November 2003

Environmental Technology Verification Report

HIDEX OY
BIOTOX™
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

Hidex Oy
BioTox™
Rapid Toxicity Testing System

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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 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 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
µL	microliter
mL	milliliter
NSDWR	National Secondary Drinking Water Regulations
%D	percent difference
PE	performance evaluation
QA	quality assurance
QC	quality control
QMP	quality management plan
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 Hidex Oy BioTox™ rapid toxicity testing system used in conjunction with the Hidex Oy Triathler™ luminometer. 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 BioTox™. Following is a description of BioTox™, based on information provided by the vendor. The information provided below was not subjected to verification in this test.

BioTox™ luminescent toxicity screening uses the Triathler™ luminometer, together with the freeze-dried BioTox™ reagent, to determine the inhibitory effect of water-soluble samples, including suspensions of solid samples. The BioTox™ reagent contains naturally luminescent *Vibrio fischeri*, which produce luciferase as a part of their metabolic pathway. Luciferase catalyzes the oxidation of a long-chain aldehyde and coenzyme, flavin mono-nucleotide. Substances affecting any part of the metabolic pathway of the bacteria directly affect the amount of light they emit. Toxic compounds interfere with this metabolic process, resulting in a reduction of light emission. To determine the toxicity of a sample, changes in light output are measured with the Triathler™ luminometer.

Figure 2-1. Triathler™ Luminometer with Injector

Sample dilutions and a control sample (2% sodium chloride) are pipetted into test tubes (500 microliters [µL] each), and the Triathler™ injector is filled with the *V. fischeri* reagent. The tube containing the control sample is placed in the Triathler™ luminometer, and 500 µL of the reagent are measured and injected. The measurement is taken after 5 seconds. The tube is set aside, and the same procedure is repeated for each sample. After a 30-minute reaction time, the tubes are shaken, and end-point readings from the control and each sample are measured. The inhibition of each sample dilution is calculated.

The BioTox™ kit, which provides for 144 measurements, contains six vials of freeze-dried *V. fischeri* reagent, six vials of reagent diluent (12.5 milliliters [mL] each), and one 50-mL bottle of concentrated sample diluent.

Reagent injection and data acquisition can be performed by a computer connected to the Triathler™ luminometer. The dimensions of the Triathler™ luminometer are 10 inches by 10 inches by 6 inches, and it weighs approximately 10 pounds.

It can only be operated on 110-volt alternating current electricity. The BioTox™ kit (which is sufficient for 150 to 250 measurements) costs $128, the Triathler™ injector costs $1,950, and the luminometer with liquid scintillation counter costs $6,950.

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, BioTox™ 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 BioTox™ 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] BioTox™ 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 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

BioTox™ was evaluated by

- Endpoint and precision—percent inhibition for all concentration levels of contaminants and potential interfering compounds and precision of replicate analyses
- Toxicity threshold for each contaminant
- False negative responses—contaminants that were reported as producing inhibition results similar to the negative control when the contaminant was present at lethal concentrations
- False positive responses—occurrence of inhibition results 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

BioTox™ 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 (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of BioTox™ to detect toxicity at various concentrations of contaminants, as well as to measure the precision of BioTox™ results.

The response of BioTox™ 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, BioTox™ was operated in the basement of a Columbus, Ohio, home to test its ability to be transported and operated in a non-laboratory setting.

3.3 Test Samples

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

3.3.1 Quality Control Samples

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

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]	17
	Positive control (zinc sulfate)	25	23
	Negative control (unspiked DDW)	NS	39
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	0.068[d]	4 per concentration level
	VX	0.22; 0.022; 0.0022; 0.00022	4 per concentration level
Field location	Cyanide	2.5	4
DDW fortified with potential interferences	Aluminum	0.36	4
	Copper	0.65	4
	Iron	0.069	4
	Manganese	0.26	4
	Zinc	3.5	4
Disinfectant by-products	Chloramination by-products	NS	4
	Chlorination by-products	NS	4

