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:	**IQ Toxicity Test™**

COMPANY:	**AquaSurvey, Inc.**		
ADDRESS:	**499 Point Breeze Rd.**	**PHONE:**	**908-788-8700**
	Flemington, NJ 08822	**FAX:**	**908-788-9165**
WEB SITE:	**http://www.aquasurvey.com/index.html**		
E-MAIL:	**Mail@AquaSurvey.com**		

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 IQ Toxicity Test™.

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 IQ Toxicity Test™. Response to interfering compounds in clean drinking water also was evaluated. Dechlorinated drinking water samples from Sergeantsville, New Jersey, (DDW) were fortified with contaminants at concentrations ranging from lethal levels to levels 10,000 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 IQ Toxicity Test™ to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the IQ Toxicity Test™ results. The response of IQ Toxicity Test™ 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 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 Sergeantsville, New Jersey, 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, which were provided by the vendor; 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 IQ Toxicity Test™ was provided by the vendor and was not subjected to verification in this test.

The IQ Toxicity Test™ allows the user to characterize the toxicity of a water sample by measuring stressor-related suppression of enzyme activity of *Daphnia magna* in one hour and 15 minutes by determining fluorescent light emittance.

The IQ Toxicity Test™ is performed in three plastic exposure chambers, each consisting of six 10-milliliter (mL) compartments. Six *Daphnia magna* are placed in each 10-mL compartment. In a single study or replicate test, 18 organisms are exposed to each concentration level by using the three exposure chambers. One compartment is filled with the negative control sample, and the other five compartments are filled with sequentially decreasing concentrations of the contaminant being tested. Three exposure chambers containing a dilution series of the contaminant being tested are analyzed for each individual sample replicate. The EC_{50} (concentration at which 50% of the organisms were affected) is calculated for each replicate. To fully characterize a contaminant, four replicates of three exposure chambers are analyzed, and the EC_{50} is calculated for each of the four replicates. After the organisms are in contact with the control and sample (drinking) water for one hour, a fluorogenically tagged sugar suspension is added to each of the six compartments. After 15 minutes, the exposure chamber is illuminated with a black light (longwave ultraviolet [UV]). The control organisms emit bright bluish-white light—indicating that they are healthy. If the organisms in the sample water are not glowing as brightly, they are scored as adversely affected. For the organisms to fluoresce, they must ingest the tagged sugar (galactose) and express the enzyme (galactosidase); then the enzyme must successfully cleave the sugar from the fluorogenic marker. This marker, although unable to fluoresce while attached to the sugar molecule, is now liberated and fluoresces as it flows through the organism's circulatory system. This is an obvious visual endpoint. The toxicity of a contaminant at a

specific concentration level was considered detectable if the organisms at that concentration level were adversely affected to a greater extent than the negative control in all four replicate tests. Within one replicate sample, the negative control was allowed to have three adversely affected organisms. Therefore, for a concentration level of contaminant to adversely affect the organisms to a larger extent than the negative control, at least four organisms had to be adversely affected in each replicate.

The Threat Detection Starter Kit™ includes the equipment needed to be purchased once and supplies to perform 30 toxicity tests and maintain a *Daphnia magna* production culture for 30 days. Supplied in the starter kits are instructions, a test scoring form, exposure chambers, fluorogenic substrate, reconstituted water stock solution, pipettes, longwave UV light, sonicator, fluorescent light box, 45-liter (L) carboy, and assorted equipment to facilitate the performance of 30 toxicity tests. This kit also includes a starter culture of live *Daphnia magna*, a 30-day supply of food, culture dishes, and equipment to initiate an ongoing *Daphnia magna* production culture. A Threat Detection Maintenance Kit™ provides the supplies needed to conduct 30 additional toxicity tests and to maintain the *Daphnia magna* culture an additional 30 days. The starter kit is packaged in four boxes, each less than 20 pounds, and is $2,400. The maintenance kit is packaged in one less-than-20-pound box and is $400.

VERIFICATION OF PERFORMANCE

Endpoint and Precision/Toxicity Threshold: The table below presents IQ Toxicity Test™ 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 Conc. (mg/L)	EC_{50} for each Replicate (mg/L)				Avg. EC_{50} (mg/L)	%RSD	Toxicity Thresh. (mg/L)
			1	2	3	4			
Contaminants in DDW	Aldicarb	280	2.4	1.2	1.3	2.0	1.7	35	3.5
	Colchicine	240	17	106	19	14	39	115	24
	Cyanide	250	0.25	0.09	0.062	0.25	0.16	63	0.25
	Dicrotophos	1,400	1.3	0.88	1.2	0.88	1.06	20	0.88
	Thallium sulfate	2,400	102	119	123	164	127	21	120
	Botulinum toxin[a]	0.30	0.095	0.062	[b]	0.095	0.084	23	0.00030
	Ricin[c]	15	1.53	0.13	0.32	0.44	0.61	103	0.015
	Soman	0.13[d]	0.0012	0.0016	0.0011	0.0016	0.0014	21	0.0013
	VX	0.077[d]	0.020	0.021	0.020	0.019	0.020	5.0	0.0095
Potential interferences in DDW	**Interference**	**Conc. (mg/L)**	**Average Inhibitions at a Single Concentration (%)**						
	Aluminum	0.36	90						
	Copper	0.65	100						
	Iron	0.069	90						
	Manganese	0.26	3						
	Zinc	3.5	7						

[a] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.

[b] EC_{50} could not be calculated because percent of organisms adversely affected did not increase with concentration.

[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 in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 44% of the expected concentration of 0.30 mg/L for soman, and 38% of the expected concentration of 0.20 mg/L for VX.

False Positive/Negative Responses: All the organisms exposed to drinking water from a system disinfected by chloramination were adversely affected, indicating that false positive responses are possible for these samples. Because drinking water from Columbus, Ohio, a system that used chlorination as its disinfecting process, did not provide an environment conducive to maintaining *Daphnia magna*, a water sample from Sergeantsville, New Jersey, was used as the sample matrix. The water sample from the New Jersey system, which uses chlorination, did not adversely affect the *Daphnia magna*. If the background water sample causes the organisms to be adversely affected in the absence of contaminants, this test should not be used because the results will be false positive. There were no false negative responses. Each contaminant caused adverse effects to the organisms below the lethal dose concentration for human toxicity.

Field Portability: A single concentration of cyanide was analyzed in the field and the laboratory. An EC_{50} of 0.16 mg/L with a relative standard deviation (RSD) of 61% was measured in the laboratory, while an EC_{50} of 0.070 mg/L with an RSD of 10% was measured in the field for the one contaminant (cyanide) that was tested at both locations. The results show that the IQ Toxicity Test™ performed similarly in both locations. A supply of *Daphnia magna* must be maintained to facilitate short-notice field testing.

Other Performance Factors: The instruction manual was easy to understand. Although the operators had scientific backgrounds, based on observations of the verification test coordinator, operators with little technical training would probably be able to successfully analyze sample sets if their fine motor skills were adequate. Operators analyzed 25 sets of three exposure chambers per day. The test did not include culturing *Daphnia magna*.

Original signed by Gabor J. Kovacs 11/13/03
Gabor J. Kovacs Date
Vice President
Environmental Sector
Battelle

Original signed by Timothy E. Oppelt 12/1/03
Timothy E. Oppelt Date
Director
National Homeland Security Research Center
U.S. Environmental Protection Agency