

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION
PROGRAM



ETV Joint Verification Statement

TECHNOLOGY TYPE:	Rapid Toxicity Testing System	
APPLICATION:	Detecting Toxicity in Drinking Water	
TECHNOLOGY NAME:	ToxScreen-II	
COMPANY:	CheckLight Ltd.	
ADDRESS:	Kölner Strasse 6 P.O. Box 72 Qiryat-Tiv'on 36000 Israel	PHONE: (972) 4 9930530 FAX: (972) 4 9533176
WEB SITE:	www.checklight.co.il	
E-MAIL:	info@checklight.co.il	

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 permittees), 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 CheckLight Ltd. ToxScreen-II 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, ToxScreen-II 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 ToxScreen-II 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.

ToxScreen-II 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. Note that CheckLight Ltd. recommends that a 50% inhibition is required for a conclusive indication of toxicity. During this test, a thorough evaluation of the toxicity threshold was performed. Therefore, the toxicity threshold was determined with respect to the negative control rather than the 50% inhibition threshold
- False positive responses—chlorination and chloramination by-product inhibition with respect to unspiked American Society for Testing and Materials Type II deionized water samples that exceeded 50%
- False negative responses—contaminants that were reported as producing less than 50% and/or were not significantly different from 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 negative background inhibition that caused falsely low inhibition
- Other performance factors (sample throughput, ease of use, reliability).

ToxScreen-II 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 one million 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 kills the bacteria within the ToxScreen II reagent 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 ToxScreen-II to detect toxicity, as well as to measure the precision of ToxScreen-II results. The response of ToxScreen-II to possible interferences 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 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.

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 ToxScreen-II Test Kit is based on information provided by the vendor. This technology description was not verified in this test.

ToxScreen-II provides on-site detection of organic and inorganic toxicants, such as heavy metals; pesticides; herbicides; chlorinated hydrocarbons; polychlorinated biphenyls; benzene, toluene, ethylbenzene, and xylenes; and phencyclidine. ToxScreen-II can be used in both field and laboratory testing. Typical applications include effluent toxicity testing; surface and ground water screening for changes in water quality; and raw drinking water monitoring for early warning of dangerous spills, accidents, and sabotage/bioterrorism.

Under proper conditions, luminous bacteria emit high and steady levels of luminescence. Chemical 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 a cell's integrity and membrane function have a strong effect on luminescence. Hence, toxicants of different characteristics such as pesticides, herbicides, chlorinated hydrocarbons, and heavy metals exert a measurable effect on a bacterial luminescence system. By comparing the luminescence level obtained in a suspected toxic sample with that obtained in a clean water control sample after a short period of incubation, very low concentrations of a broad range of toxicants can be detected. To detect toxicants in water samples, ToxScreen-II uses a highly sensitive variant of *Photobacterium leiognathi* and two assay buffers: one for detecting heavy metals (Pro-Metal Buffer) and the other for organic pollutants (Pro-Organic Buffer). When used concurrently, these buffers are designed to discriminate between the presence of organic and metal toxicants at submilligram per liter concentrations.

The ToxScreen-II luminometer is 150 millimeters (mm) wide by 280 mm deep by 170 mm high and weighs approximately two kilograms. The test kit comes with stoppered vials holding freeze-dried luminous bacteria, hydration buffer, storage buffer, Pro-Metal concentrated assay buffer, Pro-Organic concentrated assay buffer, concentrated positive control solutions, and empty test tubes. The portable luminometer costs \$3,950, and a starter kit including reagents for 1,000 single tests costs \$550.

