November 2003

Environmental Technology Verification Report

CHECKLIGHT, LTD.
TOXSCREEN-II
RAPID TOXICITY TESTING SYSTEM

Prepared by
Battelle

Battelle
. . . Putting Technology To Work

Under a cooperative agreement with

EPA **U.S. Environmental Protection Agency**

ETV ETV ETV

November 2003

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

CheckLight, Ltd.
ToxScreen-II
Rapid Toxicity Testing System

by
Ryan James
Amy Dindal
Zachary Willenberg
Karen Riggs

Battelle
Columbus, Ohio 43201

Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, has financially supported and collaborated in the extramural program described here. This document has been peer reviewed by the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use.

Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technology across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of seven environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv/.

Effective verifications of monitoring technologies are needed to assess environmental quality and to supply cost and performance data to select the most appropriate technology for that assessment. Under a cooperative agreement, Battelle has received EPA funding to plan, coordinate, and conduct such verification tests for "Advanced Monitoring Systems for Air, Water, and Soil" and report the results to the community at large. Information concerning this specific environmental technology area can be found on the Internet at http://www.epa.gov/etv/centers/center1.html.

Acknowledgments

The authors wish to acknowledge the support of all those who helped plan and conduct the verification test, analyze the data, and prepare this report. Many thanks go to Battelle's Medical Research and Evaluation Facility for providing the facilities for and personnel capable of working with chemical warfare agents and biotoxins. We would also like to thank Karen Bradham, U.S. EPA National Exposure Research Laboratory; Steve Allgeier, U.S. EPA Office of Water; Ricardo DeLeon, Metropolitan Water District of Southern California; Yves Mikol, New York City Department of Environmental Protection; and Stanley States, Pittsburgh Water and Sewer Authority, for their careful review of the test/QA plan and this verification report.

Contents

Page

Figures

Tables

List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
ATEL	Aqua Tech Environmental Laboratories
DI	deionized water
DDW	dechlorinated drinking water from Columbus, Ohio
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
HDPE	high-density polyethylene
ID	identification
LD	lethal dose
µL	microliter
mg	milligram
mL	milliliter
NSDWR	National Secondary Drinking Water Regulations
%D	percent difference
PE	performance evaluation
QA	quality assurance
QC	quality control
QMP	quality management plan
SOP	standard operating procedure
TSA	technical systems audit

Chapter 1
Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative 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 testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of 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 EPA's National Exposure Research Laboratory and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the CheckLight, Ltd. ToxScreen-II rapid toxicity testing system. Rapid toxicity testing systems were identified as a priority technology verification category through the AMS Center stakeholder process.

Chapter 2
Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This verification report provides results for the verification testing of ToxScreen-II. Following is a description of ToxScreen-II, based on information provided by the vendor. The information provided below 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 (Figure 2-1) uses a 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 may be 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.

Figure 2-1. ToxScreen-II Rapid Toxicity Testing System

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 chloro-acetate. 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 use while conducting field tests.

Chapter 3
Test Design and Procedures

3.1 Introduction

The objective of this verification test of rapid toxicity technologies was to evaluate their ability to detect certain toxins and to determine their susceptibility to interfering chemicals in a controlled experimental matrix. Rapid toxicity technologies do not identify or determine the concentration of specific contaminants, but serve as a screening tool to quickly determine whether water is potentially toxic. Rapid toxicity technologies use bacteria (e.g., *Vibrio fischeri*), enzymes (e.g., luciferase), or small crustaceans (e.g., *Daphnia magna*) that either directly, or in combination with reagents, produce a background level of light or use dissolved oxygen at a steady rate in the absence of toxic contaminants. Toxic contaminants in water are indicated by a change in the color or intensity of light produced or by a decrease in the dissolved oxygen uptake rate in the presence of the contaminants.

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 can detect the toxicity caused by each contaminant, its response to interfering compounds in clean drinking water, such as water treatment chemicals and by-products, was evaluated. Table 3-1 shows the contaminants and potential interferences that were evaluated during this verification test.

This verification test was conducted according to procedures specified in the *Test/QA Plan for Verification of Rapid Toxicity Technologies*.[1] ToxScreen-II was verified by analyzing a dechlorinated drinking water (DDW) sample from Columbus, Ohio, fortified with various concentrations of the contaminants and interferences shown in Table 3-1. Hereafter in this report, DDW will refer to dechlorinated drinking water from Columbus, Ohio. Where possible, the concentration of each contaminant or potential interference was confirmed independently by Aqua Tech Environmental Laboratories (ATEL), Marion, Ohio, or by Battelle, depending on the analyte.

Table 3-1. Contaminants and Potential Interferences

Category	Contaminant
Carbamate pesticide	aldicarb
Pharmaceutical	colchicine
Industrial chemical	cyanide
Organophosphate pesticide	dicrotophos
Rodenticide	thallium sulfate
Biological toxins	botulinum toxin, ricin
Nerve agents	soman, VX
Potential interferences	aluminum, copper, iron, manganese, zinc, chloramination by-products, and chlorination by-products

ToxScreen-II was evaluated by

- Endpoint and precision—percent inhibition for all concentration levels of contaminants and potential interfering compounds and precision of replicate analyses
- Toxicity threshold for each contaminant
- False negative responses—contaminants that were reported as producing inhibition results similar to the negative control when the contaminant was present at lethal concentrations
- False positive responses—occurrence of inhibition of dechlorinated drinking water samples significantly greater than the inhibition reported for unspiked American Society for Testing and Materials (ASTM) Type II deionized (DI) water samples (zero inhibition)
- Field portability
- Ease of use
- Throughput.

3.2 Test Design

ToxScreen-II was used to analyze the DDW sample fortified with contaminants at concentrations ranging from lethal levels to concentrations several orders of magnitude less than the lethal dose. The lethal dose of each contaminant was determined by calculating the concentration at which 250 mL of water would probably cause the death of a 154-pound person. These calculations were based on toxicological data available for each contaminant. For soman, the stock solution confirmation showed degradation in the water; therefore, the concentrations

analyzed were less than anticipated. Whether the concentration is still a lethal dose, as is the case for all contaminants, depends on the characteristics of the individual person and the amount of contaminant ingested. 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 at various concentrations of contaminants, as well as to measure the precision of ToxScreen-II results.

The response of ToxScreen-II to compounds used during the water treatment process (identified as potential interferences in Table 3-1) was evaluated by analyzing separate aliquots of DDW fortified with each potential interference at approximately one-half of the concentration limit recommended by the EPA's National Secondary Drinking Water Regulations (NSDWR)[2] 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 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. In addition to comprehensive testing in Battelle laboratories, ToxScreen-II was operated in the basement of a Columbus, Ohio, home to test its ability to be transported and operated in a non-laboratory setting.