[a] NS = Samples not fortified with any contaminant or potential interference.
[b] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[c] Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
[d] Due to the degradation of soman in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 23% of the expected concentration of 0.30 mg/L.

positive control sample. While performance limits were not placed on the results, inhibition of greater than approximately 50% indicated to the operator that BioTox™ was functioning properly. The negative control sample (unspiked DDW) 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 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 BioTox™ 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 BioTox™ 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 of 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 ASTM Type II DI water 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

The BioTox™ reagents were used in conjunction with the Hidex Triathler™ luminometer. The Triathler™ luminometer was equipped with an injector that was used to add the BioTox™ reagents to the water sample being analyzed. To analyze the test samples, the *Vibrio fischeri* were reconstituted in a 2% salt solution and placed in a beaker. The intake tubing from the injector was placed in the solution of bacteria. The test samples were prepared by adding 450 µL of sample (made in DDW) and 50 µL of 20% sodium chloride to a sample cuvette. The cuvette was then placed into the Triathler™ luminometer, and 500 µL of bacteria were injected into the cuvette. After approximately 5 seconds, the luminescence was recorded. The cuvette was inserted into the Triathler™ luminometer 30 minutes later for the final luminescence measurement. Absolute light units were reported by the Triathler™ luminometer. For each contaminant, BioTox™ 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 BioTox™, a single concentration level of cyanide, prepared in the same way as the other DDW samples, was analyzed in replicate by BioTox™ 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 BioTox™. Both held bachelor's degrees in the sciences and took part in a conference call with the vendor to become accustomed to operating BioTox™.

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 interferent 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]	0.068[d] ± 0.001	<0.05
VX	[c]	0.22 ± 0.001	<0.05
Potential Interference			
Aluminum	EPA 200.8	0.36 ± 0.01	<0.10
Copper	EPA 200.8	0.65 ± 0.01	0.011
Iron	EPA 200.8	0.069 ± 0.008	<0.04
Manganese	EPA 200.8	0.26 ± 0.01	<0.01
Zinc	EPA 200.8	3.5 ± 0.35	0.30

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

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]	1.3 NTU
Organic carbon	SM 5310[8]	2.5 mg/L	1.7 mg/L
Specific conductivity	SM 2510[8]	364 µmho	502 µmho
Alkalinity	SM 2320[8]	42 mg/L	90 mg/L
pH	EPA 150.1[9]	7.65	7.80
Hardness	EPA 130.2[9]	112 mg/L	177 mg/L
Total organic halides	SM 5320B[8]	190 µg/L	110 µg/L
Total trihalomethanes	EPA 524.2[10]	52.8 µg/L	20.1 µg/L
Total haloacetic acids	EPA 552.2[11]	75.7 µg/L	7.6 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 standard operating procedure. 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 BioTox™ 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 is 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 zero, it would indicate to the operator that BioTox™ was operating incorrectly. For 23 positive control samples, the average inhibition was 58% ± 39%, indicating the proper functioning of BioTox™.

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

The Triathler™ reports the absolute light units for each sample analyzed. Light measurements were recorded at 5 seconds after bacteria injection and then again 30 minutes later. The percent inhibition (*%I*) of each sample was calculated by comparing the inhibition of light in the test sample to that in a control sample using the following equations:

$$k = \frac{C_F}{C_I} \tag{2}$$

$$\%\, I = \left(1 - \frac{T_F}{k \cdot T_I}\right) \times 100\% \tag{3}$$

where k is the correction factor for the control sample, C_F and C_I are the final and initial light measurements of the control sample, respectively; and T_F and T_I are the final and initial light measurements of the test samples. For all but the chlorinated and chloraminated by-product samples, which used ASTM Type II DI water as the control sample, DDW was used as the control sample.

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

$$S = \left[\frac{1}{n-1}\sum_{k=1}^{n}\left(I_k - \bar{I}\right)^2\right]^{1/2} \tag{4}$$

where n is the number of replicate samples, I_k is the percent inhibition measured for the k^{th} sample, and $\bar{I}$ is the average percent inhibition of the replicate samples. Because the average inhibitions were frequently near zero for this data set, relative standard deviations often would have greatly exceeded 100%, making the results difficult to interpret. Therefore, the precision

results were left in the form of standard deviations so the reader could easily view the uncertainty around the average for results that were both near zero and significantly larger than zero.

