November 2003

Environmental Technology Verification Report

AQUA SURVEY, INC.
IQ TOXICITY TEST™
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

Aqua Survey, Inc.
IQ Toxicity Test™
Rapid Toxicity Testing System

by
Ryan James
Amy Dindal
Zachary Willenberg
Karen Riggs

Battelle
Columbus, Ohio 43201

Notice

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

Foreword

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

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

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

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Contents

Page

Notice ii

Foreword iii

Acknowledgments iv

List of Abbreviations viii

1 Background 1

2 Technology Description 2

3 Test Design and Procedures 4
- 3.1 Introduction 4
- 3.2 Test Design 5
- 3.3 Test Samples 6
  - 3.3.1 Quality Control Samples 6
  - 3.3.2 Drinking Water Fortified with Contaminants 8
  - 3.3.3 Drinking Water Fortified with Potential Interferences 8
- 3.4 Test Procedure 9
  - 3.4.1 Test Sample Preparation and Storage 9
  - 3.4.2 Test Sample Analysis Procedure 9
  - 3.4.3 Stock Solution Confirmation Analysis 11

4 Quality Assurance/Quality Control 14
- 4.1 Quality Control of Stock Solution Confirmation Methods 14
- 4.2 Quality Control of Drinking Water Samples 14
- 4.3 Audits 15
  - 4.3.1 Performance Evaluation Audit 15
  - 4.3.2 Technical Systems Audit 16
  - 4.3.3 Audit of Data Quality 16
- 4.4 QA/QC Reporting 16
- 4.5 Data Review 16

5 Statistical Methods and Reported Parameters 18
- 5.1 Endpoints and Precision 18
- 5.2 Toxicity Threshold 19
- 5.3 False Positive/Negative Responses 19
- 5.4 Field Portability 19
- 5.5 Other Performance Factors 20

6 Test Results 21
6.1 Endpoints and Precision 21
6.1.1 Contaminants 21
6.1.2 Potential Interferences 30
6.1.3 Precision 31
6.2 Toxicity Threshold 32
6.3 False Positive/Negative Responses 32
6.4 Field Portability 33
6.5 Other Performance Factors 34

7 Performance Summary 35

8 References 36

Figures

Figure 2-1. Bluish-White (Healthy) *Daphnia magna* 2

Tables

Table 3-1. Contaminants and Potential Interferences 5

Table 3-2. Summary of Quality Control and Contaminant Test Samples 7

Table 3-3. Dose Confirmation Results 12

Table 3-4. Water Quality Parameters 13

Table 4-1. Summary of Performance Evaluation Audit 15

Table 4-2. Summary of Data Recording Process 17

Table 6-1a. Aldicarb Percent Inhibition Results 22

Table 6-1b. Colchicine Percent Inhibition Results 23

Table 6-1c. Cyanide Percent Inhibition Results 24

Table 6-1d. Dicrotophos Percent Inhibition Results 25

Table 6-1e. Thallium Sulfate Percent Inhibition Results 26

Table 6-1f. Botulinum Toxin Percent Inhibition Results 27

Table 6-1g. Ricin Percent Inhibition Results 28

Table 6-1h. Soman Percent Inhibition Results 29

Table 6-1i. VX Percent Inhibition Results 30

Table 6-2. Potential Interferences Results 31

Table 6-3. Toxicity Thresholds 32

Table 6-4. False Negative Responses 33

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 Sergeantsville, New Jersey
EC_{50}	effective concentration causing 50% inhibition
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
HDPE	high-density polyethylene
ID	identification
L	liter
mg	milligram
mL	milliliter
NSDWR	National Secondary Drinking Water Regulations
%D	percent difference
PE	performance evaluation
QA	quality assurance
QC	quality control
QMP	quality management plan
RSD	relative standard deviation
SOP	standard operating procedure
TSA	technical systems audit
UV	ultraviolet

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 Aqua Survey, Inc., IQ Toxicity Test™ rapid toxicity testing system. Rapid toxicity testing systems were identified as a priority technology verification category through the AMS Center stakeholder process.

Chapter 2
Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This verification report provides results for the verification testing of the IQ Toxicity Test Kit™. The IQ Toxicity Test Kit™ is packaged in the Threat Detection Kit™. Following is a description of the IQ Toxicity Test Kit™, based on information provided by the vendor. The information provided below was not subjected to verification in this test.

Daphnia magna (Figure 2-1) are freshwater aquatic invertebrates that react rapidly when exposed to threat contaminants at toxic concentrations. The IQ Toxicity Test™ allows the user to characterize the toxicity of a water sample by measuring stressor-related suppression of enzyme activity of *Daphnia magna* in one hour and 15 minutes by determining fluorescent light emittance.

The IQ Toxicity Test™ is performed in three plastic exposure chambers, each consisting of six 10-milliliter (mL) compartments. Six *Daphnia magna* are placed in each 10-mL compartment. In a single study or replicate test, 18 organisms were exposed to each concentration level by using the three exposure chambers. One compartment is filled with the negative control sample, and the other five compartments are filled with sequentially decreasing concentrations of the contaminant being tested. Three exposure chambers containing a dilution series of the contaminant being tested are analyzed for each individual sample replicate. The EC_{50} (concentration at which 50% of the organisms were affected) is calculated for each replicate. To fully characterize a contaminant, four replicates of three exposure chambers are analyzed, and the EC_{50} is calculated for each of the four replicates.

