June 2006

Environmental Technology Verification Report

ABRAXIS
ABRATOX KIT

Prepared by
Battelle

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

ETV ETV ETV

June 2006

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

Abraxis
AbraTox Kit

by

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Notice

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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 six 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
cm	centimeter
DI	deionized water
DDW	dechlorinated drinking water from Columbus, Ohio
DPD	n,n-diethyl-p-phenylenediamine
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
HDPE	high-density polyethylene
LD	lethal dose
mM	millimolar
µL	microliter
mg/L	milligram per liter
mL	milliliter
mm	millimeter
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 Abraxis AbraTox Kit. Rapid toxicity technologies were identified as a priority 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 the kit. Following is a description of the AbraTox, based on information provided by the vendor. The information provided below was not verified in this test.

The AbraTox Kit (Figure 2-1) is an *in vitro* testing system that uses a naturally occurring and non-pathogenic bioluminescent bacteria *Vibrio fischeri* (strain NRRL-B-11177) to determine the toxicity of water-soluble samples. *Vibrio fischeri*, when properly grown, emits light as part of its metabolic pathway; the emitted light is an indication of the metabolic status of the bacterium. Differences in the amount of light produced can therefore be correlated to bacterial metabolism.

Figure 2-1. Abraxis AbraTox Kit

Toxic compounds interfere with the metabolic process, resulting in a reduction of light emission. The reduction of light emitted is proportional to the toxicity of the sample—the more toxic the sample, the greater percentage of light reduction.

The AbraTox *Vibrio fischeri* reagent vials are supplied freeze dried. To analyze the water samples, the vials are reconstituted with 2.5 milliliter (mL) of cold reconstitution solution and allowed to hydrate under refrigerated conditions for 30 minutes. Meanwhile, 800 micro-liters (µL) of the water sample to be analyzed are added to test cuvettes, followed by the addition of 100 µL of osmotic adjusting buffer, and allowed to incubate in the refrigerated incubation chamber for at least 15 minutes. Then, 100 µL of the diluted bacteria are added to a negative control and to each test sample and incubated in the refrigerated incubation chamber for 15 to 60 minutes. Luminescence is then measured using a portable luminometer. Significant changes in luminescence compared to the negative control (or reference sample) reflect the toxicity of a sample.

The AbraTox Kit contains six vials of freeze-dried bacteria, two vials of reconstitution solution, one bottle of Osmotic Adjusting Buffer, and one vial of positive and negative control. Test cuvettes, a repeater pipette (100 µL), and a 200 to 1,000 µL pipette and tips are required but not provided.

The box containing the AbraTox Kit has dimensions of 18 by 13 by 8 centimeters (cm). The AbraTox luminometer is 20 by 8 by 5 cm, uses 2 AA batteries, and weighs 0.3 kilograms. It can be integrated (although it was not during this test) with a personal computer for data acquisition, evaluation, and storage. The price of the AbraTox Kit (150 single tests) is $250, the luminometer is $2,000, and the incubation chamber is $250.

Chapter 3
Test Design

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.

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

This verification test was conducted from August to December 2005 according to procedures specified in the *Test/QA Plan for Verification of Rapid Toxicity Technologies* including Amendments 1 and 2.[(1)] The AbraTox Kit was verified by analyzing a dechlorinated drinking water sample from Columbus, Ohio (hereafter in this report referred to as DDW), fortified with various concentrations of the contaminants and interferences shown in Table 3-1. 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.

The AbraTox was evaluated by

- Endpoints and precision—percent inhibition for all concentration levels of contaminants and potential interfering compounds and precision of replicate analyses

- Toxicity threshold for each contaminant—contaminant level at which higher concentrations generate inhibition significantly greater than the negative control and lower concentrations do not

Table 3-1. Contaminants and Potential Interferences

Category	Contaminant
Biological toxins	Botulinum toxin complex B, ricin
Botanical pesticide	Nicotine
Carbamate pesticide	Aldicarb
Industrial chemical	Cyanide
Nerve agents	Soman, VX
Organophosphate pesticide	Dicrotophos
Pharmaceutical	Colchicine
Potential interferences	Aluminum, copper, iron, manganese, zinc, chloramination by-products, and chlorination by-products
Rodenticide	Thallium sulfate

- False positive responses—chlorination and chloramination by-product inhibition with respect to unspiked American Society for Testing and Materials (ASTM) Type II deionized (DI) water samples

- False negative responses—contaminants that were reported as producing inhibition similar to the negative control when present at lethal concentrations or negative inhibition that could cause falsely low inhibition

- Other performance factors (sample throughput, ease of use, reliability).

The AbraTox Kit was used to analyze the DDW samples fortified with contaminants at concentrations ranging from lethal levels to concentrations up to 100,000 times less than the lethal dose. The lethal dose of each contaminant was determined by calculating the concentration at which 250 milliliters (mL) of water would probably cause the death of a 154-pound person. These calculations were based on toxicological data available for each contaminant that are presented in Amendment 2 of the test/QA plan.[1] Inhibition results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the AbraTox to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the AbraTox results.

The response of the AbraTox Kit 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 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 was obtained from the Metropolitan Water District of Southern California (LaVerne, California), which uses chloramination as its disinfection process. The samples were analyzed after residual chlorine was removed using sodium thiosulfate. Sample throughput was measured based on the

number of samples analyzed per hour. Ease of use and reliability were determined based on documented observations of the operators.

3.1 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. The DDW containing the potential interferences was analyzed at a single concentration level, while at least four dilutions were analyzed for each contaminant using the AbraTox Kit. Mixtures of contaminants and possible interfering compounds were not analyzed.