5.2 Toxicity Threshold

The toxicity threshold was defined as the lowest concentration of contaminant to exhibit a percent inhibition significantly greater than the negative control. Also, each concentration level higher than the toxicity threshold had to be significantly greater than the negative control, and the inhibition produced by each lower concentration level had to be significantly less than that produced by the toxicity threshold concentration, based on the standard deviation around the average inhibitions. Since the inhibition of the negative control sample was subtracted from the inhibition of each sample, the percent inhibition of the negative control was always zero. A difference was considered significant if the average inhibition plus or minus the standard deviation did not encompass the value or range of values that were being compared.

5.3 False Positive/Negative Responses

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

A response was considered false negative when BioTox™ was subjected to a lethal concentration of some contaminant in the DDW and did not indicate inhibition significantly greater than the negative control (zero inhibition) and 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 BioTox™ in determining a false negative response. A difference was considered significant if the average inhibition plus or minus the standard deviation did not encompass the value or range of values that were being compared.

5.4 Field Portability

The results obtained from the measurements made on DDW samples in the laboratory and in the field were compiled independently and compared to assess the performance of the BioTox™ 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 BioTox™ 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. Samples that produced negative percent inhibition values indicated an increase in light production by the bacteria relative to the negative control for any of the concentrations.

6.1.1 Contaminants

Two contaminants that were analyzed by BioTox™ during this verification test produced inhibitions significantly different from the negative control for each sample set. The inhibition samples containing cyanide increased in proportion to the concentration in the sample, with the two highest concentrations of cyanide causing inhibition significantly greater than the negative control and the other concentration levels. For thallium sulfide, only the highest concentration sample exhibited inhibition distinguishable from the negative control and the other concentration levels. The other seven contaminants did not cause inhibition significantly greater than the negative control.

6.1.2 Potential Interferences

Iron exhibited percent inhibitions near zero (Table 6-2), indicating little or no response to these compounds; while aluminum and manganese exhibited slightly higher average inhibitions with relatively large uncertainties. Zinc exhibited a considerably higher inhibition of 48%, and copper exhibited almost complete inhibition. Copper and zinc may cause inhibitions that interfere with the BioTox™ results.

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 accounted for within the calculation of the inhibition of each sample. To investigate whether BioTox™ is sensitive to by-products of disinfecting processes, dechlorinated drinking water samples from water systems that use chlorination and chloramination were analyzed and

Table 6-1a. Aldicarb Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.28	-14 -10 -10 -7	-10	3
2.8	-17 6 -1 10	-1	12
28	-13 2 8 3	0	9
280 (Lethal Dose)	-3 4 11 0	3	6

Table 6-1b. Colchicine Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.24	-10 -11 -18 -68	-27	27
2.4	39 -10 -9 21	10	24
24	-20 -18 -23 1	-15	11
240 (Lethal Dose)	-25 -25 -2 21	-8	22

Table 6-1c. Cyanide Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.25	9 -19 5 -1	-1	12
2.5	22 2 8 7	10	9
25	70 65 70 38	61	16
250	97 98 96 94	96	2
2.5 (Field Location)	50 57 61 59	57	4

Table 6-1d. Dicrotophos Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
1.4	15 -3 12 0	6	9
14	-5 -5 15 2	2	10
140	5 -1 7 8	5	4
1,400 (Lethal Dose)	-2 -6 5 11	2	8

Table 6-1e. Thallium Sulfate Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
2.4	3 -2 -8 -10	-4	6
24	16 1 10 15	11	7
240	15 -3 26 34	18	16
2,400 (Lethal Dose)	49 41 38 37	41	6

Table 6-1f. Botulinum Toxin Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.0003	8 0 8 -8	2	8
0.003	14 12 13 1	10	6
0.03	1 5 -5 6	2	5
0.30 (Lethal Dose)	3 1 14 3	5	6

