

June 2006

Environmental Technology Verification Report

AQUA SURVEY, INC.
CHEM-IQ TOX™ TEST KIT

Prepared by
Battelle

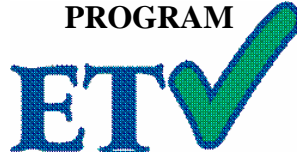
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THE ENVIRONMENTAL TECHNOLOGY VERIFICATION
PROGRAM



ETV Joint Verification Statement

| | | |
|-------------------------|--|--|
| TECHNOLOGY TYPE: | Rapid Toxicity Testing System | |
| APPLICATION: | Detecting Toxicity in Drinking Water | |
| TECHNOLOGY NAME: | Chem-IQ Tox™ | |
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The U.S. Environmental Protection Agency (EPA) has established the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative or improved 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. Information and ETV documents are available at www.epa.gov/etv.

ETV works in partnership with recognized standards and testing organizations, with stakeholder groups (consisting of buyers, vendor organizations, and permittees), and with 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 Advanced Monitoring Systems (AMS) Center, one of six technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center evaluated the performance of the Aqua Survey, Inc. Chem-IQ Tox™ Test Kit. This verification statement provides a summary of the test results.

VERIFICATION TEST DESCRIPTION

Rapid toxicity technologies use various biological organisms and chemical reactions to indicate the presence of toxic contaminants. The toxic contaminants are indicated by a change or appearance of color or a change in intensity. As part of this verification test, the Chem-IQ Tox™ Test Kit was subjected to various concentrations of contaminants such as industrial chemicals, pesticides, rodenticides, pharmaceuticals, nerve agents, and biological toxins. Each contaminant was added to separate drinking water samples and analyzed. In addition to determining whether the Chem-IQ Tox™ Test Kit could detect the toxicity caused by each contaminant, its response to interfering compounds, such as water treatment chemicals and by-products in clean drinking water, was evaluated.

The Chem-IQ Tox™ Test Kit was evaluated by

- Endpoints and precision—percent inhibition for all concentration levels of contaminants and potential interfering compounds and precision of replicate analyses
- Toxicity threshold for each contaminant—contaminant level at which higher concentrations generate inhibition significantly greater than the negative control and lower concentrations do not. Note that Aqua Survey, Inc. recommends that a 20% inhibition is required for a conclusive indication of toxicity. During this test, a thorough evaluation of the toxicity threshold was performed. Therefore, the toxicity threshold was determined with respect to the negative control rather than the 20% inhibition threshold
- False positive responses—chlorination and chloramination by-product inhibition with respect to unspiked American Society for Testing and Materials Type II deionized water samples
- False negative responses—contaminants that were reported as producing inhibition less than 20% when present at lethal concentrations (the concentration at which 250 milliliters of water would probably cause the death of a 154-pound person) or negative background inhibition that caused falsely low inhibition
- Other performance factors (sample throughput, ease of use, reliability).

The Chem-IQ Tox™ Test Kit was verified by analyzing a dechlorinated drinking water sample from Columbus, Ohio (DDW), fortified with contaminants (at concentrations ranging from lethal levels to concentrations up to 1,000 times less than the lethal dose) and interferences (metals possibly present as a result of the water treatment processes). Dechlorinated water was used because free chlorine can interfere with the performance of the test and can degrade the contaminants during storage. Inhibition (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the Chem-IQ Tox™ Test Kit to detect toxicity, as well as to measure the precision of the Chem-IQ Tox™ Test Kit results. The response of the Chem-IQ Tox™ Test Kit to possible interferents was evaluated by analyzing them at one-half of the concentration limit recommended by the EPA's National Secondary Drinking Water Regulations guidance. For analysis of by-products of the chlorination process, the unspiked DDW was analyzed because Columbus, Ohio, uses chlorination as its disinfectant procedure. For the analysis of by-products of the chloramination process, a separate drinking water sample was obtained from the Metropolitan Water District of Southern California (LaVerne, California), which uses chloramination as its disinfection process. The samples were analyzed after residual chlorine was removed using sodium thiosulfate. Sample throughput was measured based on the number of samples analyzed per hour. Ease of use and reliability were determined based on documented observations of the operators.

Quality control samples included method blank samples, which consisted of American Society for Testing and Materials Type II deionized water; positive control samples (fortified with copper chloride); and negative control samples, which consisted of the unspiked DDW.

QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted a technical systems audit, a performance evaluation audit, and a data quality audit of 10% of the test data.

This verification statement, the full report on which it is based, and the test/QA plan for this verification test are all available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

The following description of the Chem-IQ Tox™ Test Kit is based on information provided by the vendor. This technology description was not verified in this test.

The Chem-IQ Tox™ Test Kit detects toxicants in drinking water using a chemical reaction that generates fluorescence. The test can be conducted by a technician with basic laboratory skills. Sample analysis is performed by adding two reagents to test and control water samples and measuring each sample's fluorescence with a calibrated fluorometer. Percent inhibition values are calculated by comparing the light production of the control with that of the test samples. If the average percent inhibition value of the replicate test samples is greater than 20%, the test water sample is considered significantly impacted by a toxicant and considered a positive response.

The Chem-IQ Tox™ Test Kit, which costs \$250, contains 30 vials each of two reagents, 90 IQ Exposure Chambers, disposal reagent pipettes, Chem-IQ Tox™ Test Kit score cards, and a Sharpie pen. Materials and laboratory equipment required for the test include an Aquafluor™ hand-held fluorometer (Turner Design) or equivalent and a supply of non-fluorescing 4 milliliter cuvettes (10 millimeter by 10 millimeter); an automatic pipetter or equivalent with appropriate disposable tips for dispensing 10-milliliter, 250-microliter, and 50-microliter volumes; a PC3 liquid sonicator (L&R) or equivalent; a magnetic stir plate and stir bar (1/8 inch diameter); a distilled or deionized water supply; and a digital timer that displays seconds.

