THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

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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 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 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^{o}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.

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