Table 6-1g. Ricin Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.015	-8 0 -8 14	0	10
0.15	3 7 5 -6	2	6
1.5	3 -5 2 12	3	7
15 (Lethal Dose)	-6 -5 5 -13	-5	7

Table 6-1h. Soman Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.00068	4 0 7 0	3	3
0.0068	1 2 3 -1	1	2
0.068	0 0 1 -6	-1	3
0.068[(a)] (Lethal Dose)	7 2 9 9	7	3

[(a)] Due to the degradation of soman in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 23% of the expected concentration of 0.30 mg/L.

Table 6-1i. VX Percent Inhibition Results

Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
0.00022	5 4 0 0	2	2
0.0022	3 4 8 6	5	2
0.022	-4 15 2 -1	3	9
0.22 (Lethal Dose)	10 4 11 7	8	3

compared with ASTM Type II DI water as the control sample. This determination is crucial because the ability of BioTox™ to detect toxicity is dependent on the light production of the reagents in a clean drinking water matrix. If clean drinking water produces 100% inhibition of light, the detection of subsequently added contaminants would not be possible. On average, the chlorinated sample exhibited a negative and highly variable percent inhibition. When ASTM Type II DI water was used as the sample, there was an approximately 10% light loss over the 30-minute reaction time. When DDW was used as the sample, the BioTox™ reagent produced more light after the 30-minute reaction time than at the 5-second measurement, which, averaged across all 39 negative control samples, resulted in a calculated inhibition of -49% ± 33%.

For this ETV verification test, the increased background light production when analyzing DDW samples was not critical because “clean” negative control samples were analyzed with each sample set so the test samples containing the contaminants could be directly compared with a background sample that is guaranteed to be non-inhibitory. However, in a real-world scenario, a “clean” background matrix identical to the sample matrix may not be available, and ASTM Type II DI water as the control sample would be the likely replacement. This substitution would not be appropriate to obtain accurate percent inhibition data. For example, when ASTM Type II DI water was used as the control during this test, some “clean” DDW exhibited inhibitions of approximately -49%. Therefore, if a contaminant was added to that matrix to cause a 49% inhibition of the bacteria, the calculated inhibition of that sample would be zero percent,

Table 6-2. Potential Interferences Results

Interference	Concentration (mg/L)	Inhibition (%)	Average (%)	Standard Deviation (%)
Aluminum	0.36	-1 21 26 19	16	12
Copper	0.65	95 99 92 99	96	4
Iron	0.069	0 -1 -3 3	0	2
Manganese	0.26	1 9 22 10	10	9
Zinc	3.5	33 55 52 52	48	10
Chlorination By-products	NA[(a)]	[(b)]	-49	33
Chloramination By-products	NA	14 13 13 10	13	2

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

indicating no toxicity. In the absence of a negative control sample with the same makeup of the samples, it is likely that drinking water from a system that uses chlorination as its disinfection process may interfere with the measurement of percent inhibition data.

The water sample from a system that uses chloramination as its disinfection process exhibited inhibitions of 13% ± 2% with respect to ASTM Type II DI water. To a lesser extent than the chlorinated water, chloraminated water also may interfere, but the background inhibition is a relatively small positive value, so the inhibition would be slightly overestimated rather than underestimated, as is shown in the case of the chlorinated sample.

6.1.3 Precision

Across all the contaminants and potential interferences, the standard deviation was measured and reported for each set of four replicates to evaluate the BioTox™ precision. Out of 42 opportunities, the standard deviation of the four replicate measurements was under 10% for 30 concentration levels, between 10% and 20% for nine concentration levels, and over 20% for three concentration levels. Overall, the range of standard deviations was between 2 and 27%.

6.2 Toxicity Threshold

Table 6-3 gives the toxicity thresholds as defined in Section 5.2 for each contaminant. The lowest toxicity threshold concentration was for thallium sulfate at 24 mg/L, indicating that BioTox™ was most sensitive to thallium sulfate. The only other contaminant that BioTox™ was able to significantly distinguish from the negative control was cyanide at the two highest concentration levels. For aldicarb, dicrotophos, colchicine, botulinum toxin, ricin, soman, and VX, no inhibition greater than the negative control was detected, regardless of the concentration level, indicating that the technology was not responsive to these contaminants.