VERIFICATION RESULTS

| Parameter | Compound | Lethal Dose (LD) Conc. (mg/L) | Average Inhibition at Concentrations Relative to the LD Concentration (%) | | | | Range of Standard Deviations (%) | Toxicity Thresh. (mg/L) |
|-------------------------|---|-------------------------------|---|-------------------------------|--------|-------------------------------|----------------------------------|-------------------------|
| | | | LD | LD/10 | LD/100 | LD/1,000 | | |
| Contaminants in DDW | Aldicarb | 260 | -16 | 13 | -33 | 7 | 9-32 | ND |
| | Botulinum toxin complex B | 0.3 | -62 | 1 | 4 | 5 | 3-8 | ND |
| | Colchicine | 240 | 104 | 63 | 17 | 42 | 2-15 | 24 |
| | Cyanide | 250 | 63 | 47 | -21 | -18 | 2-24 | 25 |
| | Dicrotophos | 1,400 | -55 | -30 | -38 | -13 | 8-41 | ND |
| | Nicotine | 2,800 | 50 | 84 | 71 | -3 | 1-12 | 28 |
| | Ricin | 15 | -44 | -13 | 10 | -3 | 4-12 | ND |
| | Soman | 1.4 | 16 | -8 | 19 | 7 | 1-6 | ND |
| | Thallium sulfate | 2,800 | 66 | 16 | -5 | -25 | 3-13 | 2,800 |
| | VX | 2 | -44 | -36 | -14 | -13 | 8-26 | ND |
| | | Interference | Conc. (mg/L) | Average Inhibition (%) | | Standard Deviation (%) | | |
| | Aluminum | 0.5 | 4 | | 9 | | | |
| | Copper | 0.6 | 46 | | 3 | | | |
| | Iron | 0.15 | -26 | | 20 | | | |
| | Manganese | 0.25 | 11 | | 9 | | | |
| | Zinc | 2.5 | 34 | | 2 | | | |
| False positive response | Because DI water did not generate any measurable background light, the disinfection by-product samples could not be compared with the inhibition due to DI water. Therefore only the absolute light units produced by the chlorinated and chloraminated samples could be measured. Both of these samples left adequate light for subsequent inhibition due to contamination and are thus not considered to have generated false positive results. | | | | | | | |
| False negative response | False negative responses (inhibition less than 20%) were generated for aldicarb, botulinum toxin, complex B, dicrotophos, ricin, soman, and VX when they were analyzed at the lethal dose concentration. | | | | | | | |
| Ease of use | The Chem-IQ Tox™ Test Kit instructions were clearly written; but a condensed summary with only the necessary steps may be helpful. The contents of the Chem-IQ Tox™ Test Kit were well identified. The test was not difficult to perform, but analyzing several samples simultaneously required practice. No formal scientific education would be required to use the Test Kit. | | | | | | | |
| Field portability | The Chem-IQ Tox™ Test Kit was transported from a laboratory to a storage room to simulate a non-laboratory location. All materials were easily transported by one person in a small cardboard box. The Test Kit was set up in less than 10 minutes, except that Reagent Two took approximately 20 minutes to thaw. A source of electricity was required for the sonicator, while the fluorometer ran on batteries. A cooler to transport and store reagents, pipettes and tips, the sonicator and a power source, the fluorometer, and a waste container were needed for field use. Results were obtained within 10 minutes of starting the test. | | | | | | | |
| Throughput | Approximately 30 analyses were completed in one hour. The 30 analyses included method blanks, positive controls, and test samples. Approximately 130 samples could be processed per pair of Reagent One and Reagent Two vials. | | | | | | | |

ND = Significant inhibition was not detected.

| | | | |
|--|----------------|--|---------------|
| <u>Original signed by Gregory A. Mack</u> | <u>6/22/06</u> | <u>Original signed by Andrew P. Avel</u> | <u>8/7/06</u> |
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| Battelle | | Office of Research and Development | |
| | | U.S. Environmental Protection Agency | |

NOTICE: ETV verifications are based on an evaluation of technology performance under specific, predetermined criteria and the appropriate quality assurance procedures. EPA and Battelle make no expressed or implied warranties as to the performance of the technology and do not certify that a technology will always operate as verified. The end user is solely responsible for complying with any and all applicable federal, state, and local requirements. Mention of commercial product names does not imply endorsement.

June 2006

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

Aqua Survey, Inc.
Chem-IQ ToxTM Test Kit

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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 permittees, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of six environmental technology centers. Information about each of these centers can be found on the Internet at <http://www.epa.gov/etv/>.

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

Acknowledgments

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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 |
| DPD | n,n-diethyl-p-phenylenediamine |
| EPA | U.S. Environmental Protection Agency |
| ETV | Environmental Technology Verification |
| HDPE | high-density polyethylene |
| LD | lethal dose |
| mM | millimolar |
| μL | microliter |
| mg/L | milligram per liter |
| mL | milliliter |
| mm | millimeter |
| NSDWR | National Secondary Drinking Water Regulations |
| %D | percent difference |
| PE | performance evaluation |
| QA | quality assurance |
| QC | quality control |
| QMP | quality management plan |
| SOP | standard operating procedure |
| TSA | technical systems audit |

Chapter 1 Background

The U.S. Environmental Protection Agency (EPA) supports the Environmental Technology Verification (ETV) Program to facilitate the deployment of innovative environmental technologies through performance verification and dissemination of information. The goal of the ETV Program is to further environmental protection by accelerating the acceptance and use of improved and cost-effective technologies. ETV seeks to achieve this goal by providing high-quality, peer-reviewed data on technology performance to those involved in the design, distribution, financing, permitting, purchase, and use of environmental technologies.

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permittees; 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. Chem-IQ Tox™ Test Kit. Rapid toxicity technologies were identified as a priority verification category through the AMS Center stakeholder process.

Chapter 2 Technology Description

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

The Chem-IQ Tox™ Test Kit (Figure 2-1) detects toxicants in drinking water using a chemical reaction that generates fluorescence. The test can be conducted by a technician with basic



Figure 2-1 Aqua Survey, Inc. Chem-IQ Tox™ Test Kit

laboratory skills. Sample analysis is performed by adding two reagents to test and control water samples and measuring each sample's fluorescence with a calibrated fluorometer. Percent inhibition values are calculated by comparing the light production of the control with that of the test samples. If the average percent inhibition value of the replicate test samples is greater than 20%, the test water sample is considered significantly impacted by a toxicant and considered a positive response.

The Chem-IQ Tox™ Test Kit, which costs \$250, contains 30 vials each of two reagents, 90 IQ Exposure Chambers, disposal reagent pipettes, Chem-IQ Tox™ Test Kit score cards, and a Sharpie pen. Materials and laboratory equipment required for the test include an Aquafluor™ hand-held fluorometer (Turner Design) or equivalent and a supply of non-fluorescing 4-milliliter cuvettes (10 millimeter by 10 millimeter); an automatic pipetter or equivalent with appropriate disposable tips for dispensing 10-milliliter, 250-microliter, and 50-microliter volumes; a PC3 liquid sonicator (L&R) or equivalent; a magnetic stir plate and stir bar (1/8 inch diameter); a distilled or deionized water supply; and a digital timer that displays seconds.

Chapter 3 Test Design

The objective of this verification test of rapid toxicity technologies was to evaluate their ability to detect certain toxins and to determine their susceptibility to interfering chemicals in a controlled experimental matrix. Rapid toxicity technologies do not identify or determine the concentration of specific contaminants, but serve as a screening tool to quickly determine whether water is potentially toxic.

As part of this verification test, Chem-IQ Tox™ Test Kit was subjected to various concentrations of contaminants such as industrial chemicals, pesticides, rodenticides, pharmaceuticals, nerve agents, and biological toxins. Each contaminant was added to separate drinking water samples and analyzed. In addition to determining whether Chem-IQ Tox™ Test Kit can detect the toxicity caused by each contaminant, its response to interfering compounds such as water treatment chemicals and by-products in clean drinking water was evaluated. Table 3-1 shows the contaminants and potential interferences that were evaluated during this verification test.

