Section 3.2.2, the analysis procedure required that each replicate undergo the entire analysis process; therefore, the measurement of precision represents the precision of the analysis method performed on a single water sample on a given day. The precision does not reflect the repeatability of the method across more than one day or more than one preparation of reagents or more than one operator.

6.2 Toxicity Threshold

Table 6-4 gives the toxicity thresholds, as defined in Section 5.2, for each contaminant. Note the difference between detectability with respect to the negative control and the toxicity threshold with respect to the other concentration levels analyzed. A contaminant concentration level can have an inhibition significantly different from the negative control (thus detectable), but if its inhibition is not significantly different from the concentration levels below it, it would not be considered the toxicity threshold because in the context of this test, its inhibition would not be distinguishable from that of the lower concentrations. The lowest toxicity threshold concentration was for cyanide at 25 mg/L.

Table 6-4. Toxicity Thresholds

Contaminant	Concentration (mg/L)
Aldicarb	260
Botulinum toxin complex B	ND
Colchicine	ND
Cyanide	25
Dicrotophos	ND
Nicotine	280
Ricin	ND
Soman	ND
Thallium sulfate	280
VX	ND

ND = Significant inhibition was not detected.

6.3 False Positive/Negative Responses

The chlorination and chloramination by-product samples generated an inhibition that was considered false positive because it was significantly greater than the negative control. However, the inhibition was very slight, leaving enough fluorescence available for inhibition if contamination was present.

Table 6-5 shows the LuminoTox PECs Test Kit false negative responses, which are described in Section 5.3. Botulinum toxin complex B, colchicine, dicrotophos, ricin, soman, and VX did not exhibit a detectable inhibition at the lethal concentration.

6.4 Other Performance Factors

6.4.1 Ease of Use

The LuminoTox PECs Test Kit contained detailed instructions and clear illustrations. The contents of the LuminoTox PECs Test Kit were well identified with labels on the vials. Storage requirements were stated in the instructions and on the reagent vials.

Preparation of the test samples for analysis was straightforward. However, the PECs had to be stored on ice between every sample analysis to keep them from coming to room temperature. Therefore, the operators had to open and close the cooler containing the ice every time they needed to add PECs to a water sample or control, which was somewhat inconvenient because the melting ice caused the lab bench and operators' hands to be wet most of the time. The analyzer,

Table 6-5. False Negative Responses

Contaminant	Lethal Dose Concentration (mg/L)	False Negative
Aldicarb	260	no
Botulinum toxin complex B	0.30	yes
Colchicine	240	yes
Cyanide	250	no
Dicrotophos	1,400	yes
Nicotine	2,800	no
Ricin	15	yes
Soman	1.4	yes
Thallium sulfate	2,800	no
VX	2.0	yes

including a piece of foil covering the cuvette opening, was easy to use; but the necessity to record four numbers as raw data was somewhat burdensome. Lab_Bell has indicated that this is undergoing modification. After testing, the analyzer was easily wiped clean and required no routine maintenance other than selecting the PECs mode prior to the start of sample analysis.

No formal scientific education would be required to use the LuminoTox PECs Test Kit. However, good laboratory skills, especially pipetting technique, would be beneficial. Verification testing staff were able to operate the LuminoTox PECs Test Kit after a 4-hour training session with the vendor. Approximately 2 mL of liquid waste were generated per sample, along with leftover PECs and a 3-mL disposable syringe.

6.4.2 Field Portability

The LuminoTox PECs Test Kit was transported from a laboratory setting to a storage room for the field portability evaluation. The storage room contained several tables and light and power sources, but no other laboratory facilities. No carrying case was provided with the LuminoTox PECs Test Kit; however, all materials were transported by one person in a small cardboard box. The LuminoTox PECs Test Kit was set up easily in less than 10 minutes, and a source of electricity was not required since the analyzer was powered by batteries. Minimum space requirements in the field would be a mostly flat surface of approximately 45 by 60 centimeters. The only items needed for field use that were not provided in the LuminoTox PECs Test Kit were a cooler to transport and store the PECs and a timer. These two items can be purchased through Lab_Bell or are commercially available. In addition, no waste reservoir was provided. Overall, the LuminoTox PECs Test Kit was easy to transport to the non-laboratory location and was deployed in a matter of minutes. The limiting factor for testing in the field would be the approximately 30 minutes required to allow the PECs to dissolve. After dissolution, results were obtained within 10 minutes of starting the test. The LuminoTox PECs Test Kit was tested with one contaminant, cyanide, at the lethal dose concentration. The results of the test (see

