November 2003

Environmental Technology Verification Report

HACH COMPANY
TOXTRAK™
RAPID TOXICITY TESTING SYSTEM

Prepared by
Battelle

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EPA U.S. Environmental Protection Agency

ETV ETV ETV

November 2003

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

Hach Company
ToxTrak™
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.

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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
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 Hach Company ToxTrak™ 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 ToxTrak™. Following is a description of ToxTrak™, based on information provided by the vendor. The information provided below was not subjected to verification in this test.

ToxTrak™ is a colorimetric test based on resazurin dye chemistry. Resazurin is a redox-active dye that, when reduced, changes color from blue to pink. Resazurin is in the oxidized, blue state at the beginning of the test. The bacteria oxidize the glucose added to the sample with the dye and reduce the resazurin. The resazurin is first reduced by two electrons to resorufin, which is pink. Resorufin can be further reduced by two electrons to dihydroresorufin, which is colorless. Dihydroresorufin can be reoxidized by atmospheric oxygen to resorufin. To prevent interference, readings must be taken before a significant amount of resorufin has been reduced. This inhibition or acceleration of resazurin reduction is taken as an indication of toxicity in the test. Substances that are toxic to bacteria can inhibit their metabolism and thus inhibit the rate of resazurin reduction. If the reaction time is too long, the indicator is too far reduced and interference will result.

Figure 2-1. ToxTrak™ Rapid Toxicity Testing System

ToxTrak™ (Figure 2-1) uses an accelerant (gluteraldehyde) to reduce the reaction time, thus preventing oxygen interference and allowing the use of a lower level of inoculum to reduce the dye. Because of the decrease in turbidity resulting from a smaller inoculum, the absorbance of the dye can be read on a colorimeter or spectrophotometer without removing the bacterial cells from the light path. This alleviates the need for organic extraction and/or centrifugation. With the reduced turbidity, the color change of the dye can be distinguished visually. Also, the decreased reaction time eliminates the interference caused by overreduction of the dye.

ToxTrak™ works with different species of bacteria (including both Gram positive and Gram negative species) or mixed cultures. The ToxTrak™ kit includes 12 reusable sample cells with caps, several capsules of dried bacteria,

lauryl tryptose broth for culturing the bacteria, 50 ToxTrak™ Reagent Powder Pillows, 15 milliliters (mLs) of ToxTrak™ accelerator solution, 20 sterile transfer pipettes, a test tube rack, forceps, five germicidal cloths, a lab marker, illustrated instructions, and a carrying case.

The percent inhibition results are only a relative measurement. They do not represent a true quantitative measurement of toxic concentration. The percent inhibition does not necessarily increase in direct proportion to the concentration of contaminants. To determine the toxicity threshold of a toxin, it is possible to make tenfold dilutions of the sample and determine the percent inhibition for the dilutions until the sample is diluted sufficiently so that no inhibition is observed. Due to the many variables involved in the test, the limits of detection are on the order of 10% inhibition. Percent inhibition results more than 10% or more negative than -10% should be considered toxic. The percent inhibition results of several samples should be evaluated before determining whether or not a sample is toxic. Consistently detectable results indicate a high likelihood of toxicity.

For this verification test, the vendor provided a Hach DR/4000V spectrophotometer for the laboratory-based colorimeter measurements and a Hach DR/890 handheld colorimeter for the non-laboratory measurements. Any colorimeter that can analyze samples at a wavelength at or near 603 nanometers could be used in conjunction with the ToxTrak™ reagents. The ToxTrak™ kit costs $280, and reagent sets cost $100. The reagent set can be used with the test kit, a spectrophotometer, or a colorimeter. The spectrophotometer used in this verification test cost $3,950.

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, ToxTrak™ 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 ToxTrak™ 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] ToxTrak™ 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

ToxTrak™ was evaluated by

- Endpoint and precision—quantitative evaluation of the percent inhibition and precision for all concentration levels of contaminants and potential interfering compounds; also a qualitative evaluation of the presence or absence of each contaminant and potential interference at each concentration level
- 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 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

ToxTrak™ was used to analyze the DDW sample fortified with contaminants at concentrations ranging from lethal levels to concentrations 1,000 times 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 from four replicates of each contaminant at each concentration level were evaluated quantitatively to assess the ability of ToxTrak™ to detect toxicity at various concentrations of contaminants, as well as to measure the precision of ToxTrak™ results. Additionally, a qualitative evaluation of the data was performed to determine if the ToxTrak was able to indicate the presence of each contaminant at various concentration levels.