3.1.1 Quality Control Samples

QC samples included method blanks, positive controls, negative controls, and preservative blanks. The method blank samples consisted of ASTM Type II DI water and were used to ensure that no sources of contamination were introduced in the sample handling and analysis procedures. A positive control sample was included in the AbraTox Kit and was used as provided from the vendor. While performance limits were not placed on the results, significant inhibition for the positive control sample indicated to the operator that the AbraTox Kit was functioning properly. Two negative control samples were included. One was provided by the vendor. The second consisted of unspiked DDW and was used to set a background inhibition of the DDW, the matrix in which each test sample was prepared. To ensure that the preservatives in the contaminant solutions did not have an inhibitory effect, preservative blank samples were prepared. These preservative blanks consisted of DDW fortified with a concentration of preservative equivalent to that in the test solutions of botulinum toxin complex B, ricin, soman, and VX.

3.1.2 Drinking Water Fortified with Contaminants

Approximately 50 liters of Columbus, Ohio, tap water were collected in a low-density polyethylene container. The water was dechlorinated with sodium thiosulfate. Dechlorination was confirmed by adding an n,n-diethyl-p-phenylenediamine (DPD) tablet to a 10-mL aliquot of the water. Lack of color development in the presence of DPD indicated that the water was dechlorinated. All subsequent test samples were prepared from this DDW.

A stock solution of each contaminant was prepared in DDW at concentrations at or above the lethal dose level. The stock solution was further diluted 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. Additional concentrations of some contaminants were prepared and analyzed for two reasons: one was because of the large difference in response between two concentration levels. For example, if only one dilution level was almost completely inhibitory and the next dilution level was non-inhibitory, several intermediate concentrations were analyzed to better determine the toxicity threshold of that contaminant. The other reason was because sometimes the lowest concentration analyzed was mostly inhibitory,

thus, not providing even an estimate of the toxicity threshold. For these contaminants, additional tenfold dilutions were analyzed to more accurately determine the toxicity threshold. Table 3-2 lists each concentration level and the number of samples analyzed at each level.

3.1.3 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 the AbraTox Kit 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 manner, by-products of the chloramination process were evaluated using a water sample from the Metropolitan Water District of Southern California. 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.

3.2 Test Procedure

The procedures for preparing, storing, and analyzing test samples and confirming stock solutions are provided below.

3.2.1 Test Sample Preparation and Storage

A drinking water sample was collected as described in Section 3.1.2 and, because free chlorine kills the bacteria within the AbraTox reagent and can degrade the contaminants during storage, was immediately dechlorinated with sodium thiosulfate. Dechlorination of the water sample was qualitatively confirmed by adding a DPD tablet to a 10-mL aliquot of the DDW. All the contaminant samples, potential interference samples, preservative blanks, and negative control QC samples were made from this water sample, while the method blank sample was prepared from ASTM Type II DI water. The positive and negative control samples included in the AbraTox Kit were used as provided. All QC samples were prepared prior to the start of testing and stored at room temperature. The stability of each contaminant for which analytical methods are available was confirmed by analyzing it three times over a two-week period. Throughout this time, each contaminant maintained its original concentration to within approximately 25%. Therefore, the aliquots of DDW containing the contaminants were prepared within two weeks of testing and were stored at room temperature without chemical preservation. The contaminants without analytical methods were analyzed within 48 hours of their preparation. To maintain the integrity of the test, test samples provided to the operators were labeled only with sample identification numbers so that the operators did not know their content.

3.2.2 Test Sample Analysis Procedure

To analyze the test samples, 800 µL of the test sample were added to a sample cuvette. Then, 100 µL of osmotic adjusting buffer were pipetted into all cuvettes and mixed well. The cuvettes were incubated at 15°C for 30 minutes. As soon as the sample cuvettes were set aside for incubation, the bioluminescent bacteria *Vibrio fischeri* (strain NRRL-B-11177) were

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

Type of Sample	Sample Characteristics	Concentration Levels	No. of Sample Analyses
Quality control	Method blank (ASTM Type II water)	NA	15
	Positive control (distilled water)	Used as provided by the vendor	15
	Negative control (25% methanol)	Used as provided by the vendor	15
	Negative control (unspiked DDW)	NA	60
	Preservative blank: botulinum toxin complex B	0.015 millimolar (mM) sodium citrate	4
	Preservative blank: VX and soman	0.21% isopropyl alcohol	4 with VX, 4 with soman
	Preservative blank: ricin	0.00024% NaN_3, 0.00045 molar NaCl, 0.03mM phosphate	4
DDW fortified with contaminants	Aldicarb	260; 26; 2.6; 0.26 milligrams/liter (mg/L)	4 per concentration level
	Botulinum toxin complex B	0.3; 0.03; 0.003; 0.0003 mg/L	4 per concentration level
	Colchicine	240; 24; 2.4; 0.24; 0.024; 0.0024 mg/L	4 per concentration level
	Cyanide	250; 25; 2.5; 0.25 mg/L	4 per concentration level
	Dicrotophos	1,400; 140; 14; 1.4; mg/L	4 per concentration level
	Nicotine	2,800; 2,100; 1,400; 700; 280; 28; 2.8 mg/L	4 per concentration level
	Ricin	15; 1.5; 0.15; 0.015 mg/L	4 per concentration level
	Soman	1.4; 0.14; 0.014; 0.0014 mg/L	4 per concentration level
	Thallium sulfate	2,800; 280; 28; 2.8 mg/L	4 per concentration level
	VX	2.0; 0.2; 0.02; 0.002 mg/L	4 per concentration level
DDW fortified with potential interferences	Aluminum	0.5 mg/L	4
	Copper	0.6 mg/L	4
	Iron	0.15 mg/L	4
	Manganese	0.25 mg/L	4
	Zinc	2.5 mg/L	4
Disinfectant by-products	Chloramination by-products	NA	4
	Chlorination by-products	NA	60

NA = not applicable, samples not fortified with any preservative, contaminant, or potential interference.

reconstituted with reconstitution solution and equilibrated at 4°C for 30 minutes. After the 30-minute incubation period, 100 µL of the reconstituted bacteria were added to the sample cuvettes. After the bacteria were added, the samples were incubated for another 30 min at 4ºC, and the luminescence was measured and recorded. The luminescence was compared with that of the negative control to determine percent inhibition. The bacteria were prepared the day of use for all tests.