This verification test was conducted from August to December 2005 according to procedures specified in the *Test/QA Plan for Verification of Rapid Toxicity Technologies* including Amendments 1 and 2.⁽¹⁾ Chem-IQ Tox™ Test Kit was verified by analyzing a dechlorinated drinking water sample from Columbus, Ohio (hereafter in this report, referred to as DDW), fortified with various concentrations of the contaminants and interferences shown in Table 3-1. Where possible, the concentration of each contaminant or potential interference was confirmed independently by Aqua Tech Environmental Laboratories (ATEL), Marion, Ohio, or by Battelle, depending on the analyte.

Chem-IQ Tox™ Test Kit was evaluated by

- Endpoints and precision—percent inhibition for all concentration levels of contaminants and potential interfering compounds and precision of replicate analyses
- Toxicity threshold for each contaminant—contaminant level at which higher concentrations generate inhibition significantly greater than the negative control and lower concentrations do not. Note that Aqua Survey, Inc. recommends that a 20% inhibition is required for a conclusive indication of toxicity. During this test, a thorough evaluation of the toxicity threshold was performed. Therefore, the toxicity threshold was determined with respect to the negative control rather than the 20% inhibition threshold

Table 3-1. Contaminants and Potential Interferences

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

- False positive responses—chlorination and chloramination by-product inhibition exceeding 20% with respect to unspiked American Society for Testing and Materials (ASTM) Type II deionized (DI) water samples
- False negative responses—contaminants that were reported as producing inhibition less than 20% when present at lethal concentrations or negative background inhibition that could cause falsely low inhibition
- Other performance factors (sample throughput, ease of use, reliability).

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

The response of Chem-IQ Tox™ Test Kit to compounds used during the water treatment process (identified as potential interferences in Table 3-1) was evaluated by analyzing separate aliquots of DDW fortified with each potential interference at one-half of the concentration limit recommended by the EPA’s National Secondary Drinking Water Regulations (NSDWR)⁽²⁾ guidance. For analysis of by-products of the chlorination process, the unspiked DDW was analyzed because Columbus, Ohio, uses chlorination as its disinfectant procedure. For the analysis of by-products of the chloramination process, a separate drinking water sample was obtained from the Metropolitan Water District of Southern California (LaVerne, California), which uses chloramination as its disinfection process. The samples were analyzed after residual chlorine was removed using sodium thiosulfate. Sample throughput was measured based on the

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

3.1 Test Samples

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

3.1.1 Quality Control Samples

QC samples included method blanks, positive controls, negative controls, and preservative blanks. The method blank samples consisted of ASTM Type II DI water and were used to ensure that no sources of contamination were introduced in the sample handling and analysis procedures. A solution consisting of 600 mg/L copper was used as a positive control. While performance limits were not placed on the results, significant inhibition for the positive control sample indicated to the operator that Chem-IQ Tox™ Test Kit was functioning properly. The negative control sample consisted of unspiked DDW and was used to set a background inhibition of the DDW, the matrix in which each test sample was prepared. To ensure that the preservatives in the contaminant solutions did not have an inhibitory effect, preservative blank samples were prepared. These preservative blanks consisted of DDW fortified with a concentration of preservative equivalent to that in the test solutions of botulinum toxin complex B, ricin, soman, and VX.

3.1.2 Drinking Water Fortified with Contaminants

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

A stock solution of each contaminant was prepared in DDW at concentrations at or above the lethal dose level. The stock solution was further diluted to obtain one sample containing the lethal dose concentration for each contaminant and three additional samples with concentrations 10, 100, and 1,000 times less than the lethal dose. Table 3-2 lists each concentration level and the number of samples analyzed at each level.

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

| Type of Sample | Sample Characteristics | Concentration Levels | No. of Sample Analyses |
|--|---|--|---------------------------|
| Quality control | Method blank (ASTM Type II water) | NA | 15 |
| | Positive control | 600 mg/L copper | 15 |
| | Negative control (unspiked DDW) | NA | 60 |
| | Preservative blank: botulinum toxin complex B | 0.015 millimolar (mM) sodium citrate | 4 |
| | Preservative blank: VX and soman | 0.21% isopropyl alcohol | 4 with VX, 4 with soman |
| | Preservative blank: ricin | 0.00024% NaN ₃ , 0.00045 molar NaCl, 0.03mM phosphate | 4 |
| DDW fortified with contaminants | Aldicarb | 260; 26; 2.6; 0.26 milligrams/liter (mg/L) | 4 per concentration level |
| | Botulinum toxin complex B | 0.3; 0.03; 0.003; 0.0003 mg/L | 4 per concentration level |
| | Colchicine | 240; 24; 2.4; 0.24 mg/L | 4 per concentration level |
| | Cyanide | 250; 25; 2.5; 0.25 mg/L | 4 per concentration level |
| | Dicrotophos | 1,400; 140; 14; 1.4; mg/L | 4 per concentration level |
| | Nicotine | 2,800; 280; 28; 2.8 mg/L | 4 per concentration level |
| | Ricin | 15; 1.5; 0.15; 0.015 mg/L | 4 per concentration level |
| | Soman | 1.4; 0.14; 0.014; 0.0014 mg/L | 4 per concentration level |
| | Thallium sulfate | 2,800; 280; 28; 2.8 mg/L | 4 per concentration level |
| VX | 2.0; 0.2; 0.02; 0.002 mg/L | 4 per concentration level | |
| DDW fortified with potential interferences | Aluminum | 0.5 mg/L | 4 |
| | Copper | 0.6 mg/L | 4 |
| | Iron | 0.15 mg/L | 4 |
| | Manganese | 0.25 mg/L | 4 |
| | Zinc | 2.5 mg/L | 4 |
| Disinfectant by-products | Chloramination by-products | NA | 4 |
| | Chlorination by-products | NA | 60 |

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

3.1.3 Drinking Water Fortified with Potential Interferences

Individual aliquots of the DDW were fortified with one-half the concentration specified by the EPA's NSDWR for each potential interference. Table 3-2 lists the interferences, along with the concentrations at which they were tested. Four replicates of each of these samples were analyzed. To test the sensitivity of Chem-IQ Tox™ Test Kit to by-products of the chlorination process as potential interferences, the unspiked DDW (same as the negative control) was used since the water sample originated from a utility that uses chlorination as its disinfectant procedure. In a similar manner, the by-products of the chloramination process were evaluated using a water sample from the Metropolitan Water District of Southern California. The residual chlorine in both of these samples was removed using sodium thiosulfate, and then the samples were analyzed in replicate with no additional fortification of contaminants.

3.2 Test Procedure

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

3.2.1 Test Sample Preparation and Storage

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

3.2.2 Test Sample Analysis Procedure

To analyze the test samples, kit reagents were prepared as specified in the instructions. This involved diluting Reagent One with ASTM Type II water and sonicating for 60 seconds. Reagent Two was removed from freezer storage and allowed to thaw. The fluorometer was calibrated using ASTM Type II water as the blank and DDW as the control water sample prepared as a "standard sample." Five milliliters of each test sample, as well as the negative and positive control samples were placed in an individual cell in the exposure chamber. Reagent One, 125µL, was added to each cell. Then, 25 µL of Reagent Two were added to each cell, and the time was recorded. The contents of the cell were gently mixed by aspirating and dispensing using a

disposable pipette. A 2- to 3-mL aliquot from each cell was transferred to an individual cuvette. Fluorescence was measured exactly 10 minutes after the addition of Reagent Two. Aqua Survey recommends analyzing at least three replicate samples to conclusively determine inhibition.