3.3 Test Samples

Test samples used in the verification test included drinking water and quality control (QC) samples. Table 3-2 shows the number and type of samples analyzed. QC samples included method blanks and positive and negative control samples. The fortified drinking water samples were prepared from a single drinking water sample collected from the Columbus, Ohio, system. The water was dechlorinated using sodium thiosulfate and then fortified with various concentrations of contaminants and interferences. Using this DDW (Columbus, Ohio, dechlorinated drinking water), individual solutions containing each contaminant and potential interference were prepared and analyzed. The DDW containing the potential interferences was analyzed at a single concentration level, while at least four dilutions (made using the DDW) were analyzed for each contaminant using ToxScreen-II. Mixtures of contaminants and interfering compounds were not analyzed. One concentration level of cyanide was analyzed in the field setting.

3.3.1 Quality Control Samples

QC samples included method blank samples, which consisted of ASTM Type II DI water; positive control samples, which consisted of ASTM Type II DI water or DDW (depending on vendor preference) fortified with a contaminant and concentration selected by the vendor; and negative control samples, which consisted of the unspiked DDW. The method blank samples were used to help ensure that no sources of contamination were introduced in the sample handling and analysis procedures.

Table 3-2. Summary of Quality Control and Contaminant Test Samples

Type of Sample	Sample Characteristics	Concentration Levels (mg/L)	No. of Sample Analyses
Quality control	Method blank	NS[a]	9
	Positive control	5 (sodium chloroacetate) 1 (copper chloride)	10 24 24
	Negative control (unspiked DDW)	NS	44
DDW fortified with contaminants	Aldicarb	280; 28; 2.8; 0.28	4 per concentration level
	Colchicine	240; 24; 2.4; 0.24; 0.024	4 per concentration level
	Cyanide	250; 25; 2.5; 0.25; 0.025; 0.0025	4 per concentration level
	Dicrotophos	1,400; 140; 14; 1.4; 0.14	4 per concentration level
	Thallium sulfate	2,400; 240; 24; 2.4	4 per concentration level
	Botulinum toxin[b]	0.30; 0.030; 0.0030; 0.00030	4 per concentration level
	Ricin[c]	15; 1.5; 0.15; 0.015	4 per concentration level
	Soman	0.068[d]; 0.0068; 0.00068; 0.000068	4 per concentration level
	VX	0.22; 0.022; 0.0022; 0.00022	4 per concentration level
Field location	Cyanide	0.25	4
DDW fortified with potential interferences	Aluminum	0.36	4
	Copper	0.65	4
	Iron	0.069	4
	Manganese	0.26	4
	Zinc	3.5	4
Disinfectant by-products	Chloramination by-products	NS	4
	Chlorination by-products	NS	4

[a] NS = Samples not fortified with any contaminant or potential interference.
[b] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[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.

Copper chloride and sodium chloroacetate were provided by the vendor for use as positive control samples, and both were used at times throughout the verification test. While performance limits were not placed on the results, inhibition of greater than 50% for these contaminants indicated to the operator that ToxScreen-II was functioning properly. The negative control

sample was used to set a background inhibition of the DDW, the matrix in which each test sample was prepared.

3.3.2 Drinking Water Fortified with Contaminants

Approximately 150 liters of Columbus, Ohio, tap water were collected in a high-density polyethylene (HDPE) container. The sample was dechlorinated with 0.5 milliliter (mL) of 0.4 M sodium thiosulfate for every liter of water. All subsequent test samples were prepared from this DDW and stored in glass containers to avoid chlorine leaching from HDPE containers.

A stock solution of each contaminant was prepared in ASTM Type II DI water at concentrations above the lethal dose level. The stock solution was diluted in DDW to obtain one sample containing the lethal dose concentration for each contaminant and three additional samples with concentrations 10, 100, and 1,000 times less than the lethal dose. Exceptions to this were colchicine, cyanide, and dicrotophos, contaminants that had to be diluted further because of ToxScreen-II's sensitivity to them at higher concentrations. Table 3-2 lists each concentration level and the number of samples analyzed at each level.

3.3.3 Drinking Water Fortified with Potential Interferences

Individual aliquots of the DDW were fortified with one-half the concentration specified by the EPA's NSDWR for each potential interference. Table 3-2 lists the interferences, along with the concentrations at which they were tested. Four replicates of each of these samples were analyzed. To test the sensitivity of ToxScreen-II to by-products of the chlorination process as potential interferences, the unspiked DDW (same as the negative control) was used since the water sample originated from a utility that uses chlorination as its disinfectant procedure. In a similar test involving the by-products of the chloramination process, an additional water sample was obtained from St. Petersburg, Florida, a city that uses chloramination as its disinfectant procedure. The residual chlorine in both of these samples was removed using sodium thiosulfate, and then the samples were analyzed in replicate with no additional fortification of contaminants.

3.4 Test Procedure

3.4.1 Test Sample Preparation and Storage

A drinking water sample was collected as described in Section 3.3.2 and, because free chlorine kills the bacteria within the ToxScreen-II reagent and can degrade the contaminants during storage, was immediately dechlorinated with sodium thiosulfate. Prior to preparing each stock solution, dechlorination of the water sample was qualitatively confirmed by adding an n,n-diethyl-p-phenylenediamine tablet to a 25-mL aliquot of the DDW. Once dechlorination was confirmed, all the contaminant samples, potential interference samples, and negative control QC samples were made from this DDW, while the method blank sample was prepared from ASTM Type II DI water. The positive control samples were made from stock solutions provided by the vendor using DDW as the dilution matrix. All QC samples were prepared prior to the start of the testing and stored at room temperature for a maximum of 60 days. The aliquots of DDW con-

taining the contaminants were prepared within seven days of testing and stored in the dark at room temperature without chemical preservation. Aliquots to be analyzed by each technology were placed in uniquely labeled sample containers. The sample containers were assigned an identification (ID) number. A master log of the samples and sample ID numbers for each technology was kept by Battelle.