Table 6-3. Toxicity Thresholds

Contaminant	Concentration (mg/L)
Aldicarb	ND[(a)]
Colchicine	ND
Cyanide	25
Dicrotophos	ND
Thallium sulfate	24
Botulinum toxin	ND
Ricin	ND
Soman	ND
VX	ND

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

6.3 False Positive/Negative Responses

The drinking water sample disinfected by chloramination produced, on average, inhibition of 13% ± 2%. In the absence of a negative control sample of a very similar matrix, there is the possibility of a slightly exaggerated inhibition due to the baseline inhibition of the drinking water sample. As described in Section 6.1.2, BioTox™ produced false negative responses for unspiked drinking water disinfected by chlorination when it was calculated with respect to ASTM Type II DI water. The example described in that section provides an instance where contamination of the DDW caused significant inhibition of the bacteria's light production; however, if ASTM Type II

DI water was used as the control sample, the result would be reported as negligible inhibition because of the negative background inhibition produced by the DDW. Therefore, when only ASTM Type II DI water is used as the control for water samples in a different matrix, the risk for a false negative response is great. Another type of false negative response is when a lethal dose of contaminant is present in the water sample and the inhibition is not significantly different either from the negative control or the other lower concentration levels. Only one of the two criteria had to be met to be considered a false negative response. Table 6-4 gives these results. The inhibition induced by lethal doses of cyanide and thallium sulfate was detectable by BioTox™, while aldicarb, colchicine, dicrotophos, botulinum toxin, ricin, soman, and VX did not indicate inhibition greater than the negative control, indicating false negative responses.

Table 6-4. False Negative Results

Contaminant	Lethal Dose Concentration (mg/L)	False Negative Result
Aldicarb	280	yes
Colchicine	240	yes
Cyanide	250	no
Dicrotophos	1,400	yes
Thallium sulfate	2,400	no
Botulinum toxin	0.30	yes
Ricin	15	yes
Soman	0.068[(a)]	yes
VX	0.22	yes

[(a)] Due to the degradation of soman in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 23% of the expected concentration of 0.30 mg/L.

6.4 Field Portability

A single concentration of cyanide was prepared and analyzed in replicate at a field location to examine its ability to be used in a non-laboratory setting. BioTox™, the Triathler™ luminometer, and other necessary accessories were transported to the field in the hard metal carrying case provided by the vendor. Fully loaded, the case weighed about 20 pounds. At the field location, the Triathler™ luminometer was powered with electricity, because it was not equipped to operate on batteries. An optional field case with batteries may be purchased from the vendor, but was not available for use during ETV testing. Because of the need to connect a beaker containing bacteria to the injector, a flat, sturdy workspace (such as a small tabletop) is required to operate the Triathler™ luminometer. Table 6-1c shows the results for 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 2.5 mg/L. The inhibition produced in the field was 57% ± 4%, and the inhibition produced in the laboratory at the

same concentration was 10% ± 9%. While these inhibitions are not the same, the field measurements were made on freshly prepared solutions with a newly reconstituted batch of bacteria. The precision of the results suggests that the BioTox™ reagent and Triathler™ luminometer functioned similarly at the non-laboratory and laboratory locations.

The BioTox™ reagent must be kept at approximately -20°C prior to reconstitution and, after reconstitution, the reagent needs to be stabilized for at least two hours. The reconstituted reagent must be used the same day. These factors could be problematic in a long-term field deployment.

