THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

Battelle
. . . Putting Technology To Work

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**RAPID TOXICITY TESTING SYSTEM**
APPLICATION:	**DETECTING TOXICITY IN DRINKING WATER**
TECHNOLOGY NAME:	**ToxScreen-II**

COMPANY:	**CheckLight, Ltd.**		
ADDRESS:	**P.O.Box 72**	**PHONE:**	**(972) 4 9930530**
	Qiryat Tiv'on 36000, Israel	**FAX:**	**(972) 4 9533176**
WEB SITE:	**http://www.checklight.co.il/**		
E-MAIL:	**info@checklight.co.il**		

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 ToxScreen-II.

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 ToxScreen-II. 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 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 ToxScreen-II to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the ToxScreen-II results. The response of ToxScreen-II 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 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 sodium chloroacetate for the pro-organic buffer samples and copper chloride for the pro-metal buffer samples); 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 ToxScreen-II was provided by the vendor and was not subjected to verification in this test.

Luminous bacteria are self-maintained luminescent units that, under proper conditions, emit high and steady levels of luminescence. Chemo-physical and biological toxicants that affect cell respiration, electron transport systems, adenosine triphosphate generation, and the rate of protein or lipid synthesis alter the level of luminescence. Similarly, agents that affect the cell's integrity, and especially membrane function, have a strong effect on *in vivo* luminescence.

ToxScreen-II uses luminous bacteria, *Photobacterium leiognathi*, and special assay conditions to detect toxicants in water samples. When used in concurrent tests, two assay buffers (one for detecting organic pollutants [pro-organic buffer] and the other for detecting heavy metals [pro-metal buffer]) are used to discriminate between the presence of organic and metal toxicants at sub milligram-per-liter concentrations. Inhibition of greater than 50% using either buffer is considered a positive result. A positive result in the pro-organic buffer suggests that the contaminant causing the toxicity is organic, while a positive result in the pro-metal buffer suggests that the contaminant is a metal. First, a freeze-dried bioassay reagent is hydrated with the provided hydration buffer and, after five minutes, transferred into the provided storage buffer. The suspended reagent is maintained at 4°C until use (aliquots can be drawn for up to seven days). Next, pro-organic and pro-metal concentrated assay buffers are added separately to individual aliquots of source water samples, as well as to two aliquots of the reference sample. Then, aliquots of suspended reagent are rapidly dispensed into test cuvettes. Finally, after 90 minutes of incubation at ambient temperature, luminescence is measured using a portable luminometer. Changes in luminescence (compared with the reference sample) reflect water toxicity.

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 ToxScreen-II test kit contains stoppered vials holding freeze-dried luminous bacteria, empty test tubes, hydration buffer, storage buffer, pro-organic concentrated assay buffer, pro-metal concentrated assay buffer, and positive control solutions of copper chloride and sodium chloroacetate. A repeat dispenser (1 to 100 microliters [µL]) and 10- to 1,000-µL pipettes and tips are required, but not provided in the kit. The price of the ToxScreen-II test kit (1,000 single tests) is $300. The luminometer for reading the results of the bioassays costs $2,895. The luminometer can be integrated with a personal computer for data acquisition, evaluation, and storage. The luminometer is 5.9 x 11.0 x 6.7 inches and weighs approximately one pound. An insulating styrofoam case is available for an additional cost to conduct field tests.

VERIFICATION OF PERFORMANCE

Endpoint and Precision/Toxicity Threshold: The table below presents ToxScreen-II 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.

Pro-Organic Buffer

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	LD/10,000		
Contaminants in DDW	Aldicarb	280	69	38	58	11	NA[a]	2–15	0.28
	Colchicine	240	83	20	34	55	-31	3–48	0.24
	Cyanide	250	99	85	99	68	14	0–29	0.25
		0.025[b]	14	-6	NA	NA	NA	18–27	NA
	Dicrotophos	1,400	99	99	81	41	41	0–18	0.14
	Thallium sulfate	2,400	-10	52	-12	-32	NA	10–80	ND[c]
	Botulinum toxin[d]	0.30	69	47	-15	-8	NA	14–23	0.030
	Ricin[e]	15	50	10	30	-10	NA	6–28	15
	Soman	0.068[f]	5	-1	-9	-17	NA	9–31	ND
	VX	0.22	-8	-5	-13	-59	NA	6–30	ND