The response of ToxTrak™ 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, ToxTrak™ 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 four concentration levels (made using the DDW) were analyzed for each contaminant using ToxTrak™. 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

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]	21
	Positive control (formaldehyde)	5,000	24
	Negative control (unspiked DDW)	NS	38
DDW fortified with contaminants	Aldicarb	280; 28; 2.8; 0.28	4 per concentration level
	Colchicine	240; 24; 2.4; 0.24	4 per concentration level
	Cyanide	250; 25; 2.5; 0.25	4 per concentration level
	Dicrotophos	1,400; 140; 14; 1.4	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	0.15[d]; 0.015; 0.0015; 0.00015	4 per concentration level
	VX	0.22; 0.022; 0.0022; 0.00022	4 per concentration level
Field location	Cyanide	250	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 51% of the expected concentration of 0.30 mg/L.

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. Formaldehyde was suggested by the vendor for use as a positive control sample. While performance limits were not placed on the results, inhibition of at least 50% in response to this contaminant indicated to the operator that ToxTrak™ 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. 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 ToxTrak™ 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 ToxTrak™ 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 using the DDW in Class A volumetric glassware. 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 containing 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

In preparation for the analysis of test samples, a capsule containing dried bacteria was crushed at the bottom of a container filled with lauryl tryptose broth, and the solution was incubated at 35°C overnight. To analyze a test or control sample, 5 mL of sample, one ToxTrak™ Reagent Powder Pillow, two drops of accelerator solution, and 0.5 mL of the bacteria solution that had been incubated overnight were added to a sample cell. The cell was capped and mixed by shaking, and was then placed in the spectrometer for absorbance measurement. An initial measurement was made on all cells within the sample set. The spectrometer reported absorbances for each measurement. The absorbance of the control sample, unspiked DDW, was periodically measured. When its absorbance had decreased by 0.400 to 0.700 absorbance units, the absorbances of the rest of the test samples were measured a second time. The reaction time was 90 to 120 minutes.

For each contaminant, ToxTrak™ analyzed the lethal dose concentration and three additional concentration levels four times. Only one concentration of potential interference was analyzed. To test the field portability of ToxTrak™, a single concentration level of cyanide, prepared in the same way as the other DDW samples, was analyzed in replicate by ToxTrak™ 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 ToxTrak™. Both held bachelor's degrees in the sciences and spent approximately one hour with the vendor to become accustomed to performing tests using ToxTrak™ and the accompanying spectrometers.

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 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 3-3. 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]	0.15[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 51% of the expected concentration of 0.30 mg/L.

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 ToxTrak™ for approximately every 20 drinking water samples that were analyzed. These samples set a baseline response for a clean water matrix. A negative control sample (unspiked DDW) was analyzed with approximately every four samples. The inhibitions of the test samples were calculated with respect to the negative control samples analyzed within the same analysis set. Therefore, any inhibition significantly greater than zero was due to the contaminants and not the DDW matrix. 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 inhibition significantly greater than 50%, it would indicate to the operator that ToxTrak™ was operating incorrectly. In 27 positive control samples analyzed, the average inhibition was 80% with a standard deviation of 27%, indicating the proper functioning of ToxTrak™.

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 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

Initial and final absorbance readings were recorded from the spectrometer 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 background inhibition of the DDW when calculating the percent inhibition. Each test sample was compared to a negative control sample analyzed in the same set as the test sample. The percent inhibition was calculated using the following equation:

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

$$\Delta A = A_{Final} - A_{initial}$$

where A represents absorbance measurements made using the spectrometers.