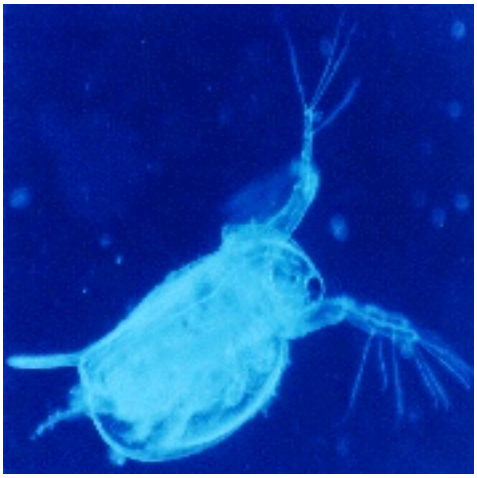

Figure 2-1. Bluish-White (Healthy) *Daphnia magna*

After the organisms are in contact with the control and sample (drinking) water for one hour, a fluorogenically tagged sugar substrate suspension is added to each of the six compartments. After 15 minutes, the exposure chamber is illuminated with a black light (longwave ultraviolet [UV]). The control organisms emitted bright bluish-white light—indicating that they were healthy. If the organisms in the sample water are not glowing as brightly, they are scored as adversely affected. For the organisms to fluoresce, they must ingest the tagged sugar

(galactose) and express the enzyme (galactosidase); then the enzyme must successfully cleave the sugar from the fluorogenic marker. This marker, although unable to fluoresce while attached to the sugar molecule, is now liberated and fluoresces as it flows through the organism's circulatory system. This is an obvious visual endpoint.

The Threat Detection Starter Kit™ includes the equipment needed to be purchased once and the supplies to perform 30 toxicity tests and maintain a *Daphnia magna* production culture for 30 days. Supplied in the starter kits are instructions, a test scoring form, exposure chambers, fluorogenic substrate, reconstituted water stock solution, pipettes, longwave UV light, sonicator, fluorescent light box, 45-liter (L) carboy, and assorted equipment to facilitate the performance of 30 toxicity tests. This kit also includes a starter culture of live *Daphnia magna*, a 30-day supply of food, culture dishes, and equipment to initiate an ongoing *Daphnia magna* production culture. A Threat Detection Maintenance Kit™ provides the supplies needed to conduct 30 additional toxicity tests and to maintain the *Daphnia magna* culture an additional 30 days. The starter kit is packaged in four boxes, each less than 20 pounds, and is $2,400. The maintenance kit is packaged in one less-than-20-pound box and is $400.

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, IQ Toxicity Test™ 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 IQ Toxicity Test™ 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] IQ Toxicity Test™ was verified by analyzing a dechlorinated drinking water (DDW) sample from Sergeantsville, New Jersey, fortified with various concentrations of the contaminants and interferences shown in Table 3-1. Hereafter in this report, DDW will refer to the dechlorinated drinking water from Sergeantsville, New Jersey. Where possible, the concentration of each contaminant or potential interference was confirmed independently by Aqua Tech Environmental Laboratories (ATEL), Marion, Ohio, or by Battelle, depending on the analyte.

Table 3-1. Contaminants and Potential Interferences

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

IQ Toxicity Test™ was evaluated by

- Endpoint and precision—percent inhibition for each concentration level and effective EC_{50} values were calculated for each contaminant; the reproducibility of the EC_{50} values was also evaluated.
- Toxicity threshold for each contaminant
- False negative responses—when the lethal concentration did not adversely affect the organisms
- False positive responses—occurrence of adversely affected organisms in unspiked dechlorinated drinking water samples
- Field portability
- Ease of use
- Throughput.

3.2 Test Design

IQ Toxicity Test™ was used to analyze the DDW sample fortified with contaminants at concentrations ranging from lethal levels to concentrations several orders of magnitude less than the lethal dose. The lethal dose of each contaminant was determined by calculating the concentration at which 250 mL of water would probably cause the death of a 154-pound person. These calculations were based on toxicological data available for each contaminant. For soman and VX, 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 dilution series for each contaminant were evaluated to assess the ability of IQ Toxicity Test™ to detect toxicity at various concentrations of contaminants, as well as to measure the precision of IQ Toxicity Test™ results.

The response of IQ Toxicity Test™ 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 Sergeantsville, New Jersey, 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, IQ Toxicity Test™ was used in the basement of a Columbus, Ohio, home to test its ability to be transported and used 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 Sergeantsville, New Jersey, system. The water was dechlorinated using sodium thiosulfate and then fortified with various concentrations of contaminants and interferences. Using this DDW (Sergeantsville, New Jersey, 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 the IQ Toxicity Test™. Mixtures of contaminants and interfering compounds were not analyzed. One dilution series of cyanide was analyzed in replicate at the field location.

