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:	**Eclox**		
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The U.S. Environmental Protection Agency (EPA) supports 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.

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 seven technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center has recently evaluated the performance of rapid toxicity testing systems used to detect toxicity in drinking water. This verification statement provides a summary of the test results for the Eclox testing system.

VERIFICATION TEST DESCRIPTION

Rapid toxicity technologies use bacteria, enzymes, or small crustaceans that produce light or use oxygen at a steady rate in the absence of toxic contaminants. Toxic contaminants in drinking water are indicated by a change in the color or intensity of light or by a change in the rate of oxygen use. As part of this verification test, which took place between July 14 and August 22, 2003, various contaminants were added to separate drinking water samples and analyzed by Eclox. Response to interfering compounds in clean drinking water also was evaluated. Dechlorinated drinking water samples from Columbus, Ohio, (DDW) were fortified with contaminants at concentrations ranging from lethal levels to concentrations several orders of magnitude less than the lethal dose and analyzed. Endpoint and precision, toxicity threshold for each contaminant, false positive/negative responses, ease of use, and sample throughput were evaluated.

Inhibition results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of Eclox to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the Eclox results. The response of Eclox to compounds used during the water treatment process (interfering compounds) was evaluated by analyzing separate aliquots of DDW fortified with each potential interferent at approximately 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, 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 from St. Petersburg, Florida, which uses chloramination as its disinfection process, was obtained. The samples were analyzed after residual chlorine was removed using the vendor-provided dechlorinating reagent. 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 and the verification test coordinator.

Quality control samples included method blank samples, which consisted of American Society for Testing and Materials (ASTM) Type II deionized (DI) water; positive control samples fortified with phenol; 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. EPA QA staff also performed a technical systems audit while testing was being conducted.

TECHNOLOGY DESCRIPTION

The following description of Eclox was provided by the vendor and was not subjected to verification in this test.

Eclox is a broadband chemiluminescence test that qualitatively assesses a water sample to determine whether it has been contaminated. The technique, used extensively in the medical field as an immunodiagnostic tool, is based upon the reaction of luminol and an oxidant in the presence of a catalyst enzyme—horseradish peroxidase (HRP). This reaction produces a flash of light (chemiluminescence) that is measured by a luminometer. An enhancer is added prior to the HRP so that the light output produced is of a steady measurable level. Free radical scavengers or antioxidants such as those contained in feces or urine interfere with the reaction, thus reducing the light emission. Substances such as phenols, amines, heavy metals, or compounds that interact with the enzyme also reduce the light output.

To analyze a water sample, 100 microliters (μL) of three reagents are added to 1 milliliter (mL) of the sample, and the sample cuvette is placed in the luminometer for four minutes. Results are compared with a contaminant-free reference, i.e., deionized water, which gives a high light output. Samples containing contaminants give lower light levels. Comparing the light output from sample water to that obtained from the reference indicates the contamination levels in the sample water. To determine whether a contaminant caused detectable inhibition, the inhibition exhibited by drinking water spiked with a contaminant was compared to the inhibition exhibited by the

unspiked drinking water. Four replicates of each spiked sample were analyzed. A result was considered positive if the inhibition of the water sample spiked with a contaminant plus or minus the standard deviation of four replicates did not include the inhibition of the unspiked drinking water.

The Eclox includes a luminometer, a 100-µL and a 1,000-µL pipette and pipette tips, cuvettes, reagent, a pre-conditioner, a cuvette holder, and a CD-ROM with software to download results. The luminometer stores a total of 60 measurements, and the data can be downloaded to a personal computer using the supplied software. The stored values are downloaded to a Microsoft Access database file and can be exported to a Microsoft Excel spreadsheet.

The complete Eclox kit weighs approximately 20 pounds. Overall dimensions are 20-½ inches x 17-½ inches x 8 inches. The luminometer contained in the system weighs a few pounds and is approximately 9 inches x 5 inches x 3 inches. The cost of the full Eclox kit is $7,900.

VERIFICATION OF PERFORMANCE

Endpoint and Precision/Toxicity Threshold: The table below presents Eclox percent inhibition data and the range of standard deviations for the contaminants and potential interferences that were tested. The toxicity thresholds also are shown for each contaminant tested.

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Average Inhibitions 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	280	35	4	7	10	2–10	280
	Colchicine	240	92	43	14	9	6–8	24
	Cyanide	250[a]	97	103	13	3	1–9	0.25
	Dicrotophos	1,400	29	4	2	-1	3–10	1,400
	Thallium sulfate	2,400	46	5	-3	-3	3–9	2,400
	Botulinum toxin[b]	0.30	-2	-3	-2	1	1–3	ND[c]
	Ricin[d]	15	8	2	2	1	2–3	15
	Soman	0.068[e]	0	2	2	3	2–5	ND
	VX	0.49	9	-4	-5	-7	2–3	0.49
	Interference	**Conc. (mg/L)**	**Average Inhibitions at a Single Concentration (%)**				**Standard Deviation (%)**	
Potential interferences in DDW	Aluminum	0.36	-2				7	
	Copper	0.65	4				9	
	Iron	0.069	2				6	
	Manganese	0.26	62				6	
	Zinc	3.5	10				4	

[a] Cyanide LD/10, LD/100, and LD/1,000 concentrations are 0.25, 0.05, and 0.025 mg/L.
[b] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[c] ND = Not detectable.
[d] Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
[e] Due to the degradation of soman in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 23% of the expected concentration of 0.30 mg/L.

False Positive/Negative Responses: Chlorinated (6% ± 5%) and chloraminated (0% ± 2%) drinking water samples were non-inhibitory with respect to ASTM DI water. This shows that there were no false positive

responses. At the lethal concentration level, inhibitions produced by botulinum toxin and soman were not significantly different from the negative control or inhibitions generated by lower concentrations of the same contaminant, indicating false negative responses for botulinum toxin and soman. Nerve agent test strips supplied with the Eclox kit were not tested, only the chemiluminescent toxicity test was conducted. The vendor states that the nerve agent test strip will detect soman.

Field Portability: A single concentration of cyanide was analyzed in the field and in the laboratory. Inhibitions for cyanide at 0.05 mg/L at the field location were 13% ± 10%, while laboratory testing at the same concentration produced an inhibition of 13% ± 9%. Eclox was easily transported and operated in the field. Detailed instructions in the carrying case and organized packaging made field analysis convenient.

Other Performance Factors: Although the operators had scientific backgrounds, based on the observations of the verification test coordinator, operators with little technical training would probably be able to operate Eclox by following the detailed written instructions provided with Eclox. Reagents and pipettes were color-coded to ensure mistake-free analysis. A waste container was included. Operators were able to analyze 15 samples per hour in this test.

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