The test/QA plan for this verification test[1] describes only a quantitative evaluation of the percent inhibition data generated by each technology. The ToxTrak™ manufacturer indicated during the review of this report that a qualitative data evaluation should also be performed to describe how a typical user is more likely to interpret and use the ToxTrak™ results. Specifically, the manufacturer suggested that the percent inhibition results for each concentration level of each contaminant also be evaluated as a qualitative indicator of whether or not a toxic contaminant is present. The manufacturer stated that the percent inhibition results for each contaminant do not necessarily increase linearly with the concentration of the contaminant but, depending on the contaminant, can at times be represented by a non-linear relationship that may exhibit parabolic functionality that increases in response, up to a certain concentration, but then begins to decrease.

Per the manufacturer's instructions, percent inhibition results greater than 10% or less than -10% are considered an indication of the presence of the contaminant. Results between and including -10% and 10% indicate the absence of toxic contaminants. The presence/absence data trend among the four replicates was evaluated to determine if ToxTrak™ consistently indicated the

presence (or absence) of the contaminants at the measured concentrations. Three out of four positive responses were required to indicate the presence of a contaminant at that concentration level. If two results were positive and two negative, the overall result was not considered a positive or a negative result.

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

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

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. A quantitative evaluation of precision was not appropriate for the qualitative results. However, reproducibility was shown, to a limited extent, by the requirement that three out of four replicates at a single concentration level must exhibit positive results for that concentration level to be identified as detectable.

5.2 Toxicity Threshold

The toxicity threshold was defined as the lowest concentration of contaminant to exhibit a percent inhibition significantly different from the negative control. For the quantitative results, the toxicity threshold was calculated as the concentration at which the results were significantly less than -10% or significantly greater than 10%, and the inhibition produced by each lower concentration level was significantly less than that produced by the toxicity threshold concentration. For the qualitative results, the toxicity threshold was defined as the lowest concentration level that exhibited percent inhibitions either more negative than -10% or more than 10% in at least three out of four replicates. For both the quantitative and qualitative results, the concentration levels higher than the toxicity threshold were required to meet their respective detectability requirements.

5.3 False Positive/Negative Responses

A response was considered false positive if an unspiked drinking water sample produced an inhibition significantly different from zero, as described above, 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. A qualitative result was considered false positive if the water

sample exhibited percent inhibitions results that were either less than -10% or more than 10% in at least 3 out of 4 replicates when analyzed with respect to ASTM Type II DI water.

A response was considered false negative when ToxTrak™ was subjected to a lethal concentration of some contaminant in the DDW and did not indicate significant inhibition as defined in Section 5.2 and significantly different from the other concentration levels analyzed. Requiring the inhibition of the lethal dose sample to be significantly greater than the negative control and the other concentration levels more thoroughly incorporated the uncertainty of all the measurements made by ToxTrak™ in determining a false negative response. A qualitative response was considered false negative if the lethal dose concentration level did not exhibit percent inhibitions that were either less than -10% or more than 10% in at least 3 out of 4 replicates.

5.4 Field Portability

The results obtained from the measurements made on DDW samples in the laboratory and in the field were compiled independently and compared to assess the performance of the ToxTrak™ 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 ToxTrak™ 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 calculated according to the test/QA plan. Samples that produced negative percent inhibition values indicated an increase in metabolism by the bacteria relative to the negative control. According to the vendor literature, any sample that causes inhibition significantly greater than 10% or more negative than -10% should be considered toxic. A qualitative interpretation of each replicate sample, although not planned, also is included, with a "+" sign, indicating the presence of a contaminant at that concentration level (i.e., percent inhibition results that are greater than 10% or less than -10%), or a "-" sign indicating the absence of a contaminant at that concentration level (i.e., percent inhibition results that are between -10% and 10%). The presence/absence data trend among the four replicates was evaluated to determine if ToxTrak™ consistently indicated the presence (or absence) of the contaminants at the measured concentrations. Three out of four positive responses were required to indicate the presence of a contaminant at that concentration level. If two results were positive and two negative, the overall result was not considered a positive or a negative result.

6.1.1 Contaminants

The contaminants that were analyzed by ToxTrak™ during this verification test produced results with a high degree of variability, making it difficult to quantitatively interpret the data. The only contaminant that met the requirements for quantitative detection as defined in Section 5.2 was cyanide. Cyanide was consistently detected at only the 250-mg/L level. The percent inhibitions of the two highest concentrations of thallium sulfate were significantly more negative than -10%, but these concentrations levels were not distinguishable from another. The rest of the contaminants were mostly not significantly more negative than -10% or significantly more positive than 10%.