3.3.1 Quality Control Samples

QC samples included method blank samples, which consisted of American Society for Testing and Materials (ASTM) Type II deionized (DI) water containing salts provided by the vendor to sustain life in the *Daphnia magna* organisms; 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

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

Type of Sample	Sample Characteristics	Concentration Levels (mg/L)	No. of Sample Analyses
Quality control	Method blank	NS[a]	9
	Positive control (copper)	0.5	10
	Negative control (unspiked DDW)	NS	44
DDW fortified with contaminants	Aldicarb	280; 28; 14; 7, 3.5; 2.8; 1.75; 0.28	3 per replicate
	Colchicine	240; 24; 2.4; 0.24; 0.024	3 per replicate
	Cyanide	250; 25; 2.5; 0.25; 0.025; 0.0025; 0.00025; 0.000025	3 per replicate
	Dicrotophos	1,400; 140; 14; 7; 3.5; 1.75; 1.4; 0.88; 0.014	3 per replicate
	Thallium sulfate	2,400; 240; 24; 2.4; 0.24	3 per replicate
	Botulinum toxin[b]	0.30; 0.030; 0.0030; 0.0030	3 per replicate
	Ricin[c]	15; 1.5; 0.15; 0.015	3 per replicate
	Soman	0.13[d]; 0.013; 0.0013; 0.00013	3 per replicate
	VX	0.077[d]; 0.038; 0.019; 0.0095; 0.0048; 0.0077; 0.00077; 0.000077;0.0000077	3 per replicate
Field location	Cyanide	0.25, 0.13, 0.060, 0.030, 0.020	3 per replicate
DDW fortified with potential interferences	Aluminum	0.36	5
	Copper	0.65	5
	Iron	0.069	5
	Manganese	0.26	5
	Zinc	3.5	5
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 in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 44% of the expected concentration of 0.30 mg/L for soman, and 38% of the expected concentration of 0.20 mg/L for VX.

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. The DDW already had an adequate salt content to sustain life in the *Daphnia magna,* so no additional salts were added. A solution of 0.5 mg/L copper was provided by the vendor for use as a positive control sample.

While performance limits were not placed on the results, if none of the organisms were adversely affected in that solution, it would have indicated to the operator that IQ Toxicity Test™ was not performing adequately. The negative control sample was used to set a background effect of the DDW on the organisms, the matrix in which each test sample was prepared.

3.3.2 Drinking Water Fortified with Contaminants

Sergeantsville, New Jersey, tap water was 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 four additional samples with concentrations 10, 100, 1,000 and 10,000 times less than the lethal dose. These concentrations were analyzed in the same test chamber. If there was a clear dividing concentration at which all the organisms were adversely affected and the next lowest concentration had no adversely affected organisms, the higher concentration was diluted by factors of 2, 4, 8, and 16 and analyzed in the test chamber to more closely determine the EC_{50}. 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. At least four replicates of each of these samples were analyzed. To test the sensitivity of IQ Toxicity Test™ 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 *Daphnia magna* organism and can degrade the contaminants during storage, was immediately dechlorinated with sodium thiosulfate. Prior to preparing each stock solution, dechlorination of the water sample was qualitatively confirmed by adding an n,n-diethyl-p-phenylenediamine tablet to a 25-mL aliquot of the DDW. Once dechlorination was confirmed, all the contaminant samples, potential interference samples, and negative control QC samples were made from this DDW, while the method blank sample was prepared from ASTM Type II DI water by adding salts, necessary for the organism's survival, provided by the vendor. The positive control samples were provided by the vendor. 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 IQ Toxicity Test™ relies on small crustaceans (*Daphnia magna)* to indicate potential toxicity in drinking water samples. Because of the large number of organisms needed for this verification test, the vendor provided organisms for use during testing. Therefore, culturing *Daphnia magna* was not included as part of this verification test, but it is something that had to be done prior to performing the IQ Toxicity Test™. The vendor provided one-gallon pails of 500 to 1,000 organisms in approximately one gallon of DDW. The organisms had to be fed daily by adding algae to the storage pails and had to be used within seven days of birth. The night prior to testing, the approximate number of organisms needed for testing on the following day were starved by not feeding them any algae. Just prior to testing, the majority of water that the organisms were being stored in was siphoned off, and the remaining water and organisms were poured into a plastic pail with shortened sides (2 inches high) to make it easy to select organisms with a bulb pipette.

To check the overall health of the organisms, 18 were selected from the bottom of the pail using a bulb pipette and placed in a watchglass containing approximately 10 mL of DDW. Twelve drops of a sugar substrate, prepared by mixing with ASTM Type II DI water in a sonicator, were added to the watchglass. After 15 minutes, the watchglass was placed under a black light. If more than three of the 18 organisms were adversely affected, indicated by not illuminating or a dimmed illumination, this would indicate that the organisms were not able to live in the clean water sample and that this test would not be able to indicate the presence of additional contamination. This exercise was called the pre-test. After the pre-test confirmed that the organisms were able to survive in the DDW, the test procedure was begun. Because pre-test confirmed that the *Daphnia magna* were not able to live successfully in the dechlorinated water sample from Columbus, Ohio (more than three out of 18 were adversely affected), a water sample from

Sergeantsville, New Jersey, which uses chlorination as its disinfectant procedure, was collected and used to store the organisms. This water sample was used for the sample matrix for all of the test samples analyzed as part of this test. The reason that the organisms could not survive in the Columbus, Ohio, water was not clear, but in evaluating the water quality parameters evaluated as part of this test, the concentrations of zinc, total haloacetic acids, total organic halides, and organic carbon were considerably higher in the Ohio water compared with the New Jersey water.

The IQ Toxicity Test™ was performed in disposable plastic exposure chambers consisting of six 10-mL compartments. Six *Daphnia magna* were placed in each 10-mL compartment. One compartment was filled with the negative control sample, and the other five compartments were filled with sequentially decreasing concentrations of the contaminant being tested. Three exposure chambers containing a dilution series of the contaminant being tested were analyzed for each individual sample replicate. In a single replicate, 18 organisms were exposed to each concentration level. The EC_{50} was calculated for each replicate. To fully characterize a contaminant, four replicates of three exposure chambers were analyzed, and the EC_{50} was calculated for each of the four replicates.