3.4.2 Test Sample Analysis Procedure

The ToxScreen-II protocol calls for the use of pro-organic and pro-metal concentrated assay buffers in the analysis of each sample. The buffers were developed to enhance the sensitivity of the test to a wide range of toxic agents. Inhibition of greater than 50% using either buffer should be considered a positive result. A positive result in the pro-organic buffer would suggest that the contaminant causing the toxicity is organic, while a positive result in the pro-metal buffer would suggest that the contaminant may be a metal. Therefore, each water sample analyzed requires two separate analyses, not including the reference samples. First, a freeze-dried bioassay reagent was hydrated with the provided hydration buffer. Next, pro-organic and pro-metal concentrated assay buffers were added separately to individual aliquots of the sample as well as to two aliquots of the reference sample. Then, aliquots of suspended reagent were rapidly dispensed into test cuvettes. Finally, after 90 minutes' incubation at ambient temperature, luminescence was measured using the portable luminometer. The luminescence in the test samples was compared with that of the reference sample to determine their relative toxicity.

For each contaminant, ToxScreen-II analyzed the lethal dose concentration and at least three additional concentration levels four times. Only one concentration of potential interference was analyzed. ToxScreen-II reported the absolute light units for each sample. To test the field portability of ToxScreen-II, a single concentration level of cyanide, prepared in the same way as the other DDW samples, was analyzed in replicate by ToxScreen-II in the basement of a Columbus, Ohio, home. Sample analysis procedures were performed in the same way as during testing in the laboratory. Two operators performed all the analyses using ToxScreen-II. Both held bachelor's degrees in the sciences and spent approximately four hours with the vendor to become accustomed to performing tests using ToxScreen-II.

3.4.3 Stock Solution Confirmation Analysis

The concentrations of the contaminant and interfering compound stock solutions were verified with standard analytical methods, with the exception of colchicine, ricin, and botulinum toxin—contaminants without standard analytical methods. Aliquots to be analyzed by standard methods were preserved as prescribed by the method. In addition, the same standard methods were used to measure the concentrations of each contaminant/potential interference in the unspiked DDW so that background concentrations of contaminants or potential interferences were accounted for within the displayed concentration of each contaminant/potential interference sample. Table 3-3 lists the standard methods used to measure each analyte; the results from the stock solution confirmation analyses (obtained by reporting the lethal dose concentration for the contaminants

Table 3-3. Dose Confirmation Results

	Method	Average Concentration ± Standard Deviation N = 4 (mg/L)	Background in DDW Sample (mg/L)
Contaminant			
Aldicarb	EPA 531.1[3]	280 ± 28	<0.0007
Colchicine	[a]	NA[b]	NA
Cyanide	EPA 335.1[4]	250 ± 15	0.008
Dicrotophos	EPA SW846 (8141A)[5]	1,400 ± 140	<0.002
Thallium sulfate	EPA 200.8[6]	2,400 ± 24	<0.001
Botulinum toxin	[a]	NA	NA
Ricin	[a]	NA	NA
Soman	[c]	0.068[d] ± 0.001	<0.05
VX	[c]	0.22 ± 0.02	<0.05
Potential Interference			
Aluminum	EPA 200.8	0.36 ± 0.01	<0.10
Copper	EPA 200.8	0.65 ± 0.01	0.011
Iron	EPA 200.8	0.069 ± 0.008	<0.04
Manganese	EPA 200.8	0.26 ± 0.01	<0.01
Zinc	EPA 200.8	3.5 ± 0.35	0.30

[a] No standard method available. QA audits and balance calibration assured accurately prepared solutions.
[b] NA = Not applicable.
[c] Purity analyses performed on chemical and biological agent materials using Battelle standard operating procedures.
[d] The result of the dose confirmation analysis for soman was 23% of the expected concentration of 0.30 mg/L.

and the single concentration that was analyzed for the potential interferences); and the background levels of the contaminants and potential interferences measured in the DDW sample, which were all non-detect or negligible.

Standard methods were also used to characterize several water quality parameters such as the concentration of trihalomethanes, haloacetic acids, and total organic halides; turbidity; dissolved organic carbon content; pH; alkalinity; specific conductivity; and hardness. Table 3-4 lists these measured water quality parameters for both the water sample collected in Columbus, Ohio, representing a water system using chlorination as the disinfecting process, and the water sample collected in St. Petersburg, Florida, representing a water system using chloramination as the disinfecting process.

Table 3-4. Water Quality Parameters

Parameter	Method	Dechlorinated Columbus, Ohio, Tap Water (disinfected by chlorination)	Dechlorinated St. Petersburg, Florida, Tap Water (disinfected by chloramination)
Turbidity	EPA 180.1[7]	0.1 NTU[a]	0.3 NTU
Organic carbon	SM 5310[8]	2.5 mg/L	2.9 mg/L
Specific conductivity	SM 2510[8]	364 μmho	460 μmho
Alkalinity	SM 2320[8]	42 mg/L	97 mg/L
pH	EPA 150.1[9]	7.65	7.95
Hardness	EPA 130.2[9]	112 mg/L	160 mg/L
Total organic halides	SM 5320B[8]	190 μg/L	83 μg/L
Total trihalomethanes	EPA 524.2[10]	52.8 μg/L	2.4 μg/L
Total haloacetic acids	EPA 552.2[11]	75.7 μg/L	13.5 μg/L

[a] NTU = nephelometric turbidity unit.

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center[12] and the test/QA plan for this verification test.[1]

4.1 Quality Control of Stock Solution Confirmation Methods

The stock solutions for aldicarb, cyanide, dicrotophos, and thallium sulfate were analyzed using a standard reference method at ATEL. As part of ATEL's standard operating procedures (SOPs), various QC samples were analyzed with each sample set. These included matrix spike, laboratory control spike, and method blank samples. According to the standard methods used for the analyses, recoveries of the QC spike samples analyzed with samples from this verification test were within acceptable limits of 75% to 125%, and the method blank samples were below the detectable levels for each analyte. For VX and soman, the confirmation analyses were performed at Battelle using a Battelle SOP. Calibration standard recoveries of VX and soman were always between 69% and 130%, and most of the time were between 90% and 100%. Standard analytical methods for colchicine, ricin, and botulinum toxin were not available and, therefore, were not performed. QA audits and balance calibrations assured that solutions for these compounds were accurately prepared.