6.5 Other Performance Factors

There was no formal manual with instructions for getting the injector to function properly in conjunction with the Triathler™ luminometer. The instructions for the BioTox™ reagent and sample preparation were clear, but initially it was not clear how to collect the data properly in the absence of an electronic data acquisition system. Two conference calls with the vendor and considerable effort by the verification test coordinator were necessary to determine the proper operational procedure for the BioTox™/Triathler™. Once operational, with help from the vendor, the button on the Triathler™ luminometer that triggers injection of the bacteria spontaneously changed to a different button for no apparent reason. A significant amount of time was required to figure out which button was the correct one to use. Once the correct procedure was determined, the BioTox™/Triathler™ was easy to use and worked correctly and consistently. Although the operators had scientific backgrounds, based upon observations of the verification test coordinator, an operator with little technical training would probably be able to analyze multiple sample sets after adequate direction on how to perform tests correctly had been provided. Such direction may come through contact with the vendor or an improved instruction manual. The operators were able to analyze approximately 50 samples per hour.

Chapter 7
Performance Summary

Parameter	Compound	Lethal Dose (LD) Conc. (mg/L)	Average Inhibitions at Concentrations Relative to the LD Concentration (%)				Range of Standard Deviations (%)	Toxicity Thresh. (mg/L)[a]
			LD	LD/10	LD/100	LD/1,000		
Contaminants in DDW	Aldicarb	280	3	0	-1	-10	3-12	ND[b]
	Colchicine	240	-8	-15	10	-27	11-27	ND
	Cyanide	250	96	61	10	-1	2-16	25
	Dicrotophos	1,400	2	5	2	6	4-10	ND
	Thallium sulfate	2,400	41	18	11	-4	6-16	24
	Botulinum toxin[c]	0.30	5	2	10	2	5–8	ND
	Ricin[d]	15	-5	3	2	0	6-10	ND
	Soman	0.068[e]	7	-1	1	3	2-3	ND
	VX	0.22	8	3	5	2	2-9	ND
Potential interferences in DDW	**Interference**	**Conc. (mg/L)**	**Average Inhibitions at a Single Concentration (%)**				**Standard Deviation (%)**	
	Aluminum	0.36	16				12	
	Copper	0.65	96				4	
	Iron	0.069	0				2	
	Manganese	0.26	10				9	
	Zinc	3.5	48				10	
False positive response	Slightly exaggerated inhibitions may result if chloraminated water, which produced 13% ± 2% inhibitions, is analyzed with respect to ASTM Type II DI water.							
False negative response	Inhibition greater than the negative control was not detected for lethal doses of aldicarb, colchicine, dicrotophos, botulinum toxin, ricin, soman[c], and VX. There was a -49% ± 33% inhibition for water from the system treated by chlorination, resulting in a risk of false negative results when using ASTM Type II DI water as the control sample.							
Field portability	The inhibition of 2.5 mg/L cyanide in the field was 57% ± 4%, and in the laboratory it was 10% ± 9%. Practically, the operation did not seem much different. However, the Triathler™ is not equipped for use with batteries, so electricity was required. A field-portable case with batteries may be purchased. A flat, sturdy surface is needed to operate BioTox™ because a beaker of bacteria must be connected to the injector.							
Other performance factors	Although determining how to operate the BioTox™/Triathler™ was difficult without an instruction manual and required significant intervention from the vendor, it was easy to use once the correct procedure was determined. Although the operators had scientific backgrounds, upon observation of the test procedures, it seems likely that an operator with little technical training would probably be able to analyze samples successfully once provided with adequate guidance in the form of contact with the vendor or an improved instruction manual. Sample throughput was 50 samples per hour.							

[a] See Tables 6-1a-i in the report for the precision around each individual inhibition result.
[b] ND = Not detectable.
[c] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.
[d] Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.
[e] Due to the degradation of soman in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 23% of the expected concentration of 0.30 mg/L.

Chapter 8
References

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

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

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

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

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

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

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

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

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

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

11. U.S. EPA Method 552.2, "Haloacetic Acids and Dalapon by Liquid-Liquid Extraction, Derivatization and GC with Electron Capture Detector," *Methods for the Determination of Organic Compounds in Drinking Water—Supplement III* EPA/600/R-95/131.

12. *Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center*, Version 4.0, U.S. EPA Environmental Technology Verification Program, Battelle, Columbus, Ohio, December 2002.