For each contaminant, a minimum of the lethal dose concentration and three additional concentration levels were analyzed four times using the AbraTox Kit. Only one concentration of each potential interference was analyzed four times. The luminescence was recorded, and the percent inhibition was calculated for each sample. Two operators performed all the analyses using the AbraTox Kit. One operator performed testing with contaminants that did not require special chemical and biological agent training and one performed testing with those that did. Both held bachelor's degrees in the sciences and were trained by the vendor to operate the AbraTox Kit.

3.2.3 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 complex B—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 concentration 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 analyzing 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 alkalinity; dissolved organic carbon content; specific conductivity; hardness; pH; concentration of haloacetic acids, total organic carbon, total organic halides, and trihalomethanes; and turbidity. 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 at the Metropolitan Water District of Southern California, representing a water system using chloramination for disinfection.

Table 3-3. Stock Solution Confirmation Results

	Method	Average Concentration ± Standard Deviation N = 4 (mg/L)[(b)]	Background in DDW (mg/L)
Contaminant			
Aldicarb	Battelle method	260 ± 7	<0.005
Botulinum toxin complex B	[(a)]	NA	NA
Colchicine	[(a)]	NA	NA
Cyanide	EPA 335.3[(3)]	249 ± 4 296 ± 26 (field portability)	0.006
Dicrotophos	Battelle method	1,168 ± 18	<3.0
Nicotine	Battelle method	2,837 ± 27	<0.01
Ricin	[(a)]	NA	NA
Soman	Battelle method	1.3 ± 0.1 (10/18/05) 1.16 ± 0.06 (10/21/05	<0.025
Thallium sulfate	EPA 200.8[(4)]	2,469 ± 31	<0.001
VX	Battelle method	1.89 ± 0.08 (10/17/05) 1.77 ± 0.03 (10/20/05)	<0.0005
Potential Interference			
Aluminum	EPA 200.7[(5)]	0.50 ± 0.02	<0.2
Copper	EPA 200.7[(5)]	0.60 ± 0.03	<0.02
Iron	EPA 200.7[(5)]	0.155 ± 0.006	<0.04
Manganese	EPA 200.7[(5)]	0.281 ± 0.008	<0.01
Zinc	EPA 200.7[(5)]	2.63 ± 0.05	0.27

NA = Not applicable.
[(a)] No standard method available. QA audits and balance calibration assured accurately prepared solutions.
[(b)] Target concentration was highest concentration for each contaminant or interference on Table 3-2.

Table 3-4. Water Quality Parameters

Parameter	Method	Dechlorinated Columbus, Ohio, Tap Water (disinfected by chlorination)	Dechlorinated Southern California Tap Water (disinfected by chloramination)
Alkalinity (mg/L)	SM 2320 B[6]	40	71
Specific conductivity (μmho)	SM 2510 B[6]	572	807
Hardness (mg/L)	EPA 130.2[7]	118	192
pH	EPA 150.1[7]	7.6	8.0
Total haloacetic acids (μg/L)	EPA 552.2[8]	32.8	17.4
Dissolved organic carbon (mg/L)	SM 5310 B[6]	2.1	2.9
Total organic carbon (mg/L)	SM 5310 B[6]	2.1	2.5
Total organic halides (μg/L)	SM 5320B[6]	220	170
Total trihalomethanes (μg/L)	EPA 524.2[9]	74.9	39.2
Turbidity (NTU)	SM 2130 [10]	0.1	0.1

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[11] and the test/QA plan for this verification test.[1]

4.1 Quality Control of Stock Solution Confirmation Methods

The stock solutions for the contaminants cyanide and thallium sulfate and for the potential interferences aluminum, magnesium, zinc, iron, and copper were analyzed at ATEL using a standard reference method. 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, soman, aldicarb, nicotine, and dicrotophos, the confirmation analyses were performed at Battelle using a Battelle SOP or method. Calibration standard recoveries of VX and soman were always between 62% and 141%, and most of the time were between 90% and 120%. Dicrotophos standard recoveries ranged from 89% to 122%. Aldicarb standard recoveries ranged from 95% to120%. Nicotine standard recoveries ranged from 96% to 99%. Standard analytical methods for colchicine, ricin, and botulinum toxin complex B were not available and, therefore, 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 the AbraTox Kit for approximately every 20 drinking water samples that were analyzed. Because inhibition has to be calculated with respect to a control sample, none were calculated for the method blank samples. The method blanks were used as the control for calculating the inhibition of the DDW for the disinfecting by-product evaluation. A positive control sample was provided by the vendor and 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 significant inhibition, it would indicate to the operator that the AbraTox Kit was not functioning properly. For 15 positive control samples, an average inhibition of 98% ± 2% was measured. These inhibition values

indicated the proper functioning of the AbraTox Kit. A negative control sample (unspiked DDW) was analyzed with approximately every four samples. The percent inhibition calculation for each sample incorporated the average inhibition of the negative control samples analyzed with that particular sample set; therefore, by definition, the average inhibition of four negative control samples was 0%. A negative control supplied by the vendor was analyzed with approximately every 20 samples. This negative control provided luminescence readings similar to the method blank and DDW negative control.

4.3 Audits

A performance evaluation (PE) audit, a technical systems audit (TSA), and an audit of data quality were performed for this verification test.

4.3.1 Performance Evaluation Audit

The accuracy of the reference method used to confirm the concentration of the stock solutions of the contaminants and potential interferences was confirmed by analyzing solutions of each analyte from two separate commercial vendors. The standards from one source were used to prepare the stock solutions during the verification test, while the standards from a second source were analyzed as the PE sample. The percent difference (%D) between the measured concentration of the PE sample, and the nominal 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 nominal concentration, and A is the nominal concentration. The %D between the measured concentration of the PE standard and the nominal 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.