A qualitative evaluation of the percent inhibition results revealed that the presence of a toxic contaminant was indicated for aldicarb at 2.8 and 280 mg/L, colchicine at 240 mg/L, cyanide at 25 and 250 mg/L, dicrotophos at 14, 140, and 1,400 mg/L, thallium sulfate and ricin at all four

Table 6-1a. Aldicarb Percent Inhibition Results

Concentration (mg/L)	Quantitative			Qualitative	
	Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/ Absence	Overall Result
0.28	7	-11	22	-	NC[a]
	3			-	
	-14			+	
	-41			+	
2.8	-3	12	24	-	+
	-13			+	
	25			+	
	39			+	
28	-3	-7	17	-	-
	-1			-	
	-32			+	
	8			-	
280 (Lethal Dose)	-11	-16	3	+	+
	-16			+	
	-17			+	
	-19			+	

[a] NC = Not consistently positive or negative.

Table 6-1b. Colchicine Percent Inhibition Results

Concentration (mg/L)	Quantitative			Qualitative	
	Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/ Absence	Overall Result
0.24	10	8	3	-	-
	11			+	
	7			-	
	4			-	
2.4	-8	-3	4	-	-
	-4			-	
	2			-	
	-5			-	
24	-15	8	24	+	NC[a]
	5			-	
	3			-	
	41			+	
240 (Lethal Dose)	11	14	5	+	+
	9			-	
	16			+	
	21			+	

[a] NC = Not consistently positive or negative.

Table 6-1c. Cyanide Percent Inhibition Results

Concentration (mg/L)	Quantitative			Qualitative	
	Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/ Absence	Overall Result
0.25	-33	-10	17	+	-
	-10			-	
	7			-	
	-5			-	
2.5	-16	-6	7	+	-
	-4			-	
	3			-	
	-6			-	
25	17	11	7	+	+
	0			-	
	14			+	
	12			+	
250 (Lethal Dose)	86	72	10	+	+
	65			+	
	65			+	
	71			+	
250[(a)] (Field Location)	76	83	11	+	+
	82			+	
	100			+	
	76			+	

[(a)] Measurements made by Hach DR890 colorimeter.

Table 6-1d. Dicrotophos Percent Inhibition Results

Concentration (mg/L)	Quantitative			Qualitative	
	Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/ Absence	Overall Result
1.4	-5	-12	14	-	NC[(a)]
	-33			+	
	-11			+	
	0			-	
14	-66	-37	28	+	+
	-50			+	
	-29			+	
	-2			-	
140	-22	-53	69	+	+
	-155			+	
	-22			+	
	-12			+	
1,400 (Lethal Dose)	-51	-60	82	+	+
	-175			+	
	-32			+	
	17			+	

[(a)] NC = Not consistently positive or negative.

Table 6-1e. Thallium Sulfate Percent Inhibition Results

Concentration (mg/L)	Quantitative			Qualitative	
	Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/Absence	Overall Result
2.4	-94	-38	44	+	+
	6			-	
	-12			+	
	-52			+	
24	-17	-21	22	+	+
	-36			+	
	9			-	
	-40			+	
240	-21	-37	22	+	+
	-16			+	
	-57			+	
	-55			+	
2,400 (Lethal Dose)	-26	-104	62	+	+
	-145			+	
	-83			+	
	-163			+	

Table 6-1f. Botulinum Toxin Percent Inhibition Results

Concentration (mg/L)	Quantitative			Qualitative	
	Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/ Absence	Overall Result
0.00030	17	18	15	+	+
	-3			-	
	25			+	
	31			+	
0.0030	3	6	14	-	-
	24			+	
	6			-	
	-10			-	
0.030	5	5	6	-	-
	12			+	
	7			-	
	-3			-	
0.30 (Lethal Dose)	5	10	16	-	NC[(a)]
	-9			-	
	17			+	
	29			+	

[(a)] NC = Not consistently positive or negative.