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

3.2.3 Stock Solution Confirmation Analysis

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

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

Table 3-3. Stock Solution Confirmation Results

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

NA = Not applicable.

^(a) No standard method available. QA audits and balance calibration assured accurately prepared solutions.

^(b) Target concentration was highest concentration for each contaminant or interference on Table 3-2.

Table 3-4. Water Quality Parameters

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

NTU = nephelometric turbidity unit.

Chapter 4

Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center⁽¹¹⁾ and the test/QA plan for this verification test.⁽¹⁾

4.1 Quality Control of Stock Solution Confirmation Methods

The stock solutions for the contaminants cyanide and thallium sulfate and for the potential interferences aluminum, magnesium, zinc, iron, and copper were analyzed at ATEL using a standard reference method. As part of ATEL's standard operating procedures (SOPs), various QC samples were analyzed with each sample set. These included matrix spike, laboratory control spike, and method blank samples. According to the standard methods used for the analyses, recoveries of the QC spike samples analyzed with samples from this verification test were within acceptable limits of 75% to 125%, and the method blank samples were below the detectable levels for each analyte. For VX, soman, aldicarb, nicotine, and dicotophos, the confirmation analyses were performed at Battelle using a Battelle SOP or method. Calibration standard recoveries of VX and soman were always between 62% and 141%, and most of the time were between 90% and 120%. Dicotophos standard recoveries ranged from 89% to 122%. Aldicarb standard recoveries ranged from 95% to 120%. Nicotine standard recoveries ranged from 96% to 99%. Standard analytical methods for colchicine, ricin, and botulinum toxin complex B were not available and, therefore, not performed. QA audits and balance calibrations assured that solutions for these compounds were accurately prepared.

4.2 Quality Control of Drinking Water Samples

A method blank sample consisting of ASTM Type II DI water was analyzed once by Chem-IQ Tox™ Test Kit for approximately every 10 drinking water samples that were analyzed. Because inhibition has to be calculated with respect to a control sample, none were calculated for the method blank samples. The method blanks were used as the control for calculating the inhibition of the DDW for the disinfecting by product evaluation. A positive control sample of 600 mg/L copper also was analyzed once for approximately every 10 drinking water samples. While performance limits were not placed on the results of the positive control sample, if the positive control samples did not cause nearly complete inhibition, it would indicate to the operator that Chem-IQ Tox™ Test Kit was not functioning properly. For 15 positive control samples, an inhibition of 88% ± 12% was measured. This inhibition indicated that Chem-IQ Tox™ was

functioning properly. A negative control sample (unspiked DDW) was analyzed with approximately every four samples. The percent inhibition calculation for each sample incorporated the average inhibition of the negative control samples analyzed with that particular sample set; therefore, by definition, the average inhibition of four negative control samples was 0%.

4.3 Audits

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

4.3.1 Performance Evaluation Audit

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

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

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

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

4.3.2 Technical Systems Audit

The Battelle Quality Manager conducted a TSA to ensure that the verification test was performed in accordance with the test/QA plan⁽¹⁾ and the AMS Center QMP.⁽¹¹⁾ As part of the audit, the Battelle Quality Manager reviewed the contaminant standard and stock solution confirmation methods, compared actual test procedures with those specified in the test/QA plan, and reviewed data acquisition and handling procedures. Observations and findings from this audit were 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

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

4.3.3 Audit of Data Quality

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

4.4 QA/QC Reporting

Each internal assessment and audit was documented in accordance with Sections 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center.⁽¹¹⁾ Once the assessment report was prepared, the Battelle Verification Test Coordinator ensured that a response was provided for each adverse finding or potential problem and implemented any necessary follow-up corrective action. The Battelle Quality Manager ensured that follow-up corrective action was taken. The results of the TSA were sent to the EPA.

4.5 Data Review

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

Table 4-2. Summary of Data Recording Process

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

^(a) All activities subsequent to data recording were carried out by Battelle.

Chapter 5

Statistical Methods and Reported Parameters

The statistical methods presented in this chapter were used to verify the performance parameters listed in Section 3.

5.1 Endpoints and Precision

The fluorometer provided with the Chem-IQ Tox™ Test Kit reported the fluorescence for each sample analyzed. Each test sample was compared with a negative control sample that, for this verification test, was unspiked DDW. This comparison was made by accounting for the inhibition of the negative control in the calculation of the percent inhibition. Therefore, the percent inhibition of the four negative control samples within each sample set always averaged zero. The percent inhibition for each sample was calculated using the following equation:

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

Where L_{sample} is the fluorescence produced for the test samples and $\bar{L}_{\text{negative control}}$ is the average fluorescence of the replicate negative control sample analyzed with each sample set. For each test sample, the negative control sample was always DDW, except when the inhibition of the disinfectant by-products was being determined, in that case, ASTM Type II DI water served as the control sample.

The standard deviation (SD) of the results for the replicate samples was calculated, as follows, and used as a measure of technology precision at each concentration. The standard deviation around the average negative control results represented the variability of the inhibition caused by the negative control water. Similarly, the SD of the rest of the contaminant concentrations represented the precision of the inhibition caused by the background water combined with the contaminant.

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

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

5.2 Toxicity Threshold

The toxicity threshold was defined as the lowest concentration of contaminant to exhibit a percent inhibition significantly greater than the negative control. Also, each concentration level higher than the toxicity threshold had to be significantly greater than the negative control, and the inhibition produced by each lower concentration analyzed had to be significantly less than that produced by the toxicity threshold concentration. Since the inhibition of the test samples was calculated with respect to the inhibition of each negative control sample, the percent inhibition of the negative control was always zero. A significant differences in the inhibition at two concentration levels required that the average inhibition at each concentration level, plus or minus its respective standard deviation, did not overlap.

Aqua Survey, Inc. suggests that a 20% inhibition be attained for a conclusive indication of toxicity; however, for this test, a more thorough evaluation of sensitivity was performed. Therefore, the toxicity threshold was determined as described here, and the 20% inhibition threshold was used for the false negative/false positive evaluation.

5.3 False Positive/Negative Responses

A response was considered false positive if an unspiked drinking water sample produced an inhibition exceeding 20% when determined with respect to DI water. Depending on the degree of inhibition in the sample, toxicity from subsequent contamination of that sample may not be detectable or could be exaggerated as a result of the baseline inhibition. Drinking water samples collected from water systems using chlorination and chloramination as the disinfecting process were analyzed in this manner.

A response was considered false negative if, when a lethal concentration of some contaminant was analyzed, the average inhibition did not exceed 20%, was not significantly different from the negative control, and was not significantly different from the other concentration levels analyzed (for a lethal dose inhibition less than 100%). The inhibition of the lethal dose sample was required to be significantly greater than the other concentration levels because it more thoroughly incorporated the uncertainty of all the measurements made by the Chem-IQ Tox™ Test Kit in determining false negative results. A difference was considered significant if the average inhibition plus or minus the standard deviation did not encompass the value or range of values that were being compared.