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; therefore, PE audits were not performed for all of the contaminants. To assure the purity of the other standards, documentation, such as certificates of analysis, was obtained for colchicine, botulinum toxin complex B, and ricin. In the cases 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 TSA to ensure that the verification test was performed in accordance with the test/QA plan[(1)] and the AMS Center QMP.[(11)] 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

Table 4-1. Summary of Performance Evaluation Audit

		Measured Concentration (mg/L)	Nominal Concentration (mg/L)	%D
Contaminant	Aldicarb	0.057	0.050	14
	Cyanide	1,025	1,000	3
	Dicrotophos	1.10	1.00	10
	Nicotine	0.120	0.100	20
	Thallium	1,010	1,000	1
Potential interference	Aluminum	960	1,000	4
	Copper	1,000	1,000	0
	Iron	960	1,000	4
	Manganese	922	1,000	8
	Zinc	1,100	1,000	10

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.

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.[11] 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 they 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 signature or 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.

5.1 Endpoints and Precision

The luminometer provided with the AbraTox Kit reported the absolute light units for each sample analyzed. Each test sample 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 four negative control samples within each sample set always averaged zero. The percent inhibition for each sample was calculated using the following equation:

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

Where L_{sample} is the absolute light units generated by each test sample, and $\overline{L}_{\text{negative control}}$ is the average number of light units produced across the four negative control samples analyzed in the same sample set as the subject test sample. For this test, the negative 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 (*SD*) of the results for the replicate samples was calculated, as follows, and used as a measure of technology precision at each concentration. The standard deviation around the average negative control results represented the variability of the inhibition caused by the negative control water. Similarly, the standard deviation of the rest of the contaminant concentrations represented the precision of the inhibition caused by the background water combined with the contaminant.

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

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 inhibition was 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 of the percent inhibition so the reader could easily view the uncertainty around the average percent inhibition 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. A significant difference in the inhibition at two concentration levels required that the average inhibition at each concentration level, plus or minus its respective standard deviation, did not overlap.

5.3 False Positive/Negative Responses

A response was considered false positive if an unspiked drinking water sample produced an inhibition significantly greater than zero when determined with respect to DI water. Depending on the degree of inhibition in the sample, toxicity from subsequent contamination of that sample may not be detectable or could be exaggerated as a result of the baseline inhibition. 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 overlap with the zero inhibition plus or minus the standard deviation.

A response was considered false negative when the AbraTox Kit, subjected to a lethal concentration of some contaminant in the DDW, did not indicate inhibition significantly greater than the negative control (zero inhibition) and the other concentration levels analyzed (for lethal dose inhibition less than 100%). The inhibition of the lethal dose sample was required to be significantly greater than the other concentration levels because it more thoroughly incorporated the uncertainty of all the measurements made by the AbraTox Kit in determining false negative results. 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. In addition, background water samples that increased the light production of the AbraTox Kit organisms (i.e., negative inhibition) were considered false negative because such samples could cancel out the effect of a contaminant that inhibits light production, making it seem that the contaminant had no toxic effect.

5.4 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 documented 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-l present the percent inhibition data for 10 contaminants; and Table 6-2 gives the percent inhibition data for preservatives with a concentration similar to what would be contained in a lethal dose of botulinum toxin complex B, ricin, soman, and VX. 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. Contaminant test samples that produced negative percent inhibition values indicated an increase in light production by the bacteria and were considered non-toxic.

6.1.1 Contaminants

All the contaminants except botulinum toxin complex B, ricin, and VX exhibited some inhibition that was significantly different from the negative control. Aldicarb and thallium sulfate generated detectable inhibition at the two highest concentration levels analyzed, while colchicine generated inhibition only at the lethal dose concentration. Dicrotophos generated detectable inhibition at the lethal dose concentration and at the thousandfold dilution level, but, considering the rather low inhibition at the two intermediate concentrations, the significance of the inhibition at the lower concentration is questionable. Upon initial analysis, nicotine generated detectable inhibition at concentrations only at the lethal dose. Additional dilutions were done to better determine the toxicity threshold of nicotine. During the additional dilutions, the degree of inhibition changed in the lethal dose sample from 43% ± 2% initially to 90% ± 5% and inhibition was determined to be detectable down to 700 mg/L. The AbraTox Kit was especially sensitive to cyanide. Inhibition at the highest three concentration levels (250, 25, and 2.5 mg/L) was significantly different from the negative control. The 0.25-mg/L concentration level generated an inhibition of 25% ± 20%, which, while a positive inhibition, was not significantly different from the negative control because of the uncertainty around the average inhibition of the negative control.

Table 6-1a. Aldicarb Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	8	0	6
	-1		
	-3		
	-5		
0.26	0	12	8
	15		
	17		
	16		
2.6	12	-3	12
	1		
	-7		
	-17		
26	33	21	12
	23		
	24		
	4		
260 (Lethal Dose)	65	63	2
	62		
	60		
	63		

Table 6-1b. Botulinum Toxin Complex B Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-40	0	27
	13		
	9		
	18		
0.0003	-28	-2	19
	8		
	-3		
	16		
0.003	-23	-40	25
	-24		
	-37		
	-77		
0.03	14	-11	34
	5		
	-3		
	-61		
0.3 (Lethal Dose)	13	-10	23
	-3		
	-11		
	-42		
Lethal Dose Preservative Blank	-66	-30	24
	-27		
	-16		
	-13		
0.3 Lethal Dose	34	15	18
	21		
	15		
	-9		
Lethal Dose Preservative Blank	-27	0	19
	3		
	11		
	13		

Shading indicates that inhibition results were calculated with respect to the preservative blank.