Table 6-1g. Ricin Percent Inhibition Results

Concentration (mg/L)	Quantitative			Qualitative	
	Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/ Absence	Overall Result
0.015	-32	-45	20	+	+
	-35			+	
	-37			+	
	-75			+	
0.15	-18	-33	27	+	+
	-13			+	
	-73			+	
	-28			+	
1.5	-22	-38	13	+	+
	-33			+	
	-44			+	
	-51			+	
15 (Lethal Dose)	-35	-32	11	+	+
	-43			+	
	-17			+	
	-34			+	

Table 6-1h. Soman Percent Inhibition Results

Concentration (mg/L)	Quantitative			Qualitative	
	Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/ Absence	Overall Result
0.00015	-5	-10	6	-	NC[a]
	-5			-	
	-15			+	
	-16			+	
0.0015	-21	-21	3	+	+
	-18			+	
	-23			+	
	-24			+	
0.015	-29	-24	6	+	+
	-28			+	
	-24			+	
	-16			+	
0.15[b] (Lethal Dose)	-1	-6	13	-	NC
	-20			+	
	-13			+	
	9			-	

[a] NC = Not consistently positive or negative.

[b] Due to the degradation of soman in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 51% of the expected concentration of 0.30 mg/L.

Table 6-1i. VX Percent Inhibition Results

Concentration (mg/L)	Quantitative			Qualitative	
	Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/ Absence	Overall Result
0.00022	7	9	9	-	-
	1			-	
	22			+	
	5			-	
0.0022	-17	-6	12	+	NC[(a)]
	10			-	
	-6			-	
	-13			+	
0.022	-3	-5	12	-	-
	2			-	
	4			-	
	-22			+	
0.22 (Lethal Dose)	-16	-16	8	+	+
	-24			+	
	-5			-	
	-19			+	

[(a)] NC = Not consistently positive or negative.

concentration levels, botulinum toxin at only the lowest concentration level (0.00030 mg/L), soman at 0.0015 and 0.015 mg/L, and VX at 0.22 mg/L. These qualitative results indicating the presence of a toxic contaminant did not consistently correlate with increasing concentration, which is a performance trend that is noted in the manufacturer's literature.

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 ToxTrak™. Quantitatively, aluminum, copper, zinc, and manganese exhibited percent inhibitions not significantly greater than 10% or significantly less than -10%, indicating little or no response to these compounds, while iron exhibited an inhibition of -36% ± 23%, indicating a slightly elevated response. Qualitative evaluation of these results revealed the presence of a toxic contaminant in response to only iron.

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 ToxTrak™ 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 ToxTrak™ to detect toxicity is dependent on the light production of the reagents in a

Table 6-2. Potential Interference Results

Compound	Concentration (mg/L)	Quantitative			Qualitative	
		Inhibition (%)	Average (%)	Standard Deviation (%)	Presence/ Absence	Overall Result
Aluminum	0.36	-17 -2 2 5	-3	10	+ - - -	-
Copper	0.65	-23 1 9 -11	-6	14	+ - - +	NC[a]
Iron	0.069	-37 -7 -38 -63	-36	23	+ - + +	+
Manganese	0.26	14 24 5 2	11	10	+ + - -	NC
Zinc	3.5	2 -19 -7 -43	-17	19	- + - +	NC
Chlorination by-products	NA[b]	[c] [d]	45 -7	14 13	NA	+ -
Chloramination by-products	NA	-14 3 -12 -23	-11	11	+ - + +	+

[a] NC = Not consistently positive or negative.
[b] NA = Not applicable.
[c] Chlorination by-product data from July.
[d] Chlorination by-product data from September.

clean drinking water matrix. Approximately half of the replicates of the water samples from the water system using chlorination were analyzed in July (with non-chem/bio agent contaminants) and half in September (with chem/bio agent contaminants). In July, this sample exhibited an average inhibition of 45% ± 14% and, in September, an average inhibition of -7% ± 13%. The reason for the difference in inhibition is not clear, given that the only difference in the analyses was the amount of time that had passed. The significantly positive inhibition measured in July indicates that the DDW could interfere with the ToxTrak™ results. For the water sample that uses chloramination as the disinfection process, the inhibition with respect to ASTM Type II DI water was -11% ± 11%, indicating no toxicity. No interference is likely when performing analyses in this drinking water matrix. Qualitative evaluation of these results revealed that the by-products of chlorination (July results) and chloramination are likely to interfere with the results from

ToxTrak™. The elevated response to these background samples indicates a potential for false results, according to both the qualitative and quantitative data evaluations.

