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 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

	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.

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