Table 6-1c. Colchicine Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	9	0	7
	2		
	-8		
	-3		
0.0024	-14	-20	6
	-17		
	-23		
	-27		
0.024	-25	-25	2
	-29		
	-23		
	-24		
0.24	-45	-51	9
	-45		
	-53		
	-63		
2.4	11	2	7
	3		
	-5		
	-1		
24	-35	-39	7
	-38		
	-49		
	-33		
240 (Lethal Dose)	16	17	2
	14		
	17		
	19		

Table 6-1d. Cyanide Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-31	0	22
	0		
	13		
	18		
0.25	38	25	20
	36		
	29		
	-4		
2.5	48	43	9
	48		
	47		
	30		
25	73	71	3
	74		
	72		
	66		
250 (Lethal Dose)	79	82	5
	80		
	79		
	89		
Field Portability Negative Control	-1	0	5
	4		
	4		
	-7		
Field Portability 250	73	76	2
	78		
	77		
	75		

Table 6-1e. Dicrotophos Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	2	0	3
	3		
	-1		
	-4		
1.4	34	26	9
	29		
	27		
	13		
14	-4	14	12
	21		
	20		
	20		
140	18	10	12
	16		
	13		
	-7		
1,400 (Lethal Dose)	47	42	8
	47		
	45		
	31		

Table 6-1f. Nicotine Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	4	0	6
	5		
	-1		
	-8		
2.8	6	4	4
	-1		
	8		
	3		
28	-4	-2	4
	-5		
	-1		
	4		
280	-3	-3	7
	-13		
	-2		
	5		
2,800 (Lethal Dose)	43	43	2
	42		
	41		
	46		

Table 6-1g. Nicotine Percent Inhibition Results—Additional Dilutions

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-5	0	9
	11		
	2		
	-9		
280	9	10	4
	11		
	5		
	14		
700	68	37	21
	27		
	27		
	25		
1,400	59	60	2
	64		
	59		
	59		
2,100	95	96	4
	100		
	98		
	91		
2,800	89	90	5
	89		
	86		
	98		

Table 6-1h. Ricin Percent Inhibition (Compared to Negative Control)

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-40	0	27
	13		
	9		
	18		
0.015	-68	-41	22
	-50		
	-30		
	-18		
Lethal Dose/1,000 Preservative Blank	-108	-52	41
	-55		
	-30		
	-16		
0.15	-14	-17	8
	-24		
	-7		
	-24		
Lethal Dose/100 Preservative Blank	-18	-16	4
	-10		
	-17		
	-20		
1.5	-1	-8	6
	-15		
	-6		
	-11		
Lethal Dose/10 Preservative Blank	-35	-17	12
	-10		
	-15		
	-8		
15	-58	-29	25
	-42		
	-12		
	-4		
Lethal Dose Preservative Blank	-51	-45	4
	-42		
	-45		
	-43		

Table 6-1i. Ricin Percent Inhibition Results (Compared to Preservative Blank)

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.015	-10	7	15
	1		
	15		
	23		
Lethal Dose/1,000 Preservative Blank	-37	0	27
	-2		
	15		
	24		
0.15	2	-1	7
	-7		
	8		
	-7		
Lethal Dose/100 Preservative Blank	-1	0	3
	5		
	-1		
	-3		
1.5	14	8	5
	2		
	9		
	5		
Lethal Dose/10 Preservative Blank	-15	0	11
	6		
	2		
	7		
15 (Lethal Dose)	-9	11	17
	2		
	23		
	29		
Lethal Dose Preservative Blank	-4	0	3
	2		
	0		
	2		

Each concentration level is shown directly above the preservative blank containing an equivalent amount of preservatives. The inhibition of each pair is calculated with respect to each preservative blank.

Table 6-1j. Soman Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-9	0	6
	-1		
	5		
	4		
0.0014	-12	-1	13
	-4		
	-4		
	18		
0.014	-11	-10	4
	-9		
	-6		
	-15		
0.14	-22	-24	7
	-31		
	-15		
	-29		
1.4 (Lethal Dose)	8	4	8
	10		
	-8		
	4		
Lethal Dose Preservative Blank	-50	-40	13
	-43		
	-45		
	-21		
1.4 (Lethal Dose)	34	31	6
	36		
	23		
	31		
Lethal Dose Preservative Blank	-8	0	9
	-4		
	-2		
	14		

Shaded inhibition calculated with respect to the preservative blank.

Table 6-1k. Thallium Sulfate Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	6	0	6
	1		
	1		
	-8		
2.8	6	6	1
	6		
	5		
	7		
28	-1	5	5
	2		
	10		
	9		
280	23	20	3
	18		
	17		
	21		
2,800 (Lethal Dose)	23	14	7
	6		
	14		
	11		

Table 6-1l. VX Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	8	0	8
	-4		
	5		
	-9		
0.002	-5	-7	6
	-13		
	-12		
	1		
0.02	-6	2	11
	-8		
	8		
	15		
0.2	-38	-15	21
	-24		
	-4		
	8		
2 (Lethal Dose)	-30	-32	6
	-30		
	-41		
	-27		
Lethal Dose Preservative Blank	-17	-9	7
	-7		
	-1		
	-11		

Table 6-2. Lethal Dose Level Preservative Blank Percent Inhibition Results

Preservative Blank	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative Control	-29	0	23
	-7		
	23		
	13		
Ricin	29	21	5
	17		
	19		
	21		
Soman/VX[a]	(b)	-4	14
	-18		
	-3		
	10		
Botulinum Toxin Complex B	-9	-18	14
	-4		
	-25		
	-33		

[a] Soman and VX use the same preservative.
[b] Removed -98% as an outlier.