6.1.3 Precision

Quantitatively, the standard deviations of the replicate samples were rather large. Out of 43 opportunities, in only 12 instances were the standard deviations of four replicates smaller than 10%. Standard deviations were as high as 82% and were often greater than 15%. Precision was not an appropriate parameter for qualitative evaluation of the data, but consistency of the reported results was interpreted as an overall positive or negative trend in Table 6-1 a-i above.

6.2 Toxicity Threshold

In Table 6-3, the quantitative toxicity threshold as defined in Section 5.2 is presented for each contaminant. The only contaminant meeting the quantitative definition was cyanide at a concentration level of 250 mg/L. For aldicarb, botulinum toxin, colchicine, dichrotophos, ricin, soman, thallium sulfate, and VX, requirements for detection were not met, regardless of the concentration level, indicating that the technology was not highly responsive to these contaminants.

Table 6-3. Toxicity Thresholds

	Toxicity Threshold Concentration (mg/L)	
Contaminant	**Quantitative Evaluation**	**Qualitative Evaluation**
Aldicarb	ND[(a)]	280
Colchicine	ND	240
Cyanide	250	25
Dicrotophos	ND	14
Thallium sulfate	ND	2.4
Botulinum toxin	ND	ND
Ricin	ND	0.015
Soman	ND	ND
VX	ND	0.22

[(a)] ND = Significant inhibition was not detected.

Table 6-3 also gives the qualitative toxicity thresholds as defined in Section 5.2 for each contaminant. ToxTrak™ indicated the presence of a toxic contaminant for botulinum toxin at 0.00030 mg/L and soman at 0.0015 mg/L, but these results were not considered to be the toxicity threshold because ToxTrak™ did not determine the higher concentration levels of those

contaminants to be toxic. ToxTrak™ detected the toxicity due to ricin at a concentration of 0.015 mg/L, indicating that ToxTrak™ was most sensitive to that contaminant.

6.3 False Positive/Negative Responses

In July, false positive responses were observed for unspiked drinking water from the system that uses chlorination as its disinfectant process. In September, that same sample was largely non-inhibitory. Therefore, there seems to be a risk of false positive responses for such samples, but it is not clear why these results were not consistent. The water sample treated by chloramination and then subsequently dechlorinated caused no detectable inhibition. These results were consistent for both quantitative and qualitative data evaluation.

A false negative response was when a lethal dose of contaminant was present in the water sample, and the inhibition was not significantly larger than 10% or more negative than -10% and was also significantly greater than the other lower concentration levels. Table 6-4 presents the quantitative interpretation of the false negative responses. The inhibition induced by the lethal dose of cyanide was detectable by ToxTrak™, while the other contaminants did not indicate significant inhibition, indicating false negative responses. Table 6-4 also presents the qualitative false negative responses. When evaluating the data qualitatively, only botulinum toxin and soman were considered not to be toxic contaminants at the lethal dose concentration level, and thus were considered false negative responses.

Table 6-4. False Negative Responses

Contaminant	Lethal Dose Concentration (mg/L)	False Negative Response	
		Quantitative Evaluation	Qualitative Evaluation
Aldicarb	280	yes	no
Colchicine	240	yes	no
Cyanide	250	no	no
Dicrotophos	1,400	yes	no
Thallium sulfate	2,400	yes	no
Botulinum toxin	0.30	yes	yes
Ricin	15	yes	no
Soman	0.15[(a)]	yes	yes
VX	0.22	yes	no

[(a)] Due to the degradation of soman in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 51% of the expected concentration of 0.30 mg/L.

