

June 2006

Environmental Technology Verification Report

LAB_BELL INC.
LUMINO_TOX SAPS TEST KIT

Prepared by
Battelle

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The Business of Innovation

Under a cooperative agreement with

 **EPA** U.S. Environmental Protection Agency

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THE ENVIRONMENTAL TECHNOLOGY VERIFICATION
PROGRAM



ETV Joint Verification Statement

TECHNOLOGY TYPE:	Rapid Toxicity Testing System		
APPLICATION:	Detecting Toxicity in Drinking Water		
TECHNOLOGY NAME:	LuminoTox SAPS		
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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 permittees), 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 stabilized aqueous photosynthetic systems (SAPS) 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 SAPS 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 SAPS 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 SAPS 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 negative background inhibition that caused falsely low inhibition
- Other performance factors (sample throughput, ease of use, reliability).

The LuminoTox SAPS 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 above 1ppm inhibits the photosynthetic process that the LuminoTox SAPS Test Kit depends on to indicate toxicity and can degrade the contaminants during storage. Inhibition (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the LuminoTox SAPS Test Kit to detect toxicity, as well as to measure the precision of the LuminoTox SAPS Test Kit results. The response of the LuminoTox SAPS 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 SAPS Test Kit Test Kit is based on information provided by the vendor. This technology description was not verified in this test.

The LuminoTox SAPS Test Kit is a portable biosensor that uses SAPS activated by light absorption to recognize toxic chemicals in water. SAPS are activated at a wavelength of 470 nanometers, and fluorescence emission is read at wavelengths longer than 700 nanometers. SAPS are whole algae (*Chlorella vulgaris*) that fluoresce when photosynthesis (the conversion of electromagnetic energy into stored chemical energy) is activated by light absorption. Some of the absorbed energy is emitted as fluorescence, which is the signal measured by the LuminoTox SAPS Test Kit. The photosynthetic electron chain is inhibited by a broad spectrum of organic molecules (ureas, azides, phenols, quinones or amide derivatives, polyaromatic hydrocarbons, polychlorinated biphenyls), redox species, cyanides, and metallic cations. The LuminoTox SAPS Test Kit measures the fluorescence produced both in background water and samples containing contaminants. Decreases in fluorescence as a result of adding toxic contamination are expressed as percent inhibition.

Although other SAPS could be used in the LuminoTox analyzer, Lab_Bell uses *Chlorella vulgaris*, which is concentrated by centrifugation in the middle of its exponential growth curve and stored at 4°C for a few weeks. Prior to analysis, SAPS must be activated in room light for 90 minutes at ambient temperature. The LuminoTox test is performed in the dark (in a covered syringe) by exposing 100 microliters of SAPS solution to 2 milliliters of test sample for 10 minutes. In this short period of time, permeable molecules acting directly on the photosynthetic electron chain are detected at low concentrations. Prolonged incubation allows the detection of less permeable molecules.