It is important to note that the botulinum toxin complex B, ricin, soman, and VX stock solutions used to prepare the test samples were stored in various preservatives that included sodium azide, sodium chloride, and sodium phosphate for ricin; sodium citrate only for botulinum toxin complex B, and isopropyl alcohol for soman and VX. During the previous ETV test of this technology category, the preservatives were not accounted for in the negative control; therefore, the results from each test should be interpreted accordingly. The results for this test are more thorough because they show the sensitivity (or lack thereof) to both the preservative and the contaminant. In the in the earlier verification test, toxicity could have been the result of either. Table 3-2 details the concentrations of preservatives in the lethal dose samples of each contaminant. These data could be evaluated in two ways to determine the sensitivity of the AbraTox Kit to contaminants stored in preservatives. The first approach would be to determine the inhibition of the test samples containing preservatives with respect to the background negative control, as was the case for the contaminants that were not stored in preservatives. This technique, however, could indicate that AbraTox Kit was sensitive to the contaminant when, in fact, it was sensitive to one of the preservatives. Since these contaminants are only available (either commercially or from the government) in aqueous formulations with the preservatives, this may be appropriate. The second approach would be to fortify negative control samples with the same concentrations of preservative contained in all the samples so that the inhibition resulting from the preservatives could be subtracted from the inhibition caused by the contaminant. This approach would greatly increase the number of samples required for analysis. Therefore, for this test, aspects of both approaches were incorporated without substantially increasing the number of samples. Negative control samples fortified with a concentration of each preservative equivalent to the concentration in the lethal dose test samples (preservative blanks) were analyzed prior to analyzing any test samples. For those sets of test samples for which it was especially difficult to determine whether inhibitory effects were from the contaminant or the preservative, the preservative blank was diluted identically to all the

contaminant samples and analyzed with them so a background subtraction could take place if necessary.

During the initial analysis of the preservative blanks (Table 6-2), the only sample that generated inhibition significantly different from the unfortified negative controls was the sample representing the ricin preservative, with an inhibition of 21% ± 5%. Subsequently, for the ricin samples, all of the preservative blanks were diluted with the same concentration of preservatives as the test samples containing ricin. For the other contaminant tests, only the preservative blank containing preservatives equivalent to those in the lethal dose sample were analyzed with the contaminant samples.

The inhibition of the botulinum toxin complex B test samples was not significantly different from the negative control. In addition, the lethal dose preservative blank was not significantly different from the negative control. However, the average inhibition of the preservative blank was somewhat more negative (-30% ± 24%) than when it was analyzed prior to analysis of the contaminant samples (-18 ± 14%) (Table 6-2). Because of this, the inhibition of the lethal dose contaminant solution was also calculated with respect to the preservative blank. Calculated in this way, the inhibition of the lethal dose of the contaminant solution was 15% ± 18% , which was not significantly different compared to the preservative blank (0% ± 19%) when calculated with respect to itself. Because the highest concentration of botulinum toxin complex B analyzed was not significantly different from the preservative blank, it is unlikely that the lower concentrations would be affected by testing against dilutions of the preservative blank. Therefore, no additional dilutions of the preservative blanks were analyzed.

As mentioned above, the ricin preservative blank generated a detectable inhibition prior to the contaminant analysis and equivalent dilutions of the preservative blank samples were analyzed with the ricin samples. The inhibition of each ricin sample was calculated with respect to the preservative blank of the appropriate concentration. As Table 6-1i shows, the ricin sample inhibition was not significantly different from the corresponding preservative blank. When analyzed with the contaminant samples, the inhibition of the lethal dose level preservative blank was -45% ± 4%, compared with 21% ± 5% during its initial analysis (see Table 6-2). There is no explanation for this result. Nonetheless, because of the rather large uncertainty in the measurements, even when the inhibition of the contaminant samples was calculated with respect to the negative control (Table 6-1h) rather than with respect to their preservative blanks, the inhibition of the ricin samples was not detectable.

For soman, the preservative blank analyzed prior to the contaminant samples yielded an inhibition of -4% ± 14% (Table 6-2)—not significantly different from that of the negative control. Thus, dilutions of the preservative blank were not analyzed with the contaminants and the contaminant inhibition was calculated only with respect to the negative control. None of the soman samples exhibited inhibition significantly different from the negative control. However, the lethal dose preservative blank that was analyzed with the contaminant samples exhibited a negative inhibition that was significantly different from both the negative control and the preservative blank analyzed prior to the contaminant samples. When the inhibition of the lethal dose solution of soman was calculated with respect to the preservative blank, the inhibition was 31% ± 6%—a slight inhibition. Because of the rather modest inhibition at the lethal dose concentration and the inconsistent results from the preservative blanks, no other dilutions of the preservative blank were analyzed.

For VX, with the exception of the lethal dose (-32% ± 6%), the average inhibition at each concentration level was not significantly different from the negative control, and none of concentrations (including the preservative blank) generated a positive inhibition that was significantly different from the negative control.

6.1.2 Potential Interferences

All of the potential interference samples were prepared in DDW and compared with the negative control to determine the level of inhibition. This determination is crucial because the ability of the AbraTox Kit to detect toxicity is dependent on the background light production in whatever drinking water matrix is being used. If the background drinking water sample completely inhibits background light, inhibition caused by contaminants could not be detected. Table 6-3 presents the results from the samples that were analyzed to test the effect of potential interferences on the AbraTox Kit. Of the five metal solutions that were evaluated as possible interferences with the AbraTox Kit, three of them, zinc (15% ± 10%), iron (7% ± 3%), and copper (32% ± 11%) exhibited inhibition that was significantly different from the negative control (0% ± 3%). Zinc and iron inhibition was only slightly detectable, and the copper inhibition was an average of only 32%, leaving more than half of the available background light for inhibition by contaminants. Therefore, water samples containing similar concentrations of metals could be analyzed for contaminants as long as a negative control sample with similar levels of metals was used to generate a representative background inhibition. Enough background light for inhibition by contaminants remains even though there is some inhibition caused by the metals.