The following routine was followed for each contaminant. One replicate (three exposure chambers) with tenfold dilutions of the lethal dose concentration of each contaminant was analyzed first. For example, the lethal dose concentration of thallium sulfate was 2,400 mg/L; therefore, the six 10-mL compartments of each of the three chambers were filled with the following: negative control, 2,400 mg/L, 240 mg/L, 24 mg/L, 2.4 mg/L, and 0.24 mg/L, all solutions of thallium sulfate. Then, since the goal of the IQ Toxicity Test™ was to determine the EC_{50} of the contaminant being tested, the smaller the concentration range that caused the organisms to be less adversely affected, the more accurate the EC_{50} value. If one of the concentration levels in the tenfold dilution series caused most or all of the organisms to be adversely affected, and if the concentration level immediately below that one caused half or less of the organisms to be adversely affected, the higher of the two concentrations was analyzed. For thallium sulfate, all of the organisms were adversely affected at the 240-mg/L concentration level, and only 44%, or eight out of 18 organisms were adversely affected at the 24-mg/L concentration. Therefore, a smaller dilution range using 240 mg/L as the highest concentration was analyzed. The 10-mL compartments of each of the three chambers were filled with the following: negative control, 240 mg/L, 120 mg/L, 60 mg/L, 30 mg/L, and 15 mg/L—all solutions of thallium sulfate. Four replicates, including three exposure chambers each, were analyzed and the EC_{50} values calculated. If there was no concentration level that clearly divided adversely affected organisms and non-affected organisms, three additional replicates of the tenfold dilution series were analyzed and no further dilutions were made.

This procedure was followed for each contaminant. Only one concentration of potential interference was analyzed by filling all five available compartments of a test chamber with the potential interference tests and evaluating its affect on the organisms. Two operators performed all the analyses using IQ Toxicity Test™. Both held bachelor's degrees in the sciences and spent approximately 12 hours with the vendor to become familiar with performing IQ Toxicity Tests™.

3.4.3 Stock Solution Confirmation Analysis

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

Standard methods were also used to characterize several water quality parameters such as the concentration of trihalomethanes, haloacetic acids, and total organic halides; turbidity; dissolved organic carbon content; pH; alkalinity; specific conductivity; and hardness. Table 3-4 lists these measured water quality parameters for the water samples collected in Sergeantsville, New Jersey, and in Columbus, Ohio, representing water systems 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 Sergeantsville, New Jersey, Sample (mg/L)	Background Columbus, Ohio, Sample (mg/L)
Contaminant				
Aldicarb	EPA 531.1[3]	280 ± 28	<0.0007	<0.0007
Colchicine	[a]	NA[b]	NA	NA
Cyanide	EPA 335.1[4]	250 ± 15	<0.005	0.008
Dicrotophos	EPA SW846 (8141A)[5]	1,400 ± 140	<0.002	<0.002
Thallium sulfate	EPA 200.8[6]	2,400 ± 24	<0.001	<0.001
Botulinum toxin	[a]	NA	NA	NA
Ricin	[a]	NA	NA	NA
Soman	[c]	0.13[d] ± 0.01	<0.05	<0.05
VX	[c]	0.077 ± 0.002	<0.05	<0.05
Potential Interference				
Aluminum	EPA 200.8	0.36 ± 0.01	<0.10	<0.10
Copper	EPA 200.8	0.65 ± 0.01	<0.01	0.011
Iron	EPA 200.8	0.069 ± 0.008	<0.04	<0.04
Manganese	EPA 200.8	0.26 ± 0.01	<0.04	<0.01
Zinc	EPA 200.8	3.5 ± 0.35	<0.01	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 44% of the expected concentration of 0.30 mg/L and for VX was 38% of the expected concentration of 0.20 mg/L.

Table 3-4. Water Quality Parameters

Parameter	Method	Dechlorinated Sergeantsville, New Jersey, Tap Water (disinfected by chlorination)	Dechlorinated Columbus, Ohio, Tap Water (disinfected by chlorination)	Dechlorinated St. Petersburg, Florida, Tap Water (disinfected by chloramination)
Turbidity	EPA 180.1[7]	0.2 NTU[a]	0.1 NTU	0.3 NTU
Organic carbon	SM 5310[8]	<0.7 mg/L	2.5 mg/L	2.9 mg/L
Specific conductivity	SM 2510[8]	348 µmho	364 µmho	460 µmho
Alkalinity	SM 2320[8]	86 mg/L	42 mg/L	97 mg/L
pH	EPA 150.1[9]	7.74	7.65	7.95
Hardness	EPA 130.2[9]	84 mg/L	112 mg/L	160 mg/L
Total organic halides	SM 5320B[8]	31 µg/L	190 µg/L	83 µg/L
Total trihalomethanes	EPA 524.2[10]	2.9 µg/L	52.8 µg/L	2.4 µg/L
Total haloacetic acids	EPA 552.2[11]	16.6 µg/L	75.7 µg/L	13.5 µg/L

[a] NTU = nephelometric turbidity unit.

Chapter 4
Quality Assurance/Quality Control

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

4.1 Quality Control of Stock Solution Confirmation Methods

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

4.2 Quality Control of Drinking Water Samples

A method blank sample consisting of ASTM Type II DI water was analyzed once by IQ Toxicity Test™ for approximately every 20 drinking water samples that were analyzed. These samples were used to confirm the health of the organisms in the absence of any contaminants. One of the test compartments of every test chamber analyzed contained a negative control sample (unspiked DDW). The organisms exposed to various concentrations of contaminants were compared with those in the negative control to determine whether they were adversely affected. 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 the undiluted solution of the positive control sample should adversely affect all of the organisms. A solution of copper was provided by the vendor as the positive control sample; and, when the organisms were exposed to the undiluted solution, all the organisms were adversely affected.