The LuminoTox SAPS Test Kit consists of the LuminoTox analyzer, a bottle of SAPS for 50 tests, two vials of organic standards (positive controls to ensure that the SAPs are fully functional), and one vial of distilled water (for blank samples). Also provided are disposable syringes in which the test is performed and fabric syringe covers to protect the reaction from light. The analyzer is 21.6 by 12.7 by 7.6 centimeters and weighs 1 kilogram. The analyzer is battery-operated, is equipped with a RS-232 serial port for transferring data, and can be connected to a printer (not done during this test). A total of 100 measurements can be stored in the internal memory. The rechargeable battery operates for eight hours. Reagents (including buffers and positive and negative controls) for approximated 50 analyses cost \$106, while the LuminoTox 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	50	14	5	0	1-3	26
	Botulinum toxin Complex B	0.3	-10	-6	-5	1	1-8	ND
	Colchicine	240	0	4	0	3	1-5	ND
	Cyanide	250	17	10	7	1	2-3	250
	Dicrotophos	1,400	4	-11	-12	-10	1-2	ND
	Nicotine	2,800	34	10	1	3	1-4	280
	Ricin	15	0	1	-4	3	2-6	ND
	Soman	1.4	-2	1	2	0	2-3	ND
	Thallium sulfate	2,800	0	1	-3	-4	2-3	ND
	VX	2	5	3	-1	2	2-5	ND
Potential interferences in DDW	Interference	Conc. (mg/L)	Average Inhibition (%)		Standard Deviation (%)			
	Aluminum	0.5	1		4			
	Copper	0.6	3		1			
	Iron	0.15	1		2			
	Manganese	0.25	1		3			
	Zinc	2.5	-1		4			
False positive response	None of the LuminoTox SAPS Test Kit responses were considered false positive. All disinfection by-product test samples left enough fluorescence for inhibition due to contamination.							
False negative response	Botulinum toxin, colchicine, dicrotophos, ricin, soman, thallium sulfate, and VX exhibited non-detectable responses at the lethal dose concentration.							
Ease of use	The LuminoTox SAPS Test Kit contained detailed instructions and clear illustrations. The contents 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. The necessity to record four numbers as raw data was somewhat burdensome; however, Lab_Bell has indicated this procedure is being modified. No formal scientific education would be required to use the LuminoTox SAPS Test Kit.							
Field portability	The LuminoTox SAPS Test Kit was transported from a laboratory setting to a storage room for the field portability evaluation. The limiting factor for testing in the field would be the approximately 90 minutes required to allow the SAPS to be exposed to light prior to testing. The LuminoTox SAPS 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 17% ± 2%, and in the non-laboratory location, 16% ± 4%.							
Throughput	Approximately 20 analyses were completed per hour, and 50 samples could be analyzed with the supplies contained in one LuminoTox SAPS Test Kit.							

ND = Significant inhibition was not detected.

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June 2006

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

**Lab_Bell Inc.
LuminoTox SAPS 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 permittees, 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>.

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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
QA	quality assurance
QC	quality control
QMP	quality management plan
SAPS	stabilized aqueous photosynthetic systems
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 permittees; 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 stabilized aqueous photosynthetic systems (SAPS), hereafter referred to as the LuminoTox SAPS 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 SAPS. Following is a description of the LuminoTox SAPS Test Kit, based on information provided by the vendor. The information provided below was not verified in this test.

The LuminoTox SAPS Test Kit (Figure 2-1) is a portable biosensor that uses SAPS activated by light absorption to recognize toxic chemicals in water. SAPS are activated at a wavelength of 470 nanometers, and fluorescence emission is read at wavelengths longer than 700 nanometers. SAPS are whole algae (*Chlorella vulgaris*) that fluoresce when photosynthesis (the conversion of electromagnetic energy into stored chemical energy) is activated by light absorption. Some of the absorbed energy is emitted as fluorescence, which is the signal measured by the LuminoTox SAPS Test Kit. The photosynthetic electron chain is inhibited by a broad spectrum of organic molecules (ureas, azides, phenols, quinones or amide derivatives, polyaromatic hydrocarbons, polychlorinated biphenyls), redox species, cyanides, and metallic cations. The LuminoTox SAPS Test Kit measures the fluorescence produced both in background water and samples containing contaminants. Decreases in fluorescence parameters as a result of adding toxic contamination are expressed as percent inhibition.

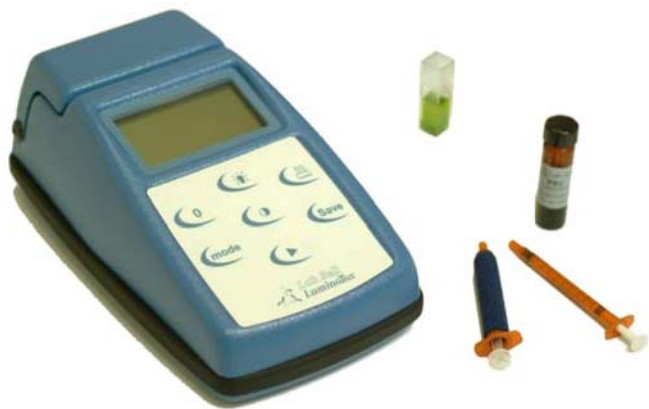


Figure 2-1. Lab_Bell Inc. LuminoTox SAPS Test Kit

Although other SAPS could be used in the LuminoTox analyzer, Lab_Bell uses *Chlorella vulgaris*, which is concentrated by centrifugation in the middle of its exponential growth curve and stored at 4°C for a few weeks. Prior to analysis, SAPS must be activated in room light for 90 minutes at ambient temperature. The LuminoTox test is performed in the dark (in a covered syringe) by exposing 100 microliters of SAPS solution to 2 milliliters of test sample for

10 minutes. In this short period of time, permeable molecules acting directly on the photosynthetic electron chain are detected at low concentrations. Prolonged incubation allows the detection of less permeable molecules.

