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:	**Microtox®**		
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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 Microtox® 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 Microtox®. Responses to interfering compounds in clean drinking water also were evaluated. Dechlorinated drinking water samples from Columbus, Ohio, (DDW) were fortified with contaminants at concentrations ranging from lethal levels to levels 1,000 times 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 the Microtox® to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the Microtox® results. The response of Microtox® 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 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 and the verification test coordinator.

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 zinc sulfate or 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 Microtox® was provided by the vendor and was not subjected to verification in this test.

Microtox® is an *in vitro* testing system that uses bioluminescent bacteria to detect toxins in air, water, soil, and sediment. Microtox® is a metabolic inhibition test that provides both acute toxicity and genotoxic analyses. Microtox® uses a strain of naturally occurring luminescent bacteria,*Vibrio fischeri*. *Vibrio fischeri* are non-pathogenic, marine, luminescent bacteria that are sensitive to a wide range of toxicants. When properly grown, luminescent bacteria produce light as a by-product of their cellular respiration. Cell respiration is fundamental to cellular metabolism and all associated life processes. Bacterial bioluminescence is tied directly to cell respiration, and any inhibition of cellular activity (toxicity) results in a decreased rate of respiration and a corresponding decrease in the rate of luminescence.

The Microtox® Model 500 Analyzer was tested as a stand-alone instrument along with the Microtox® reagent. The *Vibrio fischeri* are supplied in a standard freeze-dried (lyophilized) state and, to analyze water samples, are reconstituted in a salt solution, 2.5 milliliters (mLs) of the water sample are diluted with 250 microliter (µL) of a Microtox® reagent, then approximately 1 mL of water sample is added to 100 µL of the reconstituted bacteria. Luminescence readings are taken prior to adding the drinking water and then at 5 and 15 minutes after the addition. When analyzing unknown samples, it is recommended that inhibition data be collected at both time intervals to determine the most appropriate data collection time since the rates can vary depending on how the toxicant affects the bacteria. Results are displayed as absolute light units.

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 temperature-controlled Microtox® maintains the test organisms and samples at a standard temperature of 15ºC. As such, the Microtox® must be operated in a laboratory setting at ambient temperatures of between 15 and 30ºC. It detects light intensity at 490 nanometers, the wavelength emitted by the bacteria. Microtox® can be used with Microtox®Omni™ software and a personal computer to collect, analyze, track, and store test data. Microtox® weighs 21 pounds, measures 7-1/8 inches x 15-3/8 inches x 16-1/8 inches, and runs on 120/240 volts alternating current. Microtox® Model 500 costs $17,895, and the reagents cost $360 for approximately 200 samples.

VERIFICATION OF PERFORMANCE

Endpoint and Precision/Toxicity Threshold: The table below shows the Microtox® percent inhibition data and range of standards 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	81	31	4	3	2–5	28
	Colchicine	240	12	2	5	3	1–3	240
	Cyanide	250	100	96	46	8	0–4	0.25
	Dicrotophos	1,400	80	34	6	2	2–4	140
	Thallium sulfate	2,400	32	17	6	4	1–6	240
	Botulinum toxin[a]	0.30	-4	0	-1	-2	1–5	ND[b]
	Ricin[c]	15	-1	-4	0	-2	2–4	ND
	Soman	0.068[d]	0	-2	0	-4	3–4	ND
	VX	0.22	6	-2	9	3	2–18	ND
	Interference	**Conc. (mg/L)**	**Average Inhibitions at a Single Concentration (%)**				**Standard Deviation (%)**	
Potential interferences in DDW	Aluminum	0.36	1				5	
	Copper	0.65	61				1	
	Iron	0.069	-5				2	
	Manganese	0.26	9				3	
	Zinc sulfate	3.5	28				1	

[a] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[b] ND = Not detectable.
[c] Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
[d] 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: There was nearly complete inhibition (false positive responses) in dechlorinated water disinfected by chloramination. Therefore, the further addition of contaminants would not be detected because the background light was already inhibited by the drinking water sample matrix. Dechlorinated

water from a system disinfected by chlorination produced an inhibition that was not significantly greater than zero, allowing further contamination to be detected in that particular matrix. No inhibition (false negative responses) was detected for lethal doses of botulinum toxin, ricin, soman, and VX.

Other Performance Factors: The Microtox® pictorial manual was useful, initial light measurements served as a good check of bacterial health and instrument operation, sample handling was easy, and sample throughput was 15 samples per hour. Microtox® was not tested in a non-laboratory setting because it is designed to be only a laboratory benchtop instrument. 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 follow the instructions to analyze samples successfully.

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