VERIFICATION RESULTS
Pro-Organic Buffer

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	50	-26	0	-50	12-18	ND
	Botulinum toxin complex B	0.3	-87	14	16	-54	21-58	ND
	Colchicine	240	75	17	4	1	2-5	24
	Cyanide	250	100	100	95	72	0-2	0.25
	Dicrotophos	1,400	70	23	3	1	3-29	140
	Nicotine	2,800	83	-10	-32	-20	2-10	1,400
	Ricin	15	68 ^(a)	9	1	10	2-5	ND
	Soman	1.4	-6	-202	4	15	10-68	ND
	Thallium sulfate	2,800	66	13	9	-1	3-6	28
	VX	2	-3	-5	2	-6	3-9	ND
Potential interferences in DDW	Interference	Conc. (mg/L)	Average Inhibition (%)		Standard Deviation (%)			
			Initial Analysis	Reanalysis ^(b)	Initial Analysis	Reanalysis ^(b)		
	Aluminum	0.5	-4	-12	4	1		
	Copper	0.6	3	5	3	15		
	Iron	0.15	0	-6	1	4		
	Zinc	2.5	-1	NR	3	NR		
False positive response	None of the disinfection by-product samples produced an inhibition significantly greater than 50%, the inhibition level suggested by CheckLight Ltd. to conclusively determine toxicity.							
False negative response	Aldicarb, botulinum toxin complex B, soman, and VX produced an inhibition that either did not exceed 50% or were not significantly different from the negative control at the lethal dose concentrations. For ricin in the Pro-Organic buffer, the inhibition of the lethal dose was significantly different from the negative control, but not significantly different from the inhibition generated by the preservative blank.							
Ease of use	ToxScreen-II included clearly written instructions with good illustrations. The contents of the ToxScreen-II were well labeled, making it easy to follow the instructions. A minimum of three hours was required to rehydrate the bacteria, which must be stored at -14°C prior to rehydration. After rehydration, the bacteria can be used for up to seven days; however, the vendor suggested using them within one day. Overall, the ToxScreen-II was easy to use, making it likely that a person with no formal scientific training could conduct the tests.							
Field portability	ToxScreen-II was transported from a laboratory to a storage room to simulate operation in a non-laboratory location. It was tested with cyanide at the lethal dose concentration, and the results generated (>90% inhibition) were very similar to those obtained in the laboratory. No carrying case was provided with ToxScreen-II (one is available for purchase from Checklight Ltd.); however, all materials except the luminometer were transported in a small cardboard box. The box and luminometer were easily carried by one person, and setup for analysis took less than 10 minutes.							
Throughput	Approximately 25 analyses were completed each hour using both buffers, and approximately 1,000 samples could be processed per kit.							

ND = Significant inhibition was not detected.

NR = Not reanalyzed.

^(a) Inhibition was not significantly different from the preservative blank.

^(b) Potential interferences were reanalyzed due to four suspect negative inhibitions during the initial analysis with the Pro-Metal buffer.

Pro-Metal Buffer

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	-19	-7	33	-31	10-35	ND
	Botulinum toxin complex B	0.3	-185	-121	-91	-40	18-104	ND
	Colchicine	240	12	2	8	-9	2-6	ND
	Cyanide	250	89	64	44	19	1-7	0.25
	Dicrotophos	1,400	55	-1	-10	-3	2-4	140
	Nicotine	2,800	98	2	-10	-7	0-4	700
	Ricin	15.0	3	-2	2	2	2-4	ND
	Soman	1.4	-55	17	-66	-4	13-22	ND
	Thallium sulfate	2,800	79	53	27	4	1-4	28
	VX	2.0	5	-11	-11	-3	5-10	ND
Potential interferences in DDW	Interference	Conc. (mg/L)	Average Inhibition (%)		Standard Deviation (%)			
			Initial Analysis	Reanalysis ^(a)	Initial Analysis	Reanalysis ^(a)		
	Aluminum	0.5	-395	-13	29	3		
	Copper	0.6	-299	30	26	4		
	Iron	0.15	-399	-8	18	3		
	Zinc	2.5	86	NR	0	NR		
False positive response	Neither the chlorination nor chloramination samples generated an inhibition greater than 50%. However, the chloramination sample generated a result that indicated an enhancement in luminescence (i.e., a negative inhibition), which, according to Checklight Ltd., can also indicate toxicity.							
False negative response	The inhibition of the chloramination by-products was $-75\% \pm 20\%$ with DI water as the negative control. If a contaminant causing a 75% inhibition had been present in this water and DI water was used as the negative control, the inhibition would have been close to 0%—a false negative response. This underscores the need to use negative control samples that are as similar as possible to the samples being analyzed. A second type of false negative response occurred (for aldicarb, colchicine, botulinum toxin, ricin, soman, and VX) when the inhibition was not greater than 50% in the presence of a lethal dose of contaminant.							

ND = Significant inhibition was not detected.

NR = Not reanalyzed.

See the Pro-Organic Buffer table for descriptions for ease of use, field portability, and throughput.

^(a) Potential interferences were reanalyzed due to four suspect negative inhibitions during the initial analysis with the Pro-Metal buffer.

Original signed by Gregory A. Mack 6/22/06
Gregory A. Mack Date
Vice President
Energy, Transportation, and Environment Division
Battelle

Original signed by Andrew P. Avel 8/7/06
Andrew P. Avel Date
Acting Director
National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency

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