5.4 Other Performance Factors

Ease of use (including clarity of the instruction manual, user-friendliness of software, and overall convenience) was qualitatively assessed throughout the verification test through documented observations of the operators and Verification Test Coordinator. Sample throughput was evaluated quantitatively based on the number of samples that could be analyzed per hour.

Chapter 6 Test Results

6.1 Endpoints and Precision

Tables 6-1 a-j present the percent inhibition data for 10 contaminants; and Table 6-2 gives the percent inhibition for preservatives with concentrations similar to what would be contained in a lethal dose of botulinum toxin complex B, ricin, soman, and VX. Given in each table are the concentrations analyzed, the percent inhibition for each replicate at each concentration, and the average and standard deviation of the inhibition of the four replicates at each concentration. Contaminant test samples that produced negative percent inhibition values indicated an increase in light production by the Chem-IQ Tox reagents and were considered non-toxic.

6.1.1 Contaminants

The contaminants that generated inhibition significantly greater than the negative control included colchicine, cyanide, nicotine, soman, and thallium sulfate. Colchicine and cyanide generated detectable inhibition at the two highest concentration levels analyzed, nicotine at the three highest concentration levels, and thallium sulfate at only the highest concentration level. Alternatively, dicrotophos produced an average negative inhibition at all four concentration levels and aldicarb resulted in some negative and some positive average inhibition, depending on the concentration level; but no concentration level of aldicarb produced an average inhibition that differed significantly from the negative control.

It is important to note that the botulinum toxin complex B, ricin, soman, and VX stock solutions used to prepare the test samples were stored in various preservatives that included sodium azide, sodium chloride, and sodium phosphate for ricin; sodium citrate only for botulinum toxin complex B, and isopropyl alcohol for soman and VX. During the previous ETV test of this technology category, the preservatives were not accounted for in the negative control; therefore, the results from each test should be interpreted accordingly. The results for this test are more thorough because they show the sensitivity (or lack thereof) to both the preservative and the contaminant. In the earlier verification test, toxicity could have been the result of either. Table 3-2 details the concentrations of preservatives in the lethal dose samples of each contaminant. These data could be evaluated in two ways to determine the sensitivity of the

Table 6-1a. Aldicarb Percent Inhibition Results

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|---------------------------------|---------------------------|------------------------|---------------------------------------|
| Negative Control | 4 | 0 | 15 |
| | -23 | | |
| | 9 | | |
| | 10 | | |
| 0.26 | 32 | 7 | 32 |
| | 30 | | |
| | 1 | | |
| | -35 | | |
| 2.6 | -22 | -33 | 21 |
| | -24 | | |
| | -24 | | |
| | -65 | | |
| 26 | 4 | 13 | 9 |
| | 21 | | |
| | 19 | | |
| | 7 | | |
| 260 (Lethal Dose) | -1 | -16 | 14 |
| | -18 | | |
| | -34 | | |
| | -10 | | |

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

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|--------------------------------------|---------------------------|------------------------|---------------------------------------|
| Negative Control | 2 | 0 | 12 |
| | 9 | | |
| | -17 | | |
| | 6 | | |
| 0.0003 | 5 | 5 | 8 |
| | 9 | | |
| | -7 | | |
| | 11 | | |
| 0.003 | 5 | 4 | 3 |
| | 7 | | |
| | 1 | | |
| | 2 | | |
| 0.03 | 5 | 1 | 3 |
| | -2 | | |
| | 1 | | |
| | 0 | | |
| 0.3 (Lethal Dose) | -58 | -62 | 4 |
| | -64 | | |
| | -59 | | |
| | -66 | | |
| Lethal Dose Preservative Blank | -25 | -26 | 2 |
| | -29 | | |
| | -25 | | |
| | -25 | | |

Table 6-1c. Colchicine Percent Inhibition Results

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|---------------------------------|---------------------------|------------------------|---------------------------------------|
| Negative Control | 43 | 0 | 37 |
| | -46 | | |
| | -5 | | |
| | 8 | | |
| 0.24 | 43 | 42 | 5 |
| | 47 | | |
| | 43 | | |
| | 35 | | |
| 2.4 | 33 | 17 | 15 |
| | -2 | | |
| | 17 | | |
| | 19 | | |
| 24 | 59 | 63 | 3 |
| | 61 | | |
| | 66 | | |
| | 64 | | |
| 240 (Lethal Dose) | 106 | 104 | 2 |
| | 101 | | |
| | 104 | | |
| | 106 | | |

Table 6-1d. Cyanide Percent Inhibition Results

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|---|---------------------------|------------------------|---------------------------------------|
| Negative Control | -29 | 0 | 19 |
| | 9 | | |
| | 11 | | |
| | 9 | | |
| 0.25 | -14 | -18 | 4 |
| | -18 | | |
| | -22 | | |
| | -18 | | |
| 2.5 | -56 | -21 | 24 |
| | -17 | | |
| | -6 | | |
| | -7 | | |
| 25 | 41 | 47 | 5 |
| | 49 | | |
| | 44 | | |
| | 53 | | |
| 250 (Lethal Dose) | 64 | 63 | 2 |
| | 61 | | |
| | 62 | | |
| | 64 | | |
| Field Portability Negative Control | -6 | 0 | 4 |
| | 2 | | |
| | 1 | | |
| | 3 | | |
| Field Portability 250 | -38 | -37 | 1 |
| | -36 | | |
| | -35 | | |
| | -37 | | |

^(a) Results for cyanide at the field location were much different than in the laboratory. Results did not seem to be correlated with non-laboratory analysis.

Table 6-1e. Dicrotophos Percent Inhibition Results

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|----------------------|----------------|-------------|------------------------|
| Negative Control | -3 | 0 | 10 |
| | -10 | | |
| | 14 | | |
| | 0 | | |
| 1.4 | 17 | -13 | 21 |
| | -33 | | |
| | -14 | | |
| | -21 | | |
| 14 | -14 | -38 | 41 |
| | -5 | | |
| | -96 | | |
| | -37 | | |
| 140 | -50 | -30 | 27 |
| | -55 | | |
| | -15 | | |
| | 0 | | |
| 1,400 (Lethal Dose) | -60 | -55 | 8 |
| | -49 | | |
| | -64 | | |
| | -47 | | |

Table 6-1f. Nicotine Percent Inhibition Results

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|----------------------|----------------|-------------|------------------------|
| Negative Control | -1 | 0 | 16 |
| | 11 | | |
| | 12 | | |
| | -22 | | |
| 2.8 | 5 | -3 | 12 |
| | -20 | | |
| | -4 | | |
| | 7 | | |
| 28 | 72 | 71 | 1 |
| | 72 | | |
| | 70 | | |
| | 72 | | |
| 280 | 84 | 84 | 1 |
| | 83 | | |
| | 84 | | |
| | 84 | | |
| 2,800 (Lethal Dose) | 50 | 50 | 1 |
| | 49 | | |
| | 49 | | |
| | 51 | | |