The LuminoTox SAPS Test Kit consists of the LuminoTox analyzer, a bottle of SAPS for 50 tests, two vials of organic standards (positive controls to ensure that the SAPS are fully functional), and one vial of distilled water (for blank samples). Also provided are disposable syringes in which the test is performed and fabric syringe covers to protect the reaction from light. The analyzer is 21.6 by 12.7 by 7.6 centimeters and weighs 1 kilogram. The analyzer is battery-operated, is equipped with a RS-232 serial port for transferring data, and can be connected to a printer (not done during this test). A total of 100 measurements can be stored in the internal memory. The rechargeable battery operates for eight hours. Reagents (including buffers and positive and negative controls) for 50 analyses cost \$106, while the LuminoTox 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 SAPS 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 SAPS 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. 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 SAPS 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 SAPS 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 inhibition
- Other performance factors (sample throughput, ease of use, reliability).

The LuminoTox SAPS 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 results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the LuminoTox SAPS Test Kit to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the LuminoTox SAPS Test Kit results.

The response of the LuminoTox SAPS 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 SAPS 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 SAPS 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 SAPS 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. 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	14
	Positive control	Used as provided in kit, 0.01 mg/L atrazine	14
	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
	Thallium sulfate	2,800; 280; 28; 2.8 mg/L	4 per concentration level
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; 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	56

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 SAPS 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 over 1 ppm inhibits the photosynthetic process that the LuminoTox SAPS 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, 0.01 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 allow the LuminoTox SAPS to sit in the light at room temperature for 90 minutes. Next, 2 mL of each control and water sample (both at room temperature) were taken up into individual 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 μ L of the SAPS 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 SAPS Test Kit analyzer. The cover was closed for one minute, and the sample reading (reported in light units that were converted to percent inhibition) was taken from the LuminoTox SAPS Test Kit 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 SAPS Test Kit. 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 SAPS Test Kit.

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 SAPS 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 background inhibition of the DDW for the disinfection by-product evaluation. A positive control sample of 0.01 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, an inhibition significantly greater than zero indicated to the operator that the LuminoTox SAPS Test Kit was functioning properly. For 14 positive control samples, an inhibition of $28\% \pm 12\%$ was measured. This is further discussed in Section 6.1.1. 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:

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

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 luminometer provided with the LuminoTox SAPS Test Kit reported two values of absolute 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:

$$efficiency = \frac{F_{2\ sample} - F_{1\ sample}}{\overline{F_{2\ negative\ control}}} \quad (2)$$

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

where efficiency (E) is a measure of the fluorescence produced by the SAPS 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 efficiencies. 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 SAPS Test Kit's 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.

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

where n is the number of replicate samples, I_k is the percent inhibition measured for the k^{th} sample, and \bar{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 SAPS 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 other concentration levels because it more thoroughly incorporated the uncertainty of all the measurements made by the LuminoTox SAPs 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 SAPS 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 6-1 a-j 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 results for 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 SAPS and were considered non-toxic.

6.1.1 Contaminants

The contaminants that generated an inhibition significantly greater than the negative control included aldicarb, cyanide, and nicotine. Aldicarb and cyanide generated an inhibition significantly greater than the negative control at the highest three concentrations. In both, some of the three concentrations exhibited an inhibition that, while significantly different from the negative control, was not significantly different from the concentration level(s) below it. Consequently, the toxicity threshold, which must be significantly different from the concentration levels above and below it, was 26 mg/L for aldicarb and 250 mg/L for cyanide. Nicotine generated a detectable inhibition at the two highest concentrations analyzed (2,800 mg/L and 280 mg/L). Colchicine, dicotophos, and thallium sulfate had no 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 6-1a. Aldicarb Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	0	0	2
	0		
	3		
	-2		
Positive Control	8 ^(a)		
0.26	1	0	3
	4		
	0		
	-3		
2.6	5	5	2
	6		
	7		
	2		
26	13	14	1
	15		
	14		
	13		
260 (Lethal Dose)	49	50	1
	51		
	51		
	48		