4.2 Quality Control of Drinking Water Samples

A method blank sample consisting of ASTM Type II DI water was analyzed once by ToxScreen-II for approximately every 20 drinking water samples that were analyzed. These samples were used to set a baseline light level for a clean water matrix. A negative control sample (unspiked DDW) was analyzed for approximately every four samples. The light produced from samples fortified with contaminants were compared with the light produced from the negative control samples to calculate the percent inhibition caused by the contaminant. A positive control sample also was analyzed once for approximately every 20 drinking water samples. While performance limits were not placed on the results of the positive control sample, the vendor informed Battelle that, if the positive control samples did not cause greater than approximately 50% inhibition, it would indicate to the operator that ToxScreen-II was operating incorrectly. Sodium chloroacetate was the positive control sample for the pro-organic buffer samples and copper chloride for the pro-metal buffer samples. For 24 positive control samples of each type, the average inhibition was 90% ± 10% for the pro-metal buffer and 88% ± 10% for

the pro-organic buffer. This level of inhibition for the positive control samples indicated the proper functioning of ToxScreen-II.

4.3 Audits

4.3.1 Performance Evaluation Audit

The concentration of the standards used to prepare the contaminant and potential interferences was confirmed by analyzing solutions of each analyte prepared in ASTM Type II DI water from two separate commercial vendors using the confirmation methods. The standards from one source were used to prepare the stock solutions during the verification test, while the standards from a second source were used exclusively to confirm the accuracy of the measured concentration of the first source. The percent difference (%D) between the measured concentration of the performance evaluation (PE) sample and the prepared concentration of that sample was calculated using the following equation:

$$\%D = \frac{M}{A} \times 100\% \quad (1)$$

where M is the absolute value of the difference between the measured and the prepared concentration and A is the prepared concentration. The %D between the measured concentration of the PE standard and the prepared concentration had to be less than 25 for the measurements to be considered acceptable. Table 4-1 shows the results of the PE audit for each compound. All %D values were less than 25.

Given the lack of confirmation methodology for some of the contaminants in this verification test, PE audits were not performed for all of the contaminants. PE audits were performed when more than one source of the contaminant or potential interference was commercially available and when were methods were available to perform the confirmation. To assure the purity of the other standards, documentation, such as certificates of analysis, was obtained for colchicine, botulinum toxin, and ricin. In the case of VX and soman, which were obtained from the U.S. Army, the reputation of the source, combined with the confirmation analysis data, provided assurance of the concentration analyzed.

4.3.2 Technical Systems Audit

The Battelle Quality Manager conducted a technical systems audit (TSA) to ensure that the verification test was performed in accordance with the test/QA plan[1] and the AMS Center QMP.[12] As part of the audit, the Battelle Quality Manager reviewed the contaminant standard and stock solution confirmation methods, compared actual test procedures with those specified in the test/QA plan, and reviewed data acquisition and handling procedures. Observations and findings from this audit were documented and submitted to the Battelle verification test coordinator for response. No findings were documented that required any significant action. The records concerning the TSA are permanently stored with the Battelle Quality Manager.

Table 4-1. Summary of Performance Evaluation Audit

		Average Measured Concentration ± Standard Deviation (mg/L)	Actual Concentration (mg/L)	Percent Difference
Contaminant	Aldicarb	0.00448 ± 0.000320	0.00500	11
	Cyanide	0.207 ± 0.026	0.200	4
	Dicrotophos	0.00728 ± 0.000699	0.00748	3
	Thallium sulfate	0.090 ± 0.004	0.100	10
Potential interference	Aluminum	0.512 ± 0.013	0.500	2
	Copper	0.106 ± 0.002	0.100	6
	Iron	0.399 ± 0.004	0.400	0.30
	Manganese	0.079 ± 0.003	0.100	21
	Zinc	0.106 ± 0.016	0.100	6

The EPA Quality Manager also conducted a TSA to ensure that the verification test was performed in accordance with the test/QA plan[1] and the AMS Center QMP.[12] As part of the audit, the EPA Quality Manager compared actual test procedures with those specified in the test/QA plan and reviewed data acquisition and sample preparation records and procedures. No significant findings were observed during the EPA TSA. The records concerning the TSA are permanently stored with the EPA Quality Manager.

4.3.3 Audit of Data Quality

At least 10% of the data acquired during the verification test were audited. Battelle's Quality Manager traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting, to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

4.4 QA/QC Reporting

Each internal assessment and audit was documented in accordance with Sections 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center.[12] Once the assessment report was prepared, the Battelle verification test coordinator ensured that a response was provided for each adverse finding or potential problem and implemented any necessary follow-up corrective action. The Battelle Quality Manager ensured that follow-up corrective action was taken. The results of the TSA were sent to the EPA.

4.5 Data Review

Records generated in the verification test were reviewed before these records were used to calculate, evaluate, or report verification results. Table 4-2 summarizes the types of data recorded. The review was performed by a technical staff member involved in the verification test, but not the staff member who originally generated the record. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed.

Table 4-2. Summary of Data Recording Process

Data to be Recorded	Responsible Party	Where Recorded	How Often Recorded	Disposition of Data[a]
Dates, times of test events	Battelle	Laboratory record books	Start/end of test, and at each change of a test parameter	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Sample preparation (dates, procedures, concentrations)	Battelle	Laboratory record books	When each sample was prepared	Used to confirm the concentration and integrity of the samples analyzed, procedures entered into laboratory record books
Test parameters (contaminant concentrations, location, etc.)	Battelle	Laboratory record books	When set or changed	Used to organize/check test results, manually incorporated in data spreadsheets as necessary
Stock solution confirmation analysis, sample analysis, chain of custody, and results	Battelle or contracted laboratory	Laboratory record books, data sheets, or data acquisition system, as appropriate	Throughout sample handling and analysis process	Transferred to spreadsheets/agreed upon report

[a] All activities subsequent to data recording were carried out by Battelle.

Chapter 5
Statistical Methods and Reported Parameters

The statistical methods presented in this chapter were used to verify the performance parameters listed in Section 3.1.

5.1 Endpoints and Precision

The luminometer provided with the ToxScreen-II reported the absolute light units for each sample analyzed. Each DDW sample containing contaminants was compared with a negative control sample that, for this verification test, was unspiked DDW. This comparison was made by accounting for the inhibition of the negative control in the calculation of the percent inhibition. Therefore, the percent inhibition of the negative control sample within each sample set was defined as approximately zero percent. The percent inhibition for each sample was calculated using the following equation:

$$\%\ inhibition\ =\ \left(1-\frac{L_{control}}{L_{sample}}\right)\times 100\% \qquad (2)$$

Where L is the absolute light units produced for the control and test samples. For this test, the control sample was always DDW, except when the inhibition of the disinfectant by-products was being determined, in that case, ASTM Type II DI water served as the control sample.