4.3 Audits

4.3.1 Performance Evaluation Audit

The concentration of the standards used to prepare the contaminants 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.

Table 4-1. Summary of Performance Evaluation Audit

		AverageConcentration ± 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

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.

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

For each replicate IQ Toxicity Test™, that is, three exposure chambers of a dilution series of a contaminant, an EC_{50} value was determined. There were two ways to calculate the results. The first was to use the graph paper provided on the back of the data recording form provided by the vendor to plot the concentration versus the number of organisms adversely affected. The instructions directed that a straight line be drawn between the two data points that were separated by the "50% affected" dotted line on the graphing paper. The concentration at which the line crossed the dotted line was the EC_{50}. This would be useful for a field setting when immediate results are desired. The other method to calculate results, and the method used to calculate the results of this test, was to use the BASIC computer program, entitled LC_{50}, provided by the vendor. For each replicate, the concentration analyzed and the number of organisms adversely affected were the inputs into the program and the outputs were four calculations of the EC_{50} results—the result of three unique mathematical procedures to calculate EC_{50}. In the output file, the four results were identified as the Probit, Spearman, moving average, or binomial distribution methods. According to the vendor, the Probit method should be used as the result unless, because of the nature of the data, that result could not be calculated. In that case, the Spearman, moving average, or binomial distribution methods should be used, in that order of priority. The data in the results section are footnoted if the Probit method was not used.

The standard deviation (S) of the EC_{50} results for the replicate dilution series was calculated, as follows, and used as a measure of precision for each contaminant.

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

where n is the number of replicate dilution series, I_k is the EC_{50} measured for the k^{th} sample, and $\bar{I}$ is the average EC_{50} of the replicate dilution series. The precision results were converted to relative standard deviations (RSDs) using the following equation:

$$\%RSD = \frac{S}{A} \quad (3)$$

where A is the average of the replicate results being evaluated.

5.2 Toxicity Threshold

The toxicity threshold was defined as the lowest concentration of contaminant to cause organisms to be adversely affected to a greater extent than the negative control in all four replicate tests. Within one replicate sample, the negative control was allowed to have three adversely affected organisms. Therefore, for a concentration level of contaminant to adversely affect the organisms to a larger extent than the negative control, at least four organisms, or 22%, had to be adversely affected in each replicate.

5.3 False Positive/Negative Responses

A response would be considered false positive if an unspiked drinking water sample adversely affected more organisms than the negative control. These samples were compared only with a single negative control-filled compartment; therefore, adversely affecting more than one organism constituted a false positive response. Drinking water samples collected from water systems using chlorination and chloramination as the disinfecting process were analyzed in this manner.

A response was considered false negative when IQ Toxicity Test™ was subjected to a lethal concentration of some contaminant in the DDW and did not adversely affect more than four, or 22%, of the organisms in each replicate.

5.4 Field Portability

The results obtained from the measurements made on DDW samples in the laboratory and in the field setting were compiled independently and compared to assess the performance of the IQ Toxicity Test™ under different analysis conditions. Means and standard deviations of the end-points generated in both locations were used to make the comparison. Also, qualitative observations of IQ Toxicity Test™ 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 data describing the percent of organisms adversely affected for nine contaminants at various concentration levels, 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 of organisms adversely affected for each replicate at each concentration, the EC_{50} calculated for each replicate analysis and the average and standard deviation of the EC_{50} concentration for each contaminant. For the applicable contaminants, the percent adversely affected data for the single replicate of tenfold dilutions used to determine what concentration level to dilute and test in replicate is also presented.

6.1.1 Contaminants

The contaminants that were analyzed by the IQ Toxicity Test™ during this verification test, in general, caused the organisms to be adversely affected to a greater extent as the concentration increased. Upon initial analysis of the tenfold dilutions of the lethal dose concentration of each contaminant, aldicarb, dicrotophos, thallium sulfate, and VX adversely affected all of the organisms at one concentration level and, at the concentration level immediately below, adversely affected half or less of the organisms. For these contaminants, the higher of the two concentrations was diluted as described in Section 3.4.2 and analyzed in replicate to more accurately determine the EC_{50}. Cyanide caused all of the organisms to be adversely affected down to the 0.25-mg/L concentration level in the initial tenfold dilution, so additional tenfold dilutions were performed and analyzed in an attempt to determine an EC_{50} for cyanide. Below 0.25 mg/L, no two concentration levels exhibited adverse effects that would meet the criteria for further dilution, so the tenfold dilution of 0.25 mg/L was analyzed in replicate. Colchicine, ricin, and soman adversely affected the organisms in an increasing manner with the increasing concentration of the tenfold dilution concentrations. Therefore, the initial tenfold dilution series of each of these contaminants was analyzed in replicate to determine their EC_{50}. Botulinum toxin, while adversely affecting results at nearly all concentrations, did not exhibit the pronounced increasing effect that some of the other contaminants did. The EC_{50} of one of the replicates of botulinum toxin could not be calculated because of the lack of increasing results. Nonetheless, every contaminant adversely affected the organisms to a greater extent than the negative control at concentrations at or below that of the lethal dose concentration.