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

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

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	0	0	6
	-5		
	8		
	-3		
Positive Control	41		
0.0003	4	1	4
	3		
	1		
	-5		
0.003	0	-5	4
	-8		
	-5		
	-9		
0.03	-8	-6	1
	-5		
	-6		
	-6		
0.3 (Lethal Dose)	-6	-10	8
	-21		
	-11		
	-1		
Lethal Dose Preservative Blank	-4	-6	3
	-10		
	-6		
	-3		

Table 6-1c. Colchicine Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	2	0	2
	-3		
	0		
	1		
Positive Control	9 ^(a)		
0.24	1	3	2
	4		
	2		
	4		
2.4	-3	0	5
	-3		
	8		
	0		
24	12	4	5
	2		
	1		
	1		
240 (Lethal Dose)	0	0	1
	-2		
	0		
	0		

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

Table 6-1d. Cyanide Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-2	0	2
	-1		
	2		
	1		
Positive Control	38		
0.25	2	1	3
	2		
	2		
	-4		
2.5	5	7	3
	4		
	7		
	10		
25	11	10	3
	6		
	12		
	9		
250 (Lethal Dose)	20	17	2
	14		
	19		
	17		
Field Portability Negative Control	2	0	1
	0		
	-1		
	0		
Field Portability Positive Control	40		
Field Portability 250	12	16	4
	15		
	21		
	16		

Table 6-1e. Dicrotophos Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	6	0	4
	0		
	0		
	-5		
Positive Control	32 ^(a)		
1.4	-10	-10	1
	-10		
	-9		
	-11		
14	-12	-12	1
	-10		
	-13		
	-12		
140	-11	-11	1
	-12		
	-11		
	-10		
1,400 (Lethal Dose)	6	4	2
	1		
	6		
	3		

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

Table 6-1f. Nicotine Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-3	0	3
	-2		
	1		
	3		
Positive Control	28 ^(a)		
2.8	5	3	4
	-3		
	4		
	7		
28	2	1	3
	-2		
	-1		
	6		
280	11	10	2
	7		
	10		
	11		
2,800 (Lethal Dose)	35	34	1
	34		
	34		
	34		

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

Table 6-1g. Ricin Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-8	0	9
	9		
	6		
	-8		
Positive Control	22 ^(a)		
0.015	8	3	4
	4		
	0		
	-2		
0.15	-1	-4	4
	-8		
	0		
	-6		
1.5	2	1	2
	-1		
	-2		
	2		
15 (Lethal Dose)	0	0	6
	7		
	1		
	-7		
Lethal Dose Preservative Blank	0	4	3
	4		
	8		
	6		

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

Table 6-1h. Soman Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	0	0	1
	1		
	0		
	-1		
Positive Control	33 ^(a)		
0.0014	1	0	2
	-1		
	2		
	0		
0.014	4	2	2
	-1		
	1		
	3		
0.14	6	1	3
	0		
	1		
	-2		
1.4 (Lethal Dose)	-3	-2	3
	0		
	1		
	-5		
Lethal Dose Preservative Blank	4	3	2
	2		
	1		
	5		

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

Table 6-1i. Thallium Sulfate Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-2	0	3
	0		
	4		
	-1		
Positive Control	27 ^(a)		
2.8	-2	-4	2
	-5		
	-2		
	-6		
28	-2	-3	3
	0		
	-7		
	-3		
280	3	1	2
	-1		
	2		
	1		
2,800 (Lethal Dose)	2	0	2
	1		
	-1		
	-2		

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

Table 6-1j. VX Percent Inhibition Results

Concentration (µg/mL)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	1	0	3
	-4		
	3		
	1		
Positive Control	22 ^(a)		
0.002	5	2	3
	-2		
	4		
	0		
0.02	2	-1	5
	-1		
	-8		
	4		
0.2	5	3	3
	-1		
	3		
	4		
2 (Lethal Dose)	3	5	2
	4		
	8		
	7		
Lethal Dose Preservative Blank	7	-1	5
	-2		
	-5		
	-2		

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

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

Preservative Blank	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-1	0	3
	-2 ^(b)		
	3		
Positive Control	8 ^a		
Ricin	-14	-5	9
	-7		
	-8		
	-8		
Soman/VX ^(b)	-3	-4	4
	-8		
	-6		
	1		
Botulinum Toxin Complex B	-12	-5	9
	-6		
	7		
	-9		

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

^(b) Removed -45% because result was an obvious outlier.