Table 6-1d) were very similar to the laboratory results. Inhibition in the laboratory was 47% ± 1%, and in the non-laboratory location, 51% ± 1%, suggesting that location did not significantly impact the performance of the LuminoTox PECs Test Kit.

6.4.3 Throughput

Once the PECs were prepared, approximately 20 analyses were completed per hour. The 20 analyses included method blanks and positive and negative controls, as well as test samples. Approximately 50 samples could be analyzed with the supplies contained in one LuminoTox PECS Test Kit.

Chapter 7
Performance Summary

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Average Inhibition at Concentrations Relative to the LD Concentration (%)				Range of Standard Deviations (%)	Toxicity Thresh. (mg/L)
			LD	LD/10	LD/100	LD/1,000		
Contaminants in DDW	Aldicarb	260	26	2	0	-2	1-3	260
	Botulinum toxin complex B	0.3	0	-5	-8	-12	2-6	ND
	Colchicine	240	2	-3	-2	-6	1-5	ND
	Cyanide	250	47	31	-8	-7	1-7	25
	Dicrotophos	1,400	3	10	8	4	2-4	ND
	Nicotine	2,800	77	80	6	9	1-6	280
	Ricin	15	2	5	-7	-10	4-9	ND
	Soman	1.4	-5	-6	0	-1	4-6	ND
	Thallium sulfate	2,800	63	19	-3	-12	2-7	280
	VX	2	-5	-8	-2	3	3-5	ND

Parameter	Interference	Conc. (mg/L)	Average Inhibition (%)	Standard Deviation (%)
Potential interferences in DDW	Aluminum	0.5	0	4
	Copper	0.6	70	1
	Iron	0.15	7	6
	Manganese	0.25	5	6
	Zinc	2.5	12	5

Parameter	
False positive response	Both the chlorinated and chloraminated disinfection by-product samples produced an inhibition significantly greater than the negative control and, therefore, were considered false positive responses. However, the disinfectant by-product samples produced an inhibition of less than 15%, leaving enough fluorescence available for subsequent inhibition due to contamination.
False negative response	Botulinum toxin complex B, colchicine, dicrotophos, ricin, soman, and VX exhibited non-detectable responses at the lethal dose concentration.
Ease of Use	The LuminoTox PECs Test Kit contained detailed instructions and clear illustrations. The contents of the LuminoTox PECs Test Kit were well identified with labels on the vials. Storage requirements were stated in the instructions and on the reagent vials. Preparation of the test samples for analysis was straightforward. However, the PECs had to be stored in ice between every sample analysis to keep them from coming to room temperature, which was somewhat inconvenient because the melting ice caused the lab bench and operators' hands to be wet most of the time. The necessity to record four numbers as raw data was somewhat burdensome. Lab_Bell has indicated that it is modifying this. No formal scientific education would be required to use the LuminoTox PECs Test Kit.
Field Portability	The LuminoTox PECs Test Kit was transported from a laboratory setting to a storage room for the field portability evaluation. The LuminoTox PECs Test Kit was tested with one contaminant, cyanide, at the lethal dose concentration. The results of the test were very similar to the laboratory results. Inhibition in the laboratory was 47% ± 1%, and in the non-laboratory location, 51% ± 1%.
Throughput	Approximately 20 analyses were completed per hour, and approximately 50 samples could be analyzed with the supplies contained in one LuminoTox PECS Test Kit.

ND = Significant inhibition was not detected.

Chapter 8
References

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