Table 6-1a. Aldicarb Percent Inhibition Results

Concentration (mg/L)	Adversely Affected Organisms[(a)] (%)	EC_{50} (mg/L)	Average EC_{50} (mg/L)	%RSD
1.75	11			
3.5	44			
7.0	78	2.0		
14.0	94			
28	100			
1.75	33			
3.5	61			
7.0	89	1.3		
14.0	100			
28	100			
1.75	50		1.7	35
3.5	50			
7.0	100	1.2		
14.0	83			
28	100			
1.75	0			
3.5	61	2.4		
7.0	67			
14.0	78			
28	100			
0.028	17			
0.28	0			
2.8	0			
28	100			
280	100			

[(a)] Each percentage represents one concentration level within each replicate test.

Table 6-1b. Colchicine Percent Inhibition Results

Concentration (mg/L)	Adversely Affected Organisms[(a)] (%)	EC_{50} (mg/L)	Average EC_{50} (mg/L)	%RSD
0.024	17			
0.24	6			
2.4	17	14		
24	61			
240	83			
0.024	6			
0.24	0			
2.4	28	19		
24	56			
240	78			
0.024	11		39	115
0.24	0			
2.4	17	106		
24	39			
240	61			
0.024	11			
0.24	6			
2.4	11	17		
24	61			
240	83			

[(a)] Each percentage represents one concentration level within each replicate test.

Table 6-1c. Cyanide Percent Inhibition Results

Concentration (mg/L)	Adversely Affected Organisms[a] (%)	EC_{50} (mg/L)	Average EC_{50} (mg/L)	%RSD
0.000025	33			
0.00025	17			
0.0025	6	0.25		
0.025	17			
0.25	50			
0.000025	17			
0.00025	11			
0.0025	28	0.062		
0.025	22			
0.25	78		0.16	63
0.000025	39			
0.00025	33			
0.0025	22	0.09		
0.025	17			
0.25	83			
0.000025	0			
0.00025	0			
0.0025	6	0.25		
0.025	6			
0.25	56			
0.025	61			
0.25	94			
2.5	100			
25	100			
250	100			
Field Location				
0.020	8			
0.030	21	[a]	0.070	10
0.060	29			
0.13	72			
0.25	100			

[a] Each percentage represents one concentration level within each replicate test.

Table 6-1d. Dicrotophos Percent Inhibition Results

Concentration (mg/L)	Adversely Affected Organisms[(a)] (%)	EC_{50} (mg/L)	Average EC_{50} (mg/L)	%RSD
0.875	56			
1.75	61			
3.5	94	0.88		
7.0	100			
14	100			
0.875	39			
1.75	56			
3.5	100	1.2		
7.0	100			
14	100			
0.875	50		1.06	20
1.75	61			
3.5	89	0.88		
7.0	100			
14	100			
0.875	39			
1.75	44			
3.5	94	1.3		
7.0	100			
14	100			
0.14	11			
1.4	6			
14	100			
140	100			
1,400	100			

[(a)] Each percentage represents one concentration level within each replicate test.

Table 6-1e. Thallium Sulfate Percent Inhibition Results

Concentration (mg/L)	Adversely Affected Organisms[(a)] (%)	EC_{50} (mg/L)	Average EC_{50} (mg/L)	%RSD
15	0			
30	0			
60	17	164		
120	33			
240	67			
15	0			
30	6			
60	11	123		
120	33			
240	94			
15	0		127	21
30	22			
60	17	119		
120	39			
240	83			
15	11			
30	6			
60	28	102		
120	39			
240	94			
0.24	22			
2.4	22			
24	44			
240	100			
2,400	100			

[(a)] Each percentage represents one concentration level within each replicate test.

Table 6-1f. Botulinum Toxin Percent Inhibition Results

Concentration (mg/L)	Adversely Affected Organisms[a] (%)	EC_{50} (mg/L)	Average EC_{50} (mg/L)	%RSD
0.0000030	39			
0.00030	33			
0.0030	33	0.095[b]		
0.030	44			
0.30	56			
0.0000030	28			
0.00030	50			
0.0030	44	[c]		
0.030	33			
0.30	44			
0.0000030	11		0.084	23
0.00030	33			
0.0030	28	0.062		
0.030	44			
0.30	61			
0.0000030	22			
0.00030	39			
0.0030	28	0.095[d]		
0.030	50			
0.30	50			

[a] Each percentage represents one concentration level within each replicate test.
[b] EC_{50} calculated using moving average method.
[c] EC_{50} could not be calculated because percent of organisms adversely affected did not increase with concentration.
[d] EC_{50} calculated using Spearman method.

Table 6-1g. Ricin Percent Inhibition Results

Concentration (mg/L)	Adversely Affected Organisms[(a)] (%)	EC_{50} (mg/L)	Average EC_{50} (mg/L)	%RSD
0.00015	17			
0.015	39			
0.15	33	0.44		
1.5	56			
15	72			
0.00015	22			
0.015	33			
0.15	33	0.32		
1.5	50			
15	83		0.61	103
0.00015	28			
0.015	39			
0.15	44	0.13		
1.5	61			
15	72			
0.00015	17			
0.015	22			
0.15	33	1.53		
1.5	50			
15	67			

[(a)] Each percentage represents one concentration level within each replicate test.