Table 6-1g. Ricin Percent Inhibition Results

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|-----------------------------------|---------------------------|------------------------|---------------------------------------|
| Negative Control | 10 | 0 | 7 |
| | -2 | | |
| | -8 | | |
| | 1 | | |
| 0.015 | -1 | -3 | 4 |
| | -10 | | |
| | -1 | | |
| | 0 | | |
| 0.15 | 19 | 10 | 8 |
| | 0 | | |
| | 6 | | |
| | 15 | | |
| 1.5 | -28 | -13 | 12 |
| | -2 | | |
| | -15 | | |
| | -6 | | |
| 15 (Lethal Dose) | -48 | -44 | 8 |
| | -38 | | |
| | -54 | | |
| | -37 | | |
| Lethal Dose Preservative Blank | 16 | 12 | 5 |
| | 7 | | |
| | 17 | | |
| | 8 | | |

Table 6-1h. Soman Percent Inhibition Results

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|---|----------------|-------------|------------------------|
| Negative Control | -1 | 0 | 2 |
| | 0 | | |
| | 3 | | |
| | -2 | | |
| 0.0014 | 11 | 7 | 3 |
| | 4 | | |
| | 9 | | |
| | 5 | | |
| 0.014 | 23 | 19 | 6 |
| | 24 | | |
| | 17 | | |
| | 12 | | |
| 0.14 | -9 | -8 | 2 |
| | -8 | | |
| | -10 | | |
| | -6 | | |
| 1.4 (Lethal Dose) | 17 | 16 | 1 |
| | 15 | | |
| | 15 | | |
| | 17 | | |
| Lethal Dose Level Preservative Blank | 1 | -1 | 5 |
| | -6 | | |
| | -2 | | |
| | 4 | | |

Table 6-1i. Thallium Sulfate Percent Inhibition Results

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|------------------------|----------------|-------------|------------------------|
| Negative Control | 4 | 0 | 12 |
| | 7 | | |
| | 6 | | |
| | -17 | | |
| 2.8 | -15 | -25 | 13 |
| | -17 | | |
| | -44 | | |
| | -25 | | |
| 28 | -2 | -5 | 5 |
| | -6 | | |
| | -2 | | |
| | -11 | | |
| 280 | 29 | 16 | 12 |
| | 1 | | |
| | 20 | | |
| | 12 | | |
| 2,800 (Lethal Dose) | 62 | 66 | 3 |
| | 69 | | |
| | 67 | | |
| | 68 | | |

Table 6-1j. VX Percent Inhibition Results

| Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|--------------------------------------|----------------|-------------|------------------------|
| Negative Control | 1 | 0 | 3 |
| | -2 | | |
| | 3 | | |
| | -2 | | |
| 0.002 | -1 | -13 | 8 |
| | -17 | | |
| | -21 | | |
| | -13 | | |
| 0.02 | -28 | -14 | 11 |
| | -13 | | |
| | -15 | | |
| | 0 | | |
| 0.2 | -26 | -36 | 13 |
| | -38 | | |
| | -26 | | |
| | -53 | | |
| 2 (Lethal Dose) | -31 | -44 | 26 |
| | -33 | | |
| | -82 | | |
| | -28 | | |
| Lethal Dose Level Preservative Blank | -23 | -34 | 14 |
| | -55 | | |
| | -30 | | |
| | -26 | | |

Chem-IQ Tox™ Test Kit to contaminants stored in preservatives. The first approach would be to determine the inhibition of the test samples containing preservatives with respect to the background negative control as was the case for the contaminants that were not stored in preservatives. This technique, however, could indicate that Chem-IQ Tox™ Test Kit was sensitive to the contaminant when, in fact, it was sensitive to one of the preservatives.

Since these contaminants are only available (either commercially or from the government) in aqueous formulations with the preservatives, this may be appropriate. The second approach would be to fortify negative control samples with the same concentrations of preservative contained in all the samples so that the inhibition resulting from the preservatives could be subtracted from the inhibition caused by the contaminant. This approach would greatly increase the number of samples required for analysis. Therefore, for this test, aspects of both approaches were incorporated without substantially increasing the number of samples. Negative control samples fortified with a concentration of each preservative equivalent to the concentration in the lethal dose test samples (preservative blanks) were analyzed prior to and with every set of test samples. For those sets of test samples for which it was especially difficult to determine whether inhibitory effects were from the contaminant or the preservative, the preservative blank was diluted identically to all the contaminant samples and analyzed so a background subtraction could take place if necessary.

During the initial analysis of the preservative blanks (Table 6-2), none of the samples generated an inhibition significantly greater than the negative control or greater than 20% (Aqua Survey's suggested benchmark for significant toxicity). Because the preservatives apparently did not have toxic effects at the lethal dose concentration, no additional dilutions of preservative blanks were required to determine whether there were toxic effects from each individual concentration level; and each contaminant concentration level was evaluated and compared with the negative control to determine any toxic effects. The inhibition of the lethal dose preservative blank was determined with each contaminant sample set and is shown with each table of the contaminant inhibition.

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

| Preservative Blank | Inhibition (%) | Average (%) | Standard Deviation (%) |
|---------------------------|-----------------------|--------------------|-------------------------------|
| Negative Control | -4 | 0 | 8 |
| | 8 | | |
| | -9 | | |
| | 4 | | |
| Ricin | 24 | 13 | 12 |
| | 1 | | |
| | 4 | | |
| | 22 | | |
| Soman/VX | 11 | 10 | 2 |
| | 11 | | |
| | 7 | | |
| | 11 | | |
| Botulinum Toxin Complex B | -39 | -44 | 8 |
| | -47 | | |
| | -54 | | |
| | -36 | | |

^(a) Soman and VX use the same preservative.

Samples from three of the four botulinum toxin complex B concentrations produced an average inhibition that was not significantly different from the negative control and was less than 20% (the Aqua Survey criterion), indicating no toxic effect. The other sample, at the lethal dose concentration, was significantly different from the negative control, but in the negative direction (-62%). In addition, the lethal dose preservative blank analyzed with this sample set also produced a negative inhibition (-26%). The inhibition of the lethal dose botulinum toxin complex B sample was more negative than the lethal dose preservative blank and the negative control, indicating no toxic effect.

The ricin preservative blank analyzed prior to the contaminant analysis did not generate a detectable inhibition; therefore, as with botulinum toxin complex B, additional dilutions of the preservative blank were not required. In addition, inhibition of the lethal dose preservative blank during contaminant testing did not differ significantly from the negative control; therefore, the inhibition of each ricin sample could be compared directly to the negative control. The average inhibition of each of the three lower concentrations of ricin was not significantly different from the negative control. The lethal dose sample was significantly different, but in the negative direction, indicating the lack of a toxic effect.

For soman, the average inhibition of the preservative blank analyzed prior to the contaminant samples was $10\% \pm 2\%$, not significantly different from that of the negative control and not above the 20% threshold of toxicity suggested by the vendor. Again, dilutions of the preservative blank were not required, and the contaminant inhibition was calculated only with respect to the negative control. Each of the four soman test concentrations generated an average inhibition that was significantly different from the negative control (three in a positive direction and one in a negative direction). On average, none of them exceeded 20%, indicating no toxic effect.