To investigate whether the AbraTox Kit is sensitive to by-products of disinfecting processes, DDW samples from water systems that use chlorination and chloramination were analyzed and compared with ASTM Type II DI water as the control sample. In the absence of a background water sample, it seems likely that DI water may be used as a "clean water" control; therefore, it would be helpful to know what the results would be if this is done. The sample from the water supply disinfected by chlorination (N=60) exhibited an average inhibition of 5% ± 16%, while the sample from the water supply disinfected by chloramination exhibited an inhibition of 4% ± 7% on four replicates. The difference in the number of replicates is because the dechlorinated water was used as the negative control with each sample set; therefore, much more data were collected on that water. These inhibition data suggest that samples disinfected by either process are not likely to interfere with the AbraTox Kit results because the inhibition caused by the "clean" drinking water matrices left most of the light to potentially be inhibited by contamination.

6.1.3 Precision

Across all the contaminants and potential interferences, the standard deviation (not relative standard deviation) was measured and reported for each set of four replicates to evaluate the AbraTox Kit precision. Out of 80 opportunities, the standard deviation of the four replicate measurements was less than 10% 54 times (68%), between 10% and 20% 16 times (20%), and greater than 20% 10 times (12%). There was no consistent trend concerning when the results were repeatable. As described in Section 3.2.2, the analysis procedure required that each replicate undergo the entire analysis process; therefore, the measurement of precision represents the precision of the analysis method performed on a single water sample on a given day. The precision does not reflect the repeatability of the method across more than one day or more than one preparation of reagents or more than one operator.

Table 6-3. Potential Interferences Results

Potential Interferences	Concen-tration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Negative control (Metals)	NA	-3	0	3
		0		
		0		
		4		
Aluminum	0.5	-18	-4	10
		0		
		-2		
		4		
Copper	0.6	42	32	11
		39		
		29		
		17		
Iron	0.15	3	7	3
		9		
		9		
		6		
Manganese	0.25	2	1	6
		7		
		3		
		-7		
Zinc	2.5	4	15	10
		23		
		24		
		11		
Negative control (By-products)	NA	0	0	3%
		2		
		-4		
		1		
Chlorination by-products	NA	[a]	5	16
Chloramination by-products	NA	9	4	7
		11		
		-4		
		1		

NA = Not applicable.

(a) Average inhibition across all DDW negative control samples (N=60).

6.2 Toxicity Threshold

Table 6-4 gives the toxicity thresholds, as defined in Section 5.2, for each contaminant. Note the difference between detectability with respect to the negative control and the toxicity threshold with respect to the other concentration levels analyzed. A contaminant concentration level can have an inhibition significantly different from the negative control (thus detectable), but if its inhibition is not significantly different from the concentration levels below it, it would not be considered the toxicity threshold because, in the context of this test, the inhibition must be different from both the negative control and all lower concentrations. Specific examples include aldicarb (detectable at 26 mg/L, toxicity threshold of 260 mg/L) and cyanide (detectable at all four concentration levels, but a toxicity threshold of 25 mg/L). The lowest toxicity threshold concentration was for soman at 1.4 mg/L. Soman was not detectable when compared with the negative control, but the lethal dose of soman was detectable when the effect of the preservative was accounted for by comparing with the preservative blank containing an equivalent concentration of preservatives. Apparently, the preservative in the soman stock solution has a toxic effect on the AbraTox Kit organisms. When the effect of the preservative was subtracted from the effect of the contaminant, a significant effect of the soman remained.

Table 6-4. Toxicity Thresholds

Contaminant	Concentration (mg/L)
Aldicarb	260
Botulinum toxin complex B	ND
Colchicine	240
Cyanide	25
Dicrotophos	1,400
Nicotine	700
Ricin	ND
Soman	1.4[(a)]
Thallium sulfate	280
VX	ND

ND = Significant inhibition was not detected.
[(a)] Soman was only detectable if calculated with respect to the preservative blank.

6.3 False Positive/Negative Responses

None of the AbraTox Kit results would be considered false positive because neither the chlorination nor chloramination inhibition was, on average, significantly different from the negative control, and, therefore, light production was adequate to allow inhibition to occur if a contaminant was present that produced a detectable toxic effect. Since the background inhibition is not complete, it can be accounted for by using negative control samples that are very similar to

the water being analyzed. If samples are analyzed daily, a good practice would be to archive a negative control sample each day in case of contamination the next day.

Table 6-5 shows the false negative responses, which are described in Section 5.3. Botulinum toxin complex B, ricin, and VX did not exhibit a detectable inhibition at the lethal concentration.

Table 6-5. False Negative Responses

Contaminant	Lethal Dose Concentration (Mg/L)	False Negative
Aldicarb	260	no
Botulinum toxin complex B	0.30	yes
Colchicine	240	no
Cyanide	250	no
Dicrotophos	1,400	no
Nicotine	2,800	no
Ricin	15	yes
Soman	1.4	no[(a)]
Thallium sulfate	2,800	No
VX	2.0	yes

[(a)] Soman was not a false negative when compared to the preservative blank.

6.4 Other Performance Factors

6.4.1 Ease of Use

The AbraTox Kit contained clearly written instructions and illustrations, and the contents were clearly labeled. Storage requirements were marked on the vial labels. Overall, the packaging was easy to open. Pull-back tabs on some of the bottles occasionally had to be pried open with a spatula. The most difficult aspect of using the AbraTox Kit was keeping the incubator at 15°C because there was no temperature control on the incubator. The incubator was refrigerated before use, but warmed up quickly during the 30-minute incubation time. Therefore, the incubator was placed in the refrigerator door during the incubation time. On occasion, test readings of zero were obtained among a series of more "normal" bioluminescence readings. The vendor suggested that the *Vibrio fischeri* were not getting into the solution. The technicians were certain that the bacteria were getting into the cuvette appropriately; but, subsequently, even more care was taken during that step in the analysis.