The standard deviation (S) of the results for the replicate samples was calculated, as follows, and used as a measure of technology precision at each concentration.

$$S=\left[\frac{1}{n-1}\sum_{k=1}^{n}\left(I_k-\bar{I}\right)^2\right]^{1/2} \qquad (3)$$

where n is the number of replicate samples, I_k is the percent inhibition measured for the k^{th} sample, and $\bar{I}$ is the average percent inhibition of the replicate samples. Because the average inhibitions were frequently near zero for this data set, relative standard deviations often would have greatly exceeded 100%, making the results difficult to interpret. Therefore, the precision results were left in the form of standard deviations so the reader could easily view the uncertainty around the average for results that were both near zero and significantly larger than zero.

5.2 Toxicity Threshold

The toxicity threshold was defined as the lowest concentration of contaminant to exhibit a percent inhibition significantly greater than the negative control. Also, each concentration level higher than the toxicity threshold had to be significantly greater than the negative control, and the inhibition produced by each lower concentration analyzed had to be significantly less than that produced by the toxicity threshold concentration. Since the inhibition of the test samples was calculated with respect to the inhibition of each negative control sample, the percent inhibition of the negative control was always zero. An inhibition was significantly greater than the negative control if the average inhibition plus or minus the standard deviation did not include zero.

5.3 False Positive/Negative Responses

A response would be considered false positive if an unspiked drinking water sample produced an inhibition significantly greater than zero when determined with respect to ASTM Type II DI water. Depending on the degree of inhibition in the sample, toxicity due to subsequent contamination of that sample may not be detectable or could be exaggerated as a result of the baseline inhibition. To test for this possibility, the percent inhibition of the unspiked drinking water was determined with respect to ASTM Type II DI water. Drinking water samples collected from water systems using chlorination and chloramination as the disinfecting process were analyzed in this manner. An inhibition was considered significantly different from zero if the average inhibition, plus or minus the standard deviation, did not include zero.

A response was considered false negative when ToxScreen-II was subjected to a lethal concentration of some contaminant in the DDW and did not indicate inhibition greater than 50%. The vendor's instructions stated that a result was not positive for toxicity unless it was at least 50%. A difference was considered significant if the average inhibition plus or minus the standard deviation did not encompass the value or range of values that were being compared.

5.4 Field Portability

The results obtained from the measurements made on DDW samples in the laboratory and in the field setting were compiled independently and compared to assess the performance of the ToxScreen-II under different analysis conditions. Means and standard deviations of the endpoints generated in both locations were used to make the comparison. Also, qualitative observations of ToxScreen-II in a non-laboratory setting were made by the verification test coordinator and operators. Factors such as the ease of transport and set-up, demand for electrical power, and space requirement were documented.

5.5 Other Performance Factors

Ease of use (including clarity of the instruction manual, user-friendliness of software, and overall convenience) was qualitatively assessed throughout the verification test through observations of

the operators and verification test coordinator. Sample throughput was evaluated quantitatively based on the number of samples that could be analyzed per hour.

Chapter 6
Test Results

6.1 Endpoints and Precision

Tables 6-1a-i present the percent inhibition data for nine contaminants, and Table 6-2 presents data for five potential interferences and the drinking water samples disinfected by both chlorination and chloramination. Given in each table are the concentrations analyzed, the percent inhibition results for each replicate at each concentration, and the average and standard deviation of the inhibition of the four replicates at each concentration. Results are provided for each contaminant analyzed in both the pro-organic and pro-metal buffers. Samples that produced negative percent inhibition values indicated an increase in light production by the bacteria relative to the negative control and were considered to be non-toxic.

6.1.1 Contaminants

The contaminants that were analyzed by ToxScreen-II during this verification test produced results that differed depending on which buffer was used. Each contaminant was analyzed using each buffer at all the concentration levels. Since the buffers were developed to enhance the sensitivity of specific classes of compounds, the results were expected to show this difference. Cyanide, dicrotophos, and thallium sulfate were the only three contaminants that exhibited inhibitions that were significantly greater than the negative control in the pro-metal buffer. Cyanide exhibited significant inhibitions down to 0.025 mg/L in that buffer while dicrotophos was only significantly different than the negative control at the 1,400 mg/L level and thallium sulfate for the 240 and 2,400 mg/L concentration levels.

In the pro-organic buffer, aldicarb exhibited inhibitions significantly greater than the negative control for all of the concentration levels; but the average inhibition did not change in proportion to the concentration of alicarb in each sample. The inhibition of 2.8 mg/L sample was greater than the inhibition produced by the 28 mg/L sample and similar to the inhibition of the 280 mg/L sample. A similar situation was true for colchicine, since the 0.24 mg/L sample exhibited higher inhibitions than the 2.4 and 24 mg/L sample; however, the inhibition of the lethal dose of colchicine (83% ± 3%) was significantly greater than all of the other concentration levels analyzed. Also, in the pro-organic buffer, ToxScreen-II exhibited nearly compete inhibition (85% to 99%) for cyanide down to a concentration of 2.5 mg/L and an inhibition of 68% ± 3% for 0.25 mg/L cyanide. The inhibition of dicrotophos increased from 41% at 0.14 mg/L and 1.4 mg/L to 99% at 140 mg/L and 1,400 mg/L in the pro-organic buffer. The inhibitions of the two highest concentrations of botulinum toxin were significantly greater than the negative control, but not

Table 6-1a. Aldicarb Percent Inhibition Results

	Pro-Organic Buffer			Pro-Metal Buffer		
Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.28	10 12 3 18	11	6	-49 -51 -47 -37	-46	6
2.8	66 63 67 36	58	15	-20 -13 -15 -22	-17	4
28	41 36 39 37	38	2	-3 7 2 13	5	7
280 (Lethal Dose)	55 88 68 64	69	14	-23 -21 -21 -20	-21	1

Table 6-1b. Colchicine Percent Inhibition Results

	Pro-Organic Buffer			Pro-Metal Buffer		
Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.024	-73 -70 0 21	-31	48	0 1 -5 21	4	12
0.24	63 68 41 48	55	13	-70 -81 -85 -78	-79	7
2.4	45 39 18 35	34	11	-83 -77 -86 -88	-83	5
24	25 9 13 33	20	11	-61 -77 -70 -81	-72	9
240 (Lethal Dose)	79 82 86 84	83	3	-50 -40 -46 -34	-42	7

Table 6-1c. Cyanide Percent Inhibition Results

	Pro-Organic Buffer			Pro-Metal Buffer		
Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.0025	29 4 -30 -25	-6	27	-3 4 9 3	3	5
0.025	5 8 2 40	14	18	37 30 20 37	31	8
0.25	64 71 67 69	68	3	26 34 37 42	35	7
2.5	99 99 99 99	99	0	65 [a] 87 88	80	13
25	100 41 99 99	85	29	36 47 58 50	48	9
250 (Lethal Dose)	99 100 99 99	99	0	99 99 98 98	99	1
0.25 (Field Location)	97 97 98 96	97	1	65 58 67 54	61	6

[a] Data point removed because of transcription error.