For VX, the inhibition of the preservative blank analyzed prior to the contaminant samples was not significantly different from that of the negative control and not above the 20% threshold of toxicity suggested by the vendor. Thus, dilutions of the preservative blank were not analyzed with the contaminant samples. When the set of contaminant samples was analyzed with a lethal dose preservative blank, the preservative blank inhibition was $-34\% \pm 14\%$, indicating an enhancement of luminescence from the preservative. The reason for the difference between this result and the result obtained prior to the contaminant analysis was not clear. However, while this preservative blank result was significantly different from the negative control, it did not differ significantly from any test sample inhibition, indicating that VX had no significant toxic effect.

6.1.2 Potential Interferences

All of the potential interference samples were prepared in DDW and compared with the negative control to determine the level of inhibition. This determination is crucial because the ability of the Chem-IQ Tox™ Test Kit to detect toxicity is dependent on the background light production in whatever drinking water matrix is being used. If the background drinking water sample completely inhibits background light, inhibition caused by contaminants could not be detected. Table 6-3 presents the results from the samples analyzed to test the effect of potential interferences on the Chem IQ Tox™ Test Kit. Of the five metal solutions evaluated as possible interferences with the Chem IQ Tox™ Test, three of them, copper ($46\% \pm 3\%$), iron ($-26\% \pm 20\%$), and zinc ($34\% \pm 2\%$), exhibited an average inhibition that was significantly different from the DDW negative control ($0\% \pm 4\%$).

The iron inhibition was negative; therefore, drinking water with similar concentrations of iron would likely be amenable to the Chem-IQ Tox™ Test Kit because there would actually be an increase in background light that could potentially be inhibited by contaminants. Zinc and copper generated inhibition greater than 20% but less than 50%. While these levels of inhibition are greater than the vendor's toxicity threshold, enough background light remains that toxicity from contaminants could likely be detected provided water containing these metals were analyzed with respect to negative controls with similar background toxicity. Aluminum and manganese generated inhibition not significantly different from the negative control. These results underscore the need for negative control samples that are extremely similar to the water matrices that are suspected of having been contaminated. Small differences in the water composition can cause the appearance of toxicity.

To investigate whether the Chem-IQ Tox™ Test Kit is sensitive to by-products of disinfecting processes, DDW samples from water systems that use chlorination and chloramination were analyzed and would have been compared with ASTM Type II DI water as the control sample.

Table 6-3. Potential Interferences Results

| Potential Interferences | Concentration (mg/L) | Inhibition (%) | Average (%) | Standard Deviation (%) |
|---------------------------|----------------------|----------------|-------------|------------------------|
| Negative control (Metals) | NA | -2 | 0 | 4 |
| | | -5 | | |
| | | 2 | | |
| | | 5 | | |
| Aluminum | 0.5 | -8 | 4 | 9 |
| | | 3 | | |
| | | 11 | | |
| | | 9 | | |
| Copper | 0.6 | 44 | 46 | 3 |
| | | 51 | | |
| | | 46 | | |
| | | 44 | | |
| Iron | 0.15 | -23 | -26 | 20 |
| | | -55 | | |
| | | -15 | | |
| | | -9 | | |
| Manganese | 0.25 | 13 | 11 | 9 |
| | | 20 | | |
| | | -1 | | |
| | | 12 | | |
| Zinc | 2.5 | 32 | 34 | 2 |
| | | 34 | | |
| | | 33 | | |
| | | 36 | | |

NA = Not applicable.

However, when ASTM Type II DI water was analyzed with the Chem-IQ Tox™ Test Kit, almost all of the light was inhibited; prohibiting the calculation of inhibition with respect to the DI water. Therefore, instead of calculating inhibition, the background light generated when the two water samples were analyzed was compared (see Table 6-4). The background light units produced for the 60 DDW (chlorination by-product) samples analyzed throughout the verification test was $1,043 \pm 233$. This seemed to be adequate background light for subsequent inhibition to occur. In addition, the average number of light units produced in the sample containing chloraminated water was $2,817 \pm 201$ (N=4); thus, it seems that Chem-IQ Tox™ Test Kit could be used with either chloraminated or chlorinated water since neither sample completely inhibits the background light production and, therefore, background light remains for the subsequent detection of contaminants. The difference in the number of replicates is because the dechlorinated water was used as the negative control with each sample set; therefore, much more data were collected on that water.

Table 6-4. Disinfection By-Product Background Light Production

| Potential Interferences | N | Average Light Units | Standard Deviation |
|--------------------------------|----------|----------------------------|---------------------------|
| Chlorination by-products | 60 | 1,043 | 233 |
| Chloramination by-products | 4 | 2,817 | 201 |

6.1.3 Precision

Across all the contaminants and potential interferences, the standard deviation (not relative standard deviation) was measured for each set of four replicates to evaluate the Chem-IQ Tox™ Test Kit precision. Out of 78 opportunities, the standard deviation of the four replicate inhibition measurements was less than 10% 50 times (64% of the time), between 10% and 20% 19 times (24% of the time), and greater than 20% 9 times (11%). Overall, 88% of the time, the standard deviations were less than 20%. As described in Section 3.2.2, the analysis procedure required that each replicate undergo the entire analysis process; therefore, the measurement of precision represents the precision of the analysis method performed on a single water sample on a given day. The precision does not reflect the repeatability of the method across more than one day or more than one preparation of reagents or more than one operator.

6.2 Toxicity Threshold

Table 6-5 gives the toxicity thresholds, as defined in Section 5.2, for each contaminant. Note the difference between detectability with respect to the negative control and the toxicity threshold with respect to the other concentration levels analyzed. A contaminant concentration level can have an inhibition significantly different from the negative control (thus detectable), but if its inhibition is not significantly different from the concentration levels below it, it would not be considered the toxicity threshold because in the context of this test, its inhibition would not be distinguishable from that of the lower concentrations. The lowest toxicity threshold concentrations are for colchicine at 24 mg/L and cyanide at 25 mg/L.

6.3 False Positive/Negative Responses

Because the DI water samples did not allow detectable light to be generated, the inhibition of the chlorination and chloramination by-product samples could not be determined. This makes the availability of a non-contaminated drinking water sample for use as a negative control mandatory for analysis using the Chem-IQ Tox™ Test Kit. The absolute light units generated by both types of these samples were measured. As mentioned in Section 6.1.2, adequate background light is present to detect subsequent contamination; however, DI water may not be used as a negative control. If samples are analyzed daily, a good practice to follow would be to archive a negative control sample each day in case of contamination the next day.

Table 6-5. Toxicity Thresholds

| Contaminant | Concentration (mg/L) |
|---------------------------|-----------------------------|
| Aldicarb | ND |
| Botulinum toxin complex B | ND |
| Colchicine | 24 |
| Cyanide | 25 |
| Dicrotophos | ND |
| Nicotine | 28 |
| Ricin | ND |
| Soman | ND |
| Thallium sulfate | 2,800 |
| VX | ND |

ND = Significant inhibition was not detected.