Table 6-1h. Soman Percent Inhibition Results

Concentration (mg/L)	Adversely Affected Organisms[(a)] (%)	EC_{50} (mg/L)	Average EC_{50} (mg/L)	%RSD
0.000013	6			
0.00013	17			
0.0013	39	0.0016		
0.013	78			
0.13	100			
0.000013	11			
0.00013	28			
0.0013	44	0.0011		
0.013	72			
0.13	100			
0.000013	6		0.0014	21
0.00013	17			
0.0013	44	0.0016		
0.013	72			
0.13	100			
0.000013	6			
0.00013	28			
0.0013	39	0.0012		
0.013	78			
0.13	100			

[(a)] Each percentage represents one concentration level within each replicate test.

Table 6-1i. VX Percent Inhibition Results

Concentration (mg/L)	Adversely Affected Organisms[(a)] (%)	EC_{50} (mg/L)	Average EC_{50} (mg/L)	%RSD
0.0048	11			
0.0095	28			
0.019	39	0.019		
0.038	67			
0.077	100			
0.0048	22			
0.0095	28			
0.019	39	0.020		
0.038	61			
0.077	94		0.020	5.0
0.0048	6			
0.0095	22			
0.019	39	0.021		
0.038	67			
0.077	100			
0.0048	6			
0.0095	28			
0.019	39	0.020		
0.038	67			
0.077	100			
0.0000077	11			
0.000077	6			
0.00077	6			
0.0077	17			
0.077	100			

[(a)] Each percentage represents one concentration level within each replicate test.

6.1.2 Potential Interferences

Table 6-2 presents the results from the samples that were analyzed to test the effect of potential interferences on IQ Toxicity Test™. Aluminum, copper, and iron caused 90% to 100% of the organisms to be adversely affected. In contrast, manganese and zinc caused only one and two organisms, respectively, out of 30 to be adversely affected. It is likely that aluminum, copper, and iron at the tested concentrations would interfere with the results of the IQ Toxicity Test™.

Table 6-2. Potential Interferences Results

Potential Interferences	Concentration (mg/L)	Percent of Adversely Affected Organisms[(a)]
Aluminum	0.36	90
Copper	0.65	100
Iron	0.07	90
Manganese	0.26	3
Zinc	3.5	7
Chlorination by-products (Ohio)	NA	0
Chlorination by-products (Sergeantsville, New Jersey)	NA	0
Chloramination by-products	NA	100

[(a)] Thirty organisms were exposed to each test sample solution.

Water samples from utilities that use chlorination and chloramination as their disinfectant process were analyzed using the IQ Toxicity Test™. The Sergeantsville, New Jersey, water, from a system using chlorination, did not cause any organisms to be adversely affected; while the St. Petersburg, Florida, water, from a system that uses chloramination for disinfection, adversely affected all the organisms tested. The chloraminated water may interfere with the IQ Toxicity Test™ results. As described in Section 3.4.2, the Sergeantsville, New Jersey, water was used because the organisms failed the pre-test acceptance criteria when maintained in the Columbus, Ohio, water sample. However, during the verification test, the organisms maintained in Sergeantsville, New Jersey, water were exposed to the Columbus, Ohio, water; and no organisms were adversely affected. These results illustrate that it is necessary to know the extent to which the organisms will be able to survive in a clean water sample of the same matrix as that being tested. Otherwise, there is a definite risk of the background water interfering with the IQ Toxicity Test™.

6.1.3 Precision

For all the contaminants, the relative standard deviation of the EC_{50} was calculated and reported for each set of four replicates to evaluate the IQ Toxicity Test™ precision. The relative standard deviation of the EC_{50} for all the contaminants except colchicine, cyanide, and ricin were below 35%. These three contaminants were expected to have more variability because the tenfold dilution series were used to determine the EC_{50}.

6.2 Toxicity Threshold

Table 6-3 gives the toxicity thresholds (i.e., the lowest concentration of contaminant to cause organisms to be adversely affected to a greater extent than the negative control in all four replicate tests) for each contaminant. The lowest toxicity threshold concentration was for botulinum toxin at 0.00030 mg/L, indicating that IQ Toxicity Test™ was most sensitive to botulinum toxin.

Table 6-3. Toxicity Thresholds

Contaminant	Concentration (mg/L)
Aldicarb	3.5
Colchicine	24
Cyanide	0.25
Dicrotophos	0.88
Thallium sulfate	120
Botulinum toxin	0.00030
Ricin	0.015
Soman	0.0013
VX	0.0095

6.3 False Positive/Negative Responses

False positive responses were observed for unspiked drinking water samples from the system that uses chloramination as its disinfectant process. All the organisms were adversely affected in this water sample. The water from a system using chlorinated, Sergeantsville, New Jersey, drinking water did not cause the organisms to be adversely affected and therefore could serve as the water matrix for this verification test. However, prior to testing, another water sample from a system using chlorination (Columbus, Ohio) was used for the water matrix; and, as discussed in Section 6.1.2, it did adversely affect the organism to the extent that the water sample could not be used. Therefore, care needs to be taken, no matter what type of water sample is used, that its effect on the organisms is known prior to doing a test. If the organisms are adversely affected in "clean" drinking water, they cannot indicate the presence of contamination.

There were no false negative responses for this technology (Table 6-4). At the lethal dose concentrations, each contaminant adversely affected the organisms to a greater extent than that of the negative control.