Table 6-1d. Dicrotophos Percent Inhibition Results

	Pro-Organic Buffer			Pro-Metal Buffer		
Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.14	30 39 29 67	41	18	-76 -60 -53 -21	-52	23
1.4	30 57 43 34	41	12	-16 -25 -43 -46	-32	14
14	77 92 65 89	81	12	-38 -55 -43 -63	-50	11
140	99 99 99 99	99	0	-81 -67 -70 -86	-76	9
1,400 (Lethal Dose)	98 99 99 99	99	0	25 23 43 33	31	9

Table 6-1e. Thallium Sulfate Percent Inhibition Results

	Pro-Organic Buffer			Pro-Metal Buffer		
Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)	Inhibition (%)	Average (%)	Standard Deviation (%)
2.4	-9 -49 -26 -43	-32	18	-3 0 13 15	6	9
24	-15 -24 -5 -3	-12	10	16 11 11 26	16	7
240	31 100 26 51	52	34	66 69 70 70	69	2
2,400 (Lethal Dose)	-55 100 -82 -3	-10	80	97 99 97 97	98	1

Table 6-1f. Botulinum Toxin Percent Inhibition Results

	Pro-Organic Buffer			Pro-Metal Buffer		
Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.00030	7 15 -16 -36	-8	23	-100 -84 [(a)] -108	-97	12
0.0030	-21 5 -26 -17	-15	14	-59 -63 -62 -43	-57	9
0.030	45 48 20 76	47	23	18 5 -8 -12	1	14
0.30 (Lethal Dose)	80 81 74 41	69	19	-47 -54 -12 -62	-44	22

[(a)] Data point removed because of transcription error.

Table 6-1g. Ricin Percent Inhibition Results

	Pro-Organic Buffer			Pro-Metal Buffer		
Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.015	-52 10 4 -4	-10	28	-93 -96 -86 -100	-89	12
0.15	26 30 22 41	30	8	-74 -85 -111 -102	-93	17
1.5	27 28 -5 -11	10	21	-18 3 86 -93	-5	74
15 (Lethal Dose)	56 53 46 43	50	6	-96 -86 -100 -74	-89	12

Table 6-1h. Soman Percent Inhibition Results

	Pro-Organic Buffer			Pro-Metal Buffer		
Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.000068	-51 -35 9 10	-17	31	-55 -53 -50 -64	-55	6
0.00068	-11 -20 1 -5	-9	9	-63 -57 -64 -64	-62	3
0.0068	-10 -6 -5 16	-1	12	-39 -28 -53 -68	-47	17
0.068[(a)] (Lethal Dose)	-40 17 19 25	5	30	-50 -57 -46 91	-15	71

[(a)] 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.

Table 6-1i. VX Percent Inhibition Results

	Pro-Organic Buffer			Pro-Metal Buffer		
Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.00022	-53 -57 -60 -66	-59	6	-52 -19 -33 -21	-31	15
0.0022	-15 29 -27 -41	-13	30	-46 -13 -8 -18	-21	17
0.022	6 -17 3 -12	-5	11	-27 -14 -6 -30	-19	11
0.22 (Lethal Dose)	-26 27 3 -34	-8	28	-42 -43 4 -71	-38	31

significantly different from one another. Finally, the inhibition of ricin at 15 mg/L was significantly greater than the negative control and the other concentration levels analyzed. The inhibitions of thallium sulfate, soman, and VX did not meet the requirements of detectability, as defined in Section 5.2, in the pro-organic buffer.

In general, of the contaminants that exhibited inhibition, those with more organic makeup (aldicarb, dicrotophos, colchicine, ricin, botulinum toxin) were more likely to be detected in the pro-organic buffer compared to the pro-metal buffer. Dicrotophos was detected in both buffers, but was measured at a lower concentration in the pro-organic buffer. Thallium sulfate, a metallic contaminant, was detected only in the pro-metal buffer. Inhibition caused by cyanide was detected in both buffers because cyanide (made from potassium cyanide) has both metallic and organic characteristics.

6.1.2 Potential Interferences

Table 6-2 presents the results from the samples that were analyzed to test the effect of potential interferences on ToxScreen-II. In the pro-organic buffer, copper (59% ± 7%) and iron (29% ± 23%) were the only potential interferences that exhibited inhibitions significantly greater than the negative control. In the pro-metal buffer, copper and zinc exhibited average inhibitions of 94% and 100%, respectively, and manganese exhibited a much smaller average inhibition of 22%. Each of these metals has the potential to interfere with ToxScreen-II measurements, but especially copper and zinc in the pro-metal buffer.

Table 6-2. Potential Interferences Results

Potential Inferferences	Concen-tration (mg/L)	Pro-Organic Buffer Inhibition (%)	Pro-Organic Buffer Average (%)	Pro-Organic Buffer Standard Deviation (%)	Pro-Metal Buffer Inhibition (%)	Pro-Metal Buffer Average (%)	Pro-Metal Buffer Standard Deviation (%)
Aluminum	0.36	32 -24 65 -10	16	41	-35 2 -37 -34	-26	19
Copper	0.65	63 49 62 64	59	7	93 95 91 96	94	2
Iron	0.069	52 7 44 11	29	23	40 -6 13 -35	3	32
Manganese	0.26	-22 -15 -22 -32	-23	7	31 22 17 17	22	7
Zinc	3.5	-9 -105 3 -162	-68	79	100 100 100 100	100	0
Chlorination By-products	NA[a]	[b]	66	34	[b]	23	15
Chloramin-ation By-products	NA	81 68 92 71	78	11	-57 -19 -62 -56	-48	20

[a] NA = Not applicable.
[b] Chlorination by-product data averaged over negative control data compared to ASTM Type II DI water (N=44).