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

Table 6-6. False Negative Responses

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

6.4 Other Performance Factors

6.4.1 Ease of Use

The Chem-IQ Tox™ Test Kit instructions were clearly written; but, because they were very detailed, a condensed summary with only the necessary steps may be helpful. The contents of the Chem-IQ Tox™ Test Kit were well identified with labels on the vials. Overall, the test was not difficult to perform, but becoming efficient at analyzing several samples simultaneously required practice. The analysis procedure required one reagent to be sonicated, while another reagent was thawed after storage in a freezer. In one instance, the reagent vial broke during thawing.

A handheld fluorometer was provided by Aqua Survey. The fluorometer was easy to use, but required calibration precisely one minute prior to analysis of each sample set. The electronic readout was user-friendly, and only one number needed to be recorded. The fluorometer was easily wiped clean and required no routine maintenance other than calibration. Other miscellaneous items required include a micropipettor with various sized tips, a sonicator for use during reagent preparation, and a freezer for reagent storage.

No formal scientific education would be required to use the Chem-IQ Tox™ Test Kit, but good laboratory skills, especially pipetting, would be beneficial. Verification testing staff were able to operate the Chem-IQ Tox™ Test Kit after a training session lasting approximately two hours.

Approximately 10 mL of liquid waste were generated per sample, along with leftover reagents. In addition, one chamber per six samples, reagent vials, pipette tips, and cuvettes were generated as solid waste. It was not clear whether the reagents should be considered hazardous waste.

6.4.2 Field Portability

The Chem-IQ Tox™ Test Kit was transported from a laboratory setting to a storage room for the field portability evaluation. The storage room contained several tables and light and power sources, but no other laboratory facilities. No carrying case was provided with the Chem-IQ Tox™ Test Kit; however, all materials were transported by one person in a 38-centimeter by 38-centimeter by 38-centimeter cardboard box. The Chem-IQ Tox™ Test Kit was set up easily in less than 10 minutes, with the exception of letting one reagent thaw, which took approximately 20 minutes after removal from a freezer. A source of electricity was required for the sonicator; however, the fluorometer runs on batteries. Longer-term field deployment would require a freezer for storing reagents. The following items not provided in the Chem-IQ Tox™ Test Kit were needed for field use: a cooler to transport and store reagents, pipettes and tips, the sonicator and a power source, the fluorometer, and a waste container. Overall the Chem-IQ Tox™ Test Kit was easy to transport to the field and was deployed in a matter of minutes. The limiting factor to testing in the field would be the approximately 20 minutes required to thaw one of the reagents. Results were obtained within 10 minutes of starting the test.

The Chem-IQ Tox™ Test Kit was tested with one contaminant, cyanide, at the lethal dose concentration. Interestingly, the results obtained for the lethal dose of cyanide at the non-laboratory location (Table 6-1d) were very different from those obtained initially in the laboratory. The inhibition measured initially was $63\% \pm 2\%$, and in the non-laboratory location it was $-37\% \pm 11\%$. The positive control samples analyzed in both locations generated significant

inhibition as would be expected. There was no indication that the Chem-IQ Tox™ Test Kit was not functioning properly because the location of the analysis didn't seem to be related to these unexplainable results and more expected results had been obtained during the cyanide laboratory testing. No reanalysis was performed.

6.4.3 Throughput

Approximately 30 analyses were completed in one hour. The 30 analyses included method blanks, positive controls, and test samples. Approximately 130 samples could be processed per pair of Reagent One and Reagent Two vials.

Chapter 7 Performance Summary

| Parameter | Compound | Lethal Dose (LD) Conc. (mg/L) | Average Inhibition at Concentrations Relative to the LD Concentration (%) | | | | Range of Standard Deviations (%) | Toxicity Thresh. (mg/L) | |
|-------------------------|---|-------------------------------|---|-------------------------------|--------|-------------------------------|----------------------------------|-------------------------|--|
| | | | LD | LD/10 | LD/100 | LD/1,000 | | | |
| Contaminants in DDW | Aldicarb | 260 | -16 | 13 | -33 | 7 | 9-32 | ND | |
| | Botulinum toxin complex B | 0.3 | -62 | 1 | 4 | 5 | 3-8 | ND | |
| | Colchicine | 240 | 104 | 63 | 17 | 42 | 2-15 | 24 | |
| | Cyanide | 250 | 63 | 47 | -21 | -18 | 2-24 | 25 | |
| | Dicrotophos | 1,400 | -55 | -30 | -38 | -13 | 8-41 | ND | |
| | Nicotine | 2,800 | 50 | 84 | 71 | -3 | 1-12 | 28 | |
| | Ricin | 15 | -44 | -13 | 10 | -3 | 4-12 | ND | |
| | Soman | 1.4 | 16 | -8 | 19 | 7 | 1-6 | ND | |
| | Thallium sulfate | 2,800 | 66 | 16 | -5 | -25 | 3-13 | 2,800 | |
| | VX | 2 | -44 | -36 | -14 | -13 | 8-26 | ND | |
| | | Interference | Conc. (mg/L) | Average Inhibition (%) | | Standard Deviation (%) | | | |
| | | Aluminum | 0.5 | 4 | | 9 | | | |
| | | Copper | 0.6 | 46 | | 3 | | | |
| | | Iron | 0.15 | -26 | | 20 | | | |
| | | Manganese | 0.25 | 11 | | 9 | | | |
| | Zinc | 2.5 | 34 | | 2 | | | | |
| False positive response | Because DI water did not generate any measurable background light, the disinfection by-product samples could not be compared with the inhibition due to DI water. Therefore only the absolute light units produced by the chlorinated and chloraminated samples could be measured. Both of these samples left adequate light for subsequent inhibition due to contamination and are thus not considered to have generated false positive results. | | | | | | | | |
| False negative response | False negative responses (inhibition less than 20%) were generated for aldicarb, botulinum toxin complex B, dicrotophos, ricin, soman, and VX when they were analyzed at the lethal dose concentration. | | | | | | | | |
| Ease of use | The Chem-IQ Tox™ Test Kit instructions were clearly written; but a condensed summary with only the necessary steps may be helpful. The contents of the Chem-IQ Tox™ Test Kit were well identified. The test was not difficult to perform, but analyzing several samples simultaneously required practice. No formal scientific education would be required to use the Test Kit. | | | | | | | | |
| Field portability | The Chem-IQ Tox™ Test Kit was transported from a laboratory to a storage room to simulate a non-laboratory location. All materials were easily transported by one person in a small cardboard box. The Test Kit was set up in less than 10 minutes, except that Reagent Two took approximately 20 minutes to thaw. A source of electricity was required for the sonicator, while the fluorometer ran on batteries. A cooler to transport and store reagents, pipettes and tips, the sonicator and a power source, the fluorometer, and a waste container were needed for field use. Results were obtained within 10 minutes of starting the test. | | | | | | | | |
| Throughput | Approximately 30 analyses were completed in one hour. The 30 analyses included method blanks, positive controls, as well as test samples. Approximately 130 samples could be processed per pair of Reagent One and Reagent Two vials. | | | | | | | | |

ND = Significant inhibition was not detected.

Chapter 8

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