Table 6-4. False Negative Responses

Contaminant	Lethal Dose Concentration (mg/L)	False Negative Response
Aldicarb	280	no
Colchicine	240	no
Cyanide	250	no
Dicrotophos	1,400	no
Thallium sulfate	2,400	no
Botulinum toxin	0.30	no
Ricin	15	no
Soman[(a)]	0.13	no
VX[(a)]	0.077	no

[(a)] Due to the degradation in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose of soman was 44% of the expected concentration of 0.30 mg/L and for VX was 38% of the expected concentration of 0.20 mg/L.

6.4 Field Portability

A single solution of cyanide was prepared and diluted by factors of 2, 4, 8, and 16 and analyzed in replicate at a field location to examine the ability of IQ Toxicity Test™ to be used in a non-laboratory setting. IQ Toxicity Test™ and necessary accessories were transported to the field in a medium-sized cardboard box. Fully loaded, the box weighed about five pounds. At the field location, the organisms were selected much like they were in the laboratory. The black light supplied in the starter kit was operated with batteries on a small table in the basement of a house. Working with a table at the field location greatly facilitates the collection and transfer of organisms. Table 6-1c shows the results of the cyanide samples analyzed at the field location, along with the results of the cyanide samples analyzed in the laboratory. The EC_{50} measured for cyanide in the field was 0.07 mg/L, with a relative standard deviation of 10%; and the EC_{50} for cyanide measured in the laboratory was 0.16 mg/L, with a relative standard deviation of 63%. However, in the field setting, the smaller dilution range was used instead of the tenfold dilution series used in the laboratory. These EC_{50} results were not significantly different from one another, considering the uncertainty around the measurements. These results indicate that IQ Toxicity Test™ functioned similarly in the field and in the laboratory. To perform the tests, the *Daphnia magna* had to be cultured and less than seven days old. Culturing the organisms from the starter culture provided by the vendor takes three to four days, which may be problematic for field testing on short notice.

6.5 Other Performance Factors

The instruction manual for IQ Toxicity Test™ was easy to understand, which, in addition to approximately 12 hours with the vendor becoming familiar with performing the tests, enabled operators to become adept at analyzing multiple sample sets. Although the operators had scientific backgrounds, based on 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. Given this level of instruction, it seems that operators with little or no technical training could successfully analyze sample sets as long as their fine motor skills were adequate for capturing the organisms from a bulk solution of water using a bulb pipette. The operators analyzed 25 sets of three exposure chambers per day.

This verification test did not include the process of culturing *Daphnia magna*, which either has to be done by a vendor or by the user. The culturing process takes three to four days, and the organisms need to be used before they are seven days old.

Chapter 7
Performance Summary

Parameter	Compound	Lethal Dose Conc. (mg/L)	EC_{50} for each Replicate (mg/L)				Avg. EC_{50} (mg/L)	%RSD	Toxicity Thresh. (mg/L)
			1	2	3	4			
Contaminants in DDW	Aldicarb	280	2.4	1.2	1.3	2.0	1.7	35	3.5
	Colchicine	240	17	106	19	14	39	115	24
	Cyanide	250	0.25	0.09	0.062	0.25	0.16	63	0.25
	Dicrotophos	1,400	1.3	0.88	1.2	0.88	1.06	20	0.88
	Thallium sulfate	2,400	102	119	123	164	127	21	120
	Botulinum toxin[a]	0.30	0.095	0.062	[b]	0.095	0.084	23	0.00030
	Ricin[c]	15	1.53	0.13	0.32	0.44	0.61	103	0.015
	Soman	0.13[d]	0.0012	0.0016	0.0011	0.0016	0.0014	21	0.0013
	VX	0.077[d]	0.020	0.021	0.020	0.019	0.020	5.0	0.0095
Potential interferences in DDW	**Interference**	**Conc. (mg/L)**	**Average Inhibitions at a Single Concentration (%)**						
	Aluminum	0.36	90						
	Copper	0.65	100						
	Iron	0.069	90						
	Manganese	0.26	3						
	Zinc	3.5	7						
False positive responses	All the organisms exposed to drinking water from a system disinfected by chloramination were adversely affected, indicating that false positive responses are possible for these samples. Because drinking water from Columbus, Ohio, a system that used chlorination as its disinfecting process, did not provide an environment conducive to maintaining *Daphnia magna*, a water sample from Sergeantsville, New Jersey, was used as the sample matrix. The water sample from the New Jersey system, which uses chlorination, did not adversely affect the *Daphnia magna*. If the background water sample causes the organisms to be adversely affected in the absence of contaminants, this test should not be used because the results will be false positive.								
False negative responses	There were no false negative responses. Each contaminant caused adverse effects to the organisms below the lethal dose concentration for human toxicity.								
Field portability	An EC_{50} of 0.16 mg/L with an RSD of 61% was measured in the laboratory, while an EC_{50} of 0.070 mg/L with an RSD of 10% was measured in the field. When the uncertainties of these results are considered, the results show that the IQ Toxicity Test™ performed similarly in both locations. A supply of *Daphnia magna* must be maintained to facilitate short-notice field testing.								
Other performance factors	The instruction manual was easy to understand. Although the operators had scientific backgrounds, it seems likely that operators with little technical training would probably be able to successfully analyze sample sets if their fine motor skills were adequate. Operators analyzed 25 sets of three exposure chambers per day. The test did not include culturing *Daphnia magna*.								

[a] Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.

[b] EC_{50} could not be calculated because percent of organisms adversely affected did not increase with concentration.

[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 in water, the stock solution confirmation analysis confirmed that the concentration of the lethal dose was 44% of the expected concentration of 0.30 mg/L for soman, and 38% of the expected concentration of 0.20 mg/L for VX.

Chapter 8
References

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