Table 3-2 details the concentrations of preservatives in the lethal dose samples of each contaminant. These data could be evaluated in two ways to determine the sensitivity of the LuminoTox SAPS 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 that were not stored in preservatives. This technique, however, could indicate that the LuminoTox SAPS 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 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 do not have toxic effects at the lethal dose concentration, no additional preservative blanks were analyzed to determine whether there were toxic effects from each individual concentration level. Each concentration level was evaluated and compared with

the negative control to determine any toxic effects. The lethal dose preservative blank was determined with each contaminant sample set and is shown with each contaminant inhibition regardless of the result of the initial preservative blank analysis. Neither the contaminant test samples nor the preservative blank differed significantly from the negative control for botulinum toxin complex B, ricin, soman, or VX. Therefore, none of these contaminants caused detectable inhibition.

A positive control sample was analyzed with every set of analyses and, overall, the positive control inhibition was somewhat inconsistent throughout the test of the LuminoTox SAPS Test Kit. Prior to the verification test, Battelle had not been informed of a defined performance criterion for the positive control; so, if an inhibition greater than the negative control was generated, the testing staff considered the LuminoTox SAPS Test Kit to be operating properly. The average inhibition across all of the positive control samples was $28\% \pm 12\%$, with each positive control exhibiting more inhibition than its associated negative control. After the completion of the test, Lab_Bell expressed concern that the positive control samples did not always generate a percent inhibition as high as expected. Battelle was then provided a detailed protocol that stated that the 0.01-mg/L solution of atrazine was expected to have an inhibition of approximately $43\% \pm 5\%$. Had this information been available at the time of testing, the sample sets not meeting the required positive control inhibition would have been reanalyzed once to try to bring the control into the acceptable range as defined by Lab_Bell. However, because this was not available to Battelle until after testing, the data are being reported as collected, and the tables containing data from sample sets with positive control data less than 38% are noted as such in a footnote. Three of the positive controls had an inhibition of 40% and above, four were between 30% and 40%, and seven were less than 30%. Three sample sets contained positive controls that generated an inhibition less than 10% (aldicarb—8%, colchicine—9%, and the preservative blanks—8%). Despite the low positive control inhibition, the three highest concentration levels of aldicarb generated detectable inhibition; therefore, in that case, a positive control inhibition of 8% seemed to indicate the adequate functioning of the LuminoTox SAPS. Additionally, nicotine, with a positive control response of 28% had detectable inhibition at the top two concentrations. The low positive control inhibition may indicate a lower sensitivity of the LuminoTox SAPS Test Kit than when a positive control inhibition of greater than 40% is obtained, but at least for aldicarb and nicotine, the sensitivity seemed to be adequate.

The preservative blank inhibition also seems to suggest that the positive control inhibition was adequate for confirming the function of the technology. The lethal dose preservative blank was analyzed once prior to and once with the analysis of the contaminant samples. During the first analysis prior to contaminant testing, the positive control inhibition was 8%. During subsequent contaminant testing, every applicable positive control inhibition was higher (botulinum toxin complex B—41%, ricin—22%, soman—33%, and VX—22%) and all of the lethal dose preservative blanks generated an inhibition that was either the same as or extremely similar to what was determined during the first analysis, therefore confirming the inhibition results from the initial analysis that may have otherwise been in question because of the low positive control inhibition. Additionally, the repeatability of results across all of the contaminants was very good. Eighty-six percent of the time the standard deviation was less than 5% inhibition. This suggests that even an inhibition of 8 or 9% is likely a significant inhibition with respect to the negative control. All positive control results are reported along with their respective contaminant set in Tables 6-1a through 6-1j.