6.4 Field Portability

The Hach DR890 colorimeter was used for the inhibition measurements at the field location. A single concentration of cyanide was prepared and analyzed in replicate to examine the ability of ToxTrak™ to be used in a non-laboratory setting. ToxTrak™ and necessary accessories were transported to the field in a medium-sized cardboard box because the carrying case was not provided by the vendor. At the field location, ToxTrak™ 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 solution analyzed in the field was 250 mg/L. From a qualitative perspective, all of the cyanide results at 250 mg/L produced both in the laboratory and in the field were positive responses. The inhibition produced in the field was 83% ± 11%, and the inhibition produced in the laboratory at the same concentration was 72% ± 10%. The overlapping results indicate that ToxTrak™ functioned similarly at the laboratory and non-laboratory locations. The ToxTrak™ reagent must be incubated overnight at 35°C prior to use. This could be problematic for field deployment. According to the manufacturer, bacteria lypholyzed in the tubes and ready for immediate deployment currently are available, but were not on the market when the verification test was conducted.

6.5 Other Performance Factors

The step-by-step pictorial instruction manual for ToxTrak™ was easy to understand, which enabled operators to become quickly adept at analyzing multiple sample sets. Although the operators had scientific backgrounds, based on observations of the verification test coordinator, operators with little technical training would probably be able to analyze samples using only the instruction manual for guidance. ToxTrak™ was very straightforward to operate. The operators analyzed approximately 25 samples per hour.

Chapter 7
Performance Summary

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Average Percent Inhibitions at Concentrations Relative to the LD Concentration (Qualitative Result: "+" = Present "-" = absent)				Range of Standard Deviations (%)	Toxicity Thresh. (mg/L)[a]	
			LD	LD/10	LD/100	LD/1,000		Quan.	Qual.
Contaminants in DDW	Aldicarb	280	-16 (+)	-7 (-)	12 (+)	-11 (NC)[b]	3–24	ND[c]	280
	Colchicine	240	14 (+)	8 (NC)	-3 (-)	8 (-)	3–24	ND	240
	Cyanide	250	72 (+)	11 (+)	-6 (-)	-10 (-)	7–17	250	25
	Dicrotophos	1,400	-60 (+)	-53 (+)	-37 (+)	-12 (NC)	14–82	ND	14
	Thallium sulfate	2,400	-104 (+)	-37 (+)	-21 (+)	-38 (+)	22–62	ND	2.4
	Botulinum toxin[d]	0.30	10 (NC)	5 (-)	6 (-)	18 (+)	6–16	ND	ND
	Ricin[e]	15	-32 (+)	-38 (+)	-33 (+)	-45 (+)	11–27	ND	0.015
	Soman	0.15[f]	-6 (NC)	-24 (+)	-21 (+)	-10 (NC)	3–13	ND	ND
	VX	0.22	-16 (+)	-5 (-)	-6 (NC)	9 (-)	8–12	ND	0.22
Potential interferences in DDW	**Interference**	**Conc. (mg/L)**	**Average Inhibitions at a Single Concentration (%)**				**Standard Deviation (%)**		
	Aluminum	0.36	-3 (-)				10		
	Copper	0.65	-6 (NC)				14		
	Iron	0.069	-36 (+)				23		
	Manganese	0.26	11 (NC)				10		
	Zinc	3.5	-17 (NC)				19		
False positive response	45% ± 14% inhibition in dechlorinated water from system disinfected by chlorination for samples analyzed in July. Samples analyzed in September were non-inhibitory. The water sample from a water system disinfected by chloramination was non-inhibitory (-11% ± 11%). Qualitative results were consistent with quantitative results (i.e., both interpretation methods indicated false positive responses with these matrices).								
False negative responses	According to the quantitative data interpretation, inhibition greater than the negative control was not detected for lethal doses of any contaminant except cyanide (i.e., all contaminants except for cyanide produced false negative results). According to the qualitative data interpretation, botulinum toxin and soman exhibited false negative results.								
Field portability	ToxTrak™ performance in the field was similar to its performance in the laboratory both quantitatively and qualitatively. The carrying case was not provided by the vendor. Hach DR890 handheld colorimeter was used for field measurements. Overnight incubation of bacteria may be inconvenient for field deployment.								
Other performance factors	Pictorial manual was useful, sample handling was easy, and sample throughput was approximately 25 samples per hour. Although the operators had scientific backgrounds, operators with little technical training would probably be able to analyze sample using only instruction manual as guide.								

[a] See Tables 6-1a-i in the report for the precision around each individual inhibition result.
[b] NC = Not consistently positive or negative.
[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 51% of the expected concentration of 0.30 mg/L.

Chapter 8
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