Parameter	Interference	Conc. (mg/L)	Average Inhibitions at a Single Concentration (%)	Standard Deviation (%)
Potential interferences in DDW	Aluminum	0.36	16	41
	Copper	0.65	59	7
	Iron	0.069	29	23
	Manganese	0.26	-23	7
	Zinc	3.5	-68	79

[a] NA = Data not collected at this concentration level for this contaminant.
[b] 0.025 mg/L is not a lethal dose concentration, but it is used to describe additional concentrations of cyanide that were analyzed.
[c] ND = Not detectable.
[d] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[e] Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
[f] 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.

Pro-Metal Buffer

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	LD/10,000		
Contaminants in DDW	Aldicarb	280	-21	5	-17	-46	NA[a]	1–7	ND[b]
	Colchicine	240	-42	-72	-83	-79	4	5–12	ND
	Cyanide	250	99	48	80	35	31	1–13	0.025
		0.025[c]	31	3	NA	NA	NA	5–8	NA
	Dicrotophos	1,400	31	-76	-50	-32	-52	9–23	1,400
	Thallium sulfate	2,400	98	69	16	6	NA	1–9	240
	Botulinum toxin[d]	0.30	-44	1	-57	-97	NA	9–22	ND
	Ricin[e]	15	-89	-5	-93	-89	NA	12–74	ND
	Soman	0.068[f]	-15	-47	-62	-55	NA	3–71	ND
	VX	0.22	-38	-19	-21	-31	NA	11–31	ND

	Interference	Conc. (mg/L)	Average Inhibitions at a Single Concentration (%)	Standard Deviation (%)
Potential interferences in DDW	Aluminum	0.36	-26	19
	Copper	0.65	94	2
	Iron	0.069	3	32
	Manganese	0.26	22	7
	Zinc	3.5	100	0

[a] NA = Data not collected at this concentration level for this contaminant.
[b] ND = Not detectable.
[c] 0.025 mg/L is not a lethal dose concentration, but it is used to describe additional concentrations of cyanide that were analyzed.
[d] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[e] Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
[f] 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: False positive responses were observed for both chlorinated and chloraminated water samples with the pro-organic buffer. To avoid such results, reference samples similar to the test samples should be used. However, since the light emitted for background water samples is greatly inhibited (66 ± 34% for the chlorinated drinking water and 78% ± 11% for the chloraminated drinking water), a false positive response in these matrices may occur. The inhibition at the lethal concentration level was significantly larger than the negative control for all contaminants except VX, soman, and thallium sulfate (false negative responses were observed).

With the pro-metal buffer, the sample from the chlorinated water supply exhibited an inhibition of 23% ± 15%. This could cause slightly exaggerated results. Using a reference sample similar to the drinking water sample being tested would protect from this possibility. Inhibition was -48% ± 20% for the sample from the chloraminated water supply, indicating the possibility of false negative results. Adding a contaminant that caused 50% inhibition would result in an inhibition of approximately 0%. Using a reference sample similar to the water sample treated by dechloramination may avoid false negative results. Aldicarb, colchicine, botulinum toxin, ricin, soman, and VX were indistinguishable from the negative control.

Field Portability: A single concentration of cyanide was analyzed in the field and the laboratory. Similar performance was observed at both locations. Inhibition in the field was 97% ± 1% and in the laboratory was 68% ± 3% for the pro-organic buffer. The pro-metal buffer inhibition in the field was 61% ± 6% and in the laboratory was 35% ± 7%. ToxScreen-II was transported to the field in a vinyl bag that was provided by the vendor, but was not included as part of the test kit.

Other Performance Factors: The pictorial instruction manual was useful, and instrument operation was straightforward. Although the operators for this test had scientific backgrounds, based on the observations of the verification test coordinator, operators with little technical training would probably be able to successfully analyze sample sets. The operators analyzed approximately 25 samples per hour.

Original signed by Gabor J. Kovacs 11/19/03
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