After adding osmotic adjusting solution to the freeze-dried bacteria, the bacteria stock had to be refrigerated for 30 minutes. Therefore, the AbraTox Kit could be used with only 30 minutes advance notice. The *Vibrio fischeri* needed to be stored at -20°C prior to re-hydration. All other reagents required refrigerator storage at 4°C. Expiration dates were listed on vial labels. The *Vibrio fischeri* were consumed the day of use, other reagents were used until the vial was empty.

All necessary supplies were provided with the AbraTox Kit except for pipettes with tips and the ASTM Type II water used to prepare reagents. The luminometer was easy to use and required no special preparation before use. The electronic readout was user-friendly, with only one number needing to be recorded. The luminometer was easily wiped clean and did not require any routine maintenance after three months of use.

No formal scientific training would be required to use the AbraTox Kit, but good laboratory skills would be beneficial. Verification testing staff were able to operate the AbraTox Kit after a brief training session. Contact information for technical assistance was included in the instructions. One cuvette per sample, reagent vials, and pipette tips were generated as solid waste. No guidance was provided as to whether the waste generated was hazardous or not.

6.4.2 Field Portability

The AbraTox Kit was transported from a laboratory to a storage room to simulate a situation in which it would be operated in a non-laboratory location. The storage room contained several tables and light and power sources, but no other laboratory facilities. The luminometer was transported in a small box, and a small cooler was used to transport the reagents. One person could easily carry the basic equipment provided by the vendor (luminometer, small cooler, reagents). The AbraTox Kit was easy to set up and was operational as soon as all equipment was laid out and the luminometer was turned on. No source of electricity was required for this short-term field deployment since the luminometer operated on batteries and the reagents were kept in a cooler. A long-term field deployment would need controlled temperature storage for reagents. Minimum space requirements in the field would be a flat surface of approximately 1.5 feet by 2 feet to keep the cuvette tray level and to keep the sample solution level during luminometer readings. A cooler was required to transport and store the reagents. Maintaining a controlled 15°C incubation temperature was a challenge. The following items not provided in the AbraTox Kit were needed for field use: a cooler to transport and store reagents, ice packs, high-purity water to prepare solutions, a timer, pipettes and tips, and a waste container. Overall the AbraTox Kit was easy to transport to the field and was deployed in a matter of minutes. Results were obtained within 30 minutes of starting the test and were very similar to those obtained in the laboratory. The AbraTox Kit was tested with cyanide at the lethal dose concentration. In the laboratory, the inhibition was 82% ± 5%; while at the non-laboratory location, the inhibition was 76% ± 2%, suggesting that the performance of the AbraTox Kit was not dependent on where the analysis was performed.

6.4.3 Throughput

Approximately 25 sample analyses plus method blanks and controls were completed in one hour. Approximately 25 samples could be processed per vial of *Vibrio fischeri*.

Chapter 7
Performance Summary

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Average Inhibition at Concentrations Relative to the LD Concentration (%)				Range of Standard Deviations (%)	Toxicity Thresh. (mg/L)
			LD	LD/10	LD/100	LD/1,000		
Contaminants in DDW	Aldicarb	260	63	21	-3	12	2-12	260
	Botulinum toxin complex B	0.3	-10	-11	-40	-2	19-34	ND
	Colchicine	240	17	-39	2	-51	2-9	240
	Cyanide	250	82	71	43	25	3-20	25
	Dicrotophos	1,400	42	10	14	26	8-12	1,400
	Nicotine	2,800	43	-3	-2	4	2-7	700
	Ricin	15	11[(a)]	8[(a)]	-1[(a)]	7[(a)]	5-17	ND
	Soman	1.4	31[(a)]	-24	-10	-1	4-13	1.4[(a)]
	Thallium sulfate	2,800	14	20	5	6	1-7	280
	VX	2	-32	-15	2	-7	6-21	ND

	Interference	Conc. (mg/L)	Average Inhibition (%)	Standard Deviation (%)
Potential interferences in DDW	Aluminum	0.5	-4	10
	Copper	0.6	32	11
	Iron	0.15	7	3
	Manganese	0.25	1	6
	Zinc	2.5	15	10

False positive response	No false positive results were obtained because the inhibition of the chlorination and chloramination by-product water samples was not significantly different from that of the negative control samples.
False negative response	The AbraTox Kit generated false negative responses at the lethal dose concentration for botulinum toxin complex B, ricin, and VX.
Ease of use	The AbraTox Kit contained clearly written instructions and illustrations, and the contents were clearly labeled. Storage requirements were marked on the vial labels. The packaging was easy to open except for the pull-back tabs on some of the bottles. The most difficult aspect of using the AbraTox Kit was keeping the incubator at 15°C because there was no temperature control on the incubator. Because bacteria stock had to be refrigerated for 30 minutes, at least 30 minutes of advance notice is necessary before using the AbraTox Kit. No formal scientific training would be required to use the AbraTox Kit.
Field portability	The AbraTox Kit was transported from a laboratory to a storage room to simulate a situation in which it would be operated in a non-laboratory location. The luminometer was transported in a small box, and a small cooler was used to transport the reagents. Overall the AbraTox Kit was easy to transport to the field and was deployed in a matter of minutes. The AbraTox Kit was tested with cyanide at the lethal dose concentration. Results were obtained within 30 minutes of starting the test and were very similar to those obtained in the laboratory. In the laboratory, the inhibition for the lethal dose concentration of cyanide was 82% ± 5%; while at the non-laboratory location, the inhibition was 76% ± 2%.
Throughput	Approximately 25 sample analyses plus method blanks and controls were completed in one hour. Approximately 25 samples could be processed per vial of *Vibrio fischeri*.

ND = Significant inhibition was not detected.
[(a)] Inhibition calculated with respect to the preservative blank.

Chapter 8
References

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