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 SAPS Test Kit to detect toxicity is dependent on the background fluorescence production in whatever drinking water matrix is being used. If the background drinking water sample completely inhibits background fluorescence, inhibition caused by contaminants could not be detected. Table 6-3 presents the results from the samples that were analyzed to test the effect of potential interferences on the LuminoTox SAPS Test Kit. Of the five metal solutions that were evaluated as possible interferences, none exhibited an inhibition that was significantly different from the DDW negative control. Therefore, it seems that there is little risk of interference for these metals because enough fluorescence is produced for inhibition as a result of contamination.

To investigate whether the LuminoTox SAPS 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 by chlorination (N=56) exhibited an average inhibition of $-8\% \pm 23\%$, while the sample from the water supply disinfected by chloramination exhibited an inhibition of $0\% \pm 5\%$ on four replicates. The difference in the 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 water. These inhibition data suggest that samples disinfected by either process are not likely to interfere with the LuminoTox SAPS Test Kit results.

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 LuminoTox SAPS Test Kit precision. Out of 78 opportunities, the standard deviation of the four replicate inhibition measurements was less than 5% inhibition 67 times (86% of the time), between 5% and 10% inhibition 10 times (13% of the time), and greater than 10% inhibition just 1 time (1%). 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

Table 6-3. Potential Interferences Results

Potential Interferences	Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative control (Metals)	NA	-3 0 1 3	0	3
Positive Control (Metals)	NA	45		
Aluminum	0.5	-3 2 0 5	1	4
Copper	0.6	2 3 2 3	3	1
Iron	0.15	2 2 -1 -1	1	2
Manganese	0.25	3 4 -3 -2	1	3
Zinc	2.5	-4 -1 5 -2	-1	4
Negative control (By-products)	NA	1 0 -3 2	0	2
Positive control (By-products)	NA	34 ^(a)		
Chlorination by-products	NA	(b)	-8	23
Chloramination by-products	NA	-4 0 -3 7	0	5

NA = Not applicable.

^(a) Positive control percent inhibition less than suggested by Lab_Bell.

^(b) Average inhibition across all DDW negative control samples (N=56).

Table 6-4. Toxicity Thresholds

Contaminant	Concentration (mg/L)
Aldicarb	26
Botulinum toxin complex B	ND
Colchicine	ND
Cyanide	250
Dicrotophos	ND
Nicotine	280
Ricin	ND
Soman	ND
Thallium sulfate	ND
VX	ND

ND = Significant inhibition was not detected.

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 aldicarb at 26 mg/L.

6.3 False Positive/Negative Responses

None of the LuminoTox SAPS Test Kit results would be considered false positive because neither the chlorination nor chloramination by-product samples were inhibitory and, therefore, fluorescence production was adequate to allow inhibition to occur if a contaminant was present that produced a detectable toxic effect. Since the background inhibition is not complete, it can be accounted for by using negative control samples that are very similar to the water being analyzed. 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.

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

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	yes
VX	2.0	yes

6.4 Other Performance Factors

6.4.1 Ease of Use

The LuminoTox SAPS Test Kit contained detailed instructions and clear illustrations. The contents of the LuminoTox SAPS Test Kit were well identified with labels on the vials. Storage requirements were stated in the instructions and on the reagent vials. Overall, the test was easy to perform; but additional practice helped the operators become accustomed to the timing involved with running a large number of test samples.

Preparation of the test samples for analysis was straightforward. The analyzer, 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 fluorometer was easily wiped clean and required no routine maintenance other than selecting the SAPS mode prior to the start of sample analysis.

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

6.4.2 Field Portability

The LuminoTox SAPS 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 SAPS Test Kit; however, all materials were transported by one person in a small cardboard box. The LuminoTox SAPS Test Kit was set up easily in less than 10 minutes, and a source of electricity was not required since the fluorometer ran on 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 not provided in the LuminoTox SAPS Test Kit was a timer and a waste reservoir. Overall, the LuminoTox SAPS Test Kit was easy to transport to the field and was deployed in a matter of minutes. The limiting factor for testing in the field would be the approximately 90 minutes required to expose the SAPS to light prior to testing. After the light exposure, results were obtained within 10 minutes of starting the test. The LuminoTox SAPS 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 $17\% \pm 2\%$, and in the non-laboratory location, $16\% \pm 4\%$, suggesting that location did not impact the performance of the LuminoTox SAPS Test Kit.