All of the contaminant and potential interference samples were prepared in the DDW and compared with unspiked DDW. Therefore, any background inhibition in the DDW was corrected by subtracting the inhibition caused by the negative control sample. To investigate whether ToxScreen-II is sensitive to by-products of disinfecting processes, dechlorinated drinking water samples from water systems that use chlorination and chloramination were analyzed and compared with ASTM Type II DI water as the control sample. This determination is crucial because the ability of ToxScreen-II to detect toxicity is dependent on the bacteria's background light production in a clean drinking water matrix. If clean drinking water produces 100% inhibition of light, inhibition caused by contaminants could not be detected. In the pro-organic buffer (over 42 samples), the sample from the water supply disinfected with chlorination exhibited inhibitions of 66% ± 34%, while the sample from the water supply disinfected by chloramination exhibited inhibitions of 78% ± 11% on four replicates. This suggests that samples that have been disinfected using either process are likely to interfere with ToxScreen-II results if a

reference sample very similar to the sample matrix is not used because the inhibition caused by the "clean" drinking water matrix left only one quarter to one third of the light to potentially be inhibited by contamination. For the pro-metal buffer, the inhibition of the sample from the water supply disinfected by chlorination was 23% ± 15%, and the inhibition of the sample from the water supply disinfected by chloramination was -48% ± -20%. In the former case, this interference could cause slightly exaggerated inhibitions and, in the latter case, inhibitions could be underestimated. For example, if a contaminant that exhibited approximately 50% inhibition was placed in water from a chloraminated system, and ASTM Type II DI water was used as the reference sample, the percent inhibition would be approximately zero percent.

Overall, because of the low level of light production when using the pro-organic buffer in the DDW, water disinfected by chlorination may interfere with the ToxScreen-II results even if a similar reference sample is used. As long as a similar reference sample is used when using the pro-metal buffer, water disinfected using either process is not likely to interfere with the ToxScreen-II results. However, if ASTM Type II DI water has to be used as the reference sample, all the water types could potentially interfere with the results from ToxScreen-II.

6.1.3 Precision

Across all the contaminants and potential interferences, the standard deviation was measured and reported for each set of four replicates to evaluate ToxScreen-II precision. Out of 96 opportunities, the standard deviation of the four replicate measurements was less than 10% 42 times, between 10% and 20% 31 times, and greater than 20% 23 times. There was no clear reason for the high variability of the results. The same two operators analyzed all the samples using the techniques described in the instructions and provided personally during the vendor training.

6.2 Toxicity Threshold

Table 6-3 gives the toxicity thresholds, as defined in Section 5.2, for each contaminant. The lowest toxicity threshold concentration was for cyanide at 0.025 mg/L when the pro-metal buffer was used and botulinum toxin at 0.03 mg/L when the pro-organic buffer was used, indicating that ToxScreen-II was most sensitive to cyanide and botulinum toxin, depending on the buffer used.

6.3 False Positive/Negative Responses

False positive responses were observed for unspiked drinking water samples from systems that use chlorination and chloramination as their disinfectant processes when the pro-organic buffer was used. As described in Section 6.1.2, for a clean water sample that had been disinfected using each process, ToxScreen-II reported inhibitions of 66% ± 34% for chlorinated drinking water and 78 ± 11% for chloraminated drinking water. By-products of these processes apparently inhibited the ToxScreen-II reagent in the pro-organic buffer. At times, the background inhibition caused by the drinking water sample inhibited the light to the extent that it was questionable whether

Table 6-3. Toxicity Thresholds

Contaminant	Concentration (mg/L)	
	Pro-Organic Buffer	Pro-Metal Buffer
Aldicarb	0.28	ND[a]
Colchicine	0.24	ND
Cyanide	0.25	0.025
Dicrotophos	0.14	1,400
Thallium sulfate	ND	240
Botulinum toxin	0.030	ND
Ricin	15	ND
Soman	ND	ND
VX	ND	ND

[a] ND = Significant inhibition was not detected.

enough light remained to detect inhibition due to contamination. Generally, the negative control sample for the pro-organic buffer generated about 500 light units; in contrast, the negative control in the pro-metal buffer typically would generate approximately 100,000 light units. However, even with the low number of light units, the positive control sample always had an inhibition of greater than 50% with respect to the negative control. At a minimum, to avoid the risk of false positive responses, reference samples very similar to the test samples need to be used. However, with inhibition to this extent, false positive responses are still possible. When using the pro-metal buffer, the inhibition due to the sample from the water system disinfected by chlorination was just 23% ± 15%. Therefore, there is potential for exaggerated inhibition, but only if dissimilar reference samples are used. Since testing, the vendor has determined that residual sodium thiosulfate from dechlorination may be the reason for the high inhibition in the unspiked DDW. At any rate, the vendor has altered the storage buffer to sustain a higher light level even in the presence of the disinfectant by-products.

The inhibition due to the sample from the water system disinfected by chloramination was -48% ± 20% when using the pro-metal buffer. This scenario introduces the possibility of a false negative response if a reference sample similar to the water sample is not used. Because the chloraminated water exhibited less inhibition than ASTM Type II DI water, a negative inhibition was calculated. If ASTM Type II DI water was used as the reference sample, and the chloraminated water sample was contaminated with cyanide or another contaminant that could cause approximately a 50% inhibition, that inhibition would be calculated to an approximately zero inhibition, therefore a false result. In this case, using a similar reference would solve the problem, but the possibility of false negative responses must be considered if ASTM Type II water has to be used as the reference.

A second type of false negative response was considered when a lethal dose of contaminant is present in the water sample and the inhibition is not at least 50%, the lower limit for a positive

response according to the vendor. Table 6-4 gives these results. Only soman and VX did not exhibit at least 50% inhibitions at the lethal concentration in at least one of the two buffers used by ToxScreen-II.

Table 6-4. False Negative Responses

Contaminant	Lethal Dose Concentration (mg/L)	False Negative Response	
		Inhibition > 50% with Pro-organic Buffer	Inhibition > 50% with Pro-metal Buffer
Aldicarb	280	no	yes
Colchicine	240	no	yes
Cyanide	250	no	no
Dicrotophos	1,400	no	yes
Thallium sulfate	2,400	yes	no
Botulinum toxin	0.30	no	yes
Ricin	15	no	yes
Soman	0.068[a]	yes	yes
VX	0.22	yes	yes

[a] 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.