6.4.3 Throughput

Once the SAPS 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 SAPS 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	50	14	5	0	1-3	26
	Botulinum toxin complex B	0.3	-10	-6	-5	1	1-8	ND
	Colchicine	240	0	4	0	3	1-5	ND
	Cyanide	250	17	10	7	1	2-3	250
	Dicrotophos	1,400	4	-11	-12	-10	1-2	ND
	Nicotine	2,800	34	10	1	3	1-4	280
	Ricin	15	0	1	-4	3	2-6	ND
	Soman	1.4	-2	1	2	0	2-3	ND
	Thallium sulfate	2,800	0	1	-3	-4	2-3	ND
VX	2	5	3	-1	2	2-5	ND	
Potential interferences in DDW	Interference	Conc. (mg/L)	Average Inhibition (%)		Standard Deviation (%)			
	Aluminum	0.5	1		4			
	Copper	0.6	3		1			
	Iron	0.15	1		2			
	Manganese	0.25	1		3			
	Zinc	2.5	-1		4			
False positive response	None of the LuminoTox SAPS Test Kit responses were considered false positive. All disinfection by-product test samples left enough fluorescence for inhibition due to contamination.							
False negative response	Botulinum toxin complex B, colchicine, dicrotophos, ricin, soman, thallium sulfate, and VX exhibited non-detectable responses at the lethal dose concentration.							
Ease of use	The LuminoTox SAPS Test Kit contained detailed instructions and clear illustrations. The contents 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. The necessity to record four numbers as raw data was somewhat burdensome; however, this feature is being modified according to Lab_Bell. No formal scientific education would be required to use the LuminoTox SAPS Test Kit.							
Field portability	The LuminoTox SAPS Test Kit was transported from a laboratory setting to a storage room for the field portability evaluation. The limiting factor for testing in the field would be the approximately 90 minutes required to allow the SAPS to be exposed to light prior to testing. The LuminoTox SAPS 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 17% ± 2%, and in the non-laboratory location, 16% ± 4%.							
Throughput	Approximately 20 analyses were completed per hour, and 50 samples could be analyzed with the supplies contained in one LuminoTox SAPS Test Kit.							

ND = Significant inhibition was not detected.

Chapter 8

References

1. *Test/QA Plan for Verification of Rapid Toxicity Technologies*, Battelle, Columbus, Ohio, June 2003; Amendment 1: June 9, 2005; Amendment 2: August 19, 2005.
2. United States Environmental Protection Agency, *National Secondary Drinking Water Regulations: Guidance for Nuisance Chemicals*, EPA/810/K-92/001, July 1992.
3. U.S. EPA Method 335.3, “Cyanide, Total—Colorimetric, Automated UV,” in *Methods for the Chemical Analysis of Water and Wastes*, EPA/600/4-79/020, March 1983.
4. U.S. EPA Method 200.8, “Determination of Trace Elements in Waters and Wastes by Inductively-Coupled Plasma Mass Spectrometry,” in *Methods for the Determination of Metals in Environmental Samples*, Supplement I, EPA/600/R-94/111, 1994.
5. U.S. EPA Method 200.7, “Trace Elements in Water, Solids, and Biosolids by Inductively Coupled Plasma—Atomic Emission Spectrometry,” EPA-821-R-01-010, January 2001.
6. American Public Health Association, et al. *Standard Methods for the Examination of Water and Wastewater*. 19th Edition, 1997. Washington, DC.
7. U.S. EPA, *Methods for Chemical Analysis of Water and Wastes*, EPA/600/4-79/020.
8. U.S. EPA Method 552.2, “Haloacetic Acids and Dalapon by Liquid-Liquid Extraction, Derivatization and GC with Electron Capture Detector,” *Methods for the Determination of Organic Compounds in Drinking Water—Supplement III* EPA/600/R-95/131.
9. U.S. EPA Method 524.2, “Purgeable Organic Compounds by Capillary Column GC/Mass Spectrometry,” *Methods for the Determination of Organic Compounds in Drinking Water—Supplement III*, EPA/600/R-95/131.
10. American Public Health Association, et al. *Standard Methods for the Examination of Water and Wastewater*, 20th edition, 1998, Washington, DC.
11. *Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center*, Version 5.0, U.S. EPA Environmental Technology Verification Program, Battelle, Columbus, Ohio, December 2004.