6.4 Field Portability

A single concentration of cyanide was prepared and analyzed in replicate at a field location to examine the ability of ToxScreen-II to be used in a non-laboratory setting. ToxScreen-II and necessary accessories were conveniently transported to the field in the nylon duffle bag provided by the vendor, but not included as part of the test kit. Fully loaded, the bag weighed about five pounds. At the field location, the luminometer supplied with ToxScreen-II was operated with batteries on a small table in the basement of a house. Table 6-1c shows the results of the cyanide samples analyzed at the field location, along with the results of the cyanide samples analyzed in the laboratory. The concentration of the samples analyzed in the field was 0.25 mg/L. Using the pro-organic buffer, the inhibition produced in the field was 97% ± 1%, and the inhibition produced in the laboratory at the same concentration was 68% ± 3%. Using the pro-metal buffer, the inhibition produced in the field was 61% ± 6%, and the inhibition produced in the laboratory at the same concentration was 35% ± 7%. While these inhibitions are not the same, the field measurements were made on freshly prepared solutions with a newly reconstituted batch of bacteria, which had been incubated for three hours in the storage buffer. Therefore, some difference in inhibition should be expected. ToxScreen-II seemed to function similarly at the laboratory and non-laboratory locations.

The ToxScreen-II reagent must be kept at approximately -20°C prior to reconstitution and, once reconstituted and stored in storage buffer, can be maintained for up to one week at approximately 4°C. These factors could be problematic in a long-term field deployment.

6.5 Other Performance Factors

The step-by-step pictorial instruction manual for ToxScreen-II was easy to understand, which enabled operators to become quickly adept at analyzing multiple sample sets. ToxScreen-II was straightforward to operate. 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 using only the instruction manual. The operators analyzed 25 samples per hour.

Chapter 7
Performance Summary

Table 7-1 shows the performance verification results for the ToxScreen-II 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)[a]
			LD	LD/10	LD/100	LD/1,000	LD/10,000		
Contaminants in DDW	Aldicarb	280	69	38	58	11	NA[b]	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[c]	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[d]
	Botulinum toxin[e]	0.30	69	47	-15	-8	NA	14–23	0.030
	Ricin[f]	15	50	10	30	-10	NA	6–28	15
	Soman	0.068[g]	5	-1	-9	-17	NA	9–31	ND
	VX	0.22	-8	-5	-13	-59	NA	6–30	ND

	Interference	Conc. (mg/L)	Average Inhibitions at a Single Concentration (%)	Standard Deviation (%)
Potential interferents 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

False positive responses	False positive responses were observed for both chlorinated and chloraminated water samples. 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 chlorinated drinking water and 78% ± 11% for chloraminated drinking water), a false positive response in these matrices may occur. Residual sodium thiosulfate from dechlorination may have caused these responses. Storage buffer has been altered to increase light output in presence of disinfectant by-products.
False negative responses	The inhibition at the lethal concentration level was significantly larger than the negative control for all contaminants except VX, soman, and thallium sulfate.
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%. ToxScreen-II was transported to the field in a vinyl bag provided by the vendor, but 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, operators with little technical training would probably be able to successfully analyze sample sets. The operators analyzed approximately 25 samples per hour.

(a) See Tables 6-1a-I in the report for the precision around each individual inhibition result.
(b) NA = Data not collected at this concentration level for this contaminant.
(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) ND = Not detectable.
(e) Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
(f) Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
(g) 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.

Table 7-2 shows the performance verification results for the ToxScreen-II 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)[a]
			LD	LD/10	LD/100	LD/1,000	LD/10,000		
Contaminants in DDW	Aldicarb	280	-21	5	-17	-46	NA[b]	1–7	ND[c]
	Colchicine	240	-42	-72	-83	-79	4	5–12	ND
	Cyanide	250	99	48	80	35	31	1–13	0.025
		0.025[d]	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[e]	0.30	-44	1	-57	-97	NA	9–22	ND
	Ricin[f]	15	-89	-5	-93	-89	NA	12–74	ND
	Soman	0.068[g]	-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 interferents 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

False positive responses	The sample from the chlorinated water supply exhibited 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. Residual sodium thiosulfate from dechlorination may have caused these responses. Storage buffer has been altered to increase light output in presence of disinfectant by-products.
False negative responses	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 61% ± 6% and in the laboratory was 35% ± 7%. ToxScreen-II was transported to the field in a vinyl bag provided by the vendor, but 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, operators with little technical training would probably be able to successfully analyze sample sets. The operators analyzed approximately 25 samples per hour.

(a) See Tables 6-1a-I in the report for the precision around each individual inhibition result.
(b) NA = Data not collected at this concentration level for this contaminant.
(c) ND = Not detectable.
(d) 0.025 mg/L is not a lethal dose concentration, but it is used to describe additional concentrations of cyanide that were analyzed.
(e) Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
(f) 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.

Chapter 8
References

1. *Test/QA Plan for Verification of Rapid Toxicity Technologies,* Battelle, Columbus, Ohio, June 2003.

2. United States Environmental Protection Agency, *National Secondary Drinking Water Regulations: Guidance for Nuisance Chemicals,* EPA/810/K-92/001, July 1992.

3. U.S. EPA Method 531.1, "Measurement of n-Methylcarbamoyloximes and n-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization," in *Methods for the Determination of Organic Compounds in Drinking Water—Supplement III,* EPA/600/R-95/131, 1995.

4. U.S. EPA Method 335.1, "Cyanides, Amenable to Chlorination," in *Methods for the Chemical Analysis of Water and Wastes,* EPA/600/4-79/020, March 1983.

5. SW846 Method 8141A, "Organophosphorous Compounds by Gas Chromatography: Capillary Column Technique," Revision 1, September 1994.

6. U.S. EPA Method 200.8, "Determination of Trace Elements in Waters and Wastes by Inductively-Coupled Plasma Mass Spectrometry," in *Methods for the Determination of Organic Compounds in Drinking Water,* Supplement I, EPA/600/R-94/111, 1994.

7. U.S. EPA Method 180.1, "Turbidity (Nephelometric)," *Methods for the Determination of Inorganic Substances in Environmental Samples,* EPA/600/R-93/100, 1993.

8. American Public Health Association, et al. S*tandard Methods for the Examination of Water and Wastewater*. 19th Edition, 1997. Washington, DC.

9. U.S. EPA, *Methods for Chemical Analysis of Water and Wastes,* EPA/600/4-79/020.

10. U.S. EPA Method 524.2, "Purgeable Organic Compounds by Capillary Column GC/Mass Spectrometry," *Methods for the Determination of Organic Compounds in Drinking Water—Supplement III,* EPA/600/R-95/131.

11. U.S. EPA Method 552.2, “Haloacetic Acids and Dalapon by Liquid-Liquid Extraction, Derivatization and GC with Electron Capture Detector,” *Methods for the Determination of Organic Compounds in Drinking Water—Supplement III* EPA/600/R-95/131.
12. *Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center*, Version 4.0, U.S. EPA Environmental Technology Verification Program, Battelle, Columbus, Ohio, December 2002.