

US EPA ARCHIVE DOCUMENT

**Environmental Technology  
Verification Program**  
Advanced Monitoring  
Systems Center

Test/QA Plan for  
Verification of  
Rapid Toxicity Technologies

US EPA ARCHIVE DOCUMENT

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## **Test/QA Plan**

# **Verification of Rapid Toxicity Technologies**

**June 11, 2003**

**Prepared by**

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ETV Advanced Monitoring Systems Center

Test/QA Plan for Verification of  
Rapid Toxicity Technologies

Version 1

June 11, 2003

APPROVAL:

Name \_\_\_\_\_

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Date \_\_\_\_\_

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## **1.0 INTRODUCTION**

### **1.1 Test Objective**

This test/quality assurance (QA) plan provides procedures for a verification test of rapid analysis technologies that measure toxicity in drinking water. The verification test will be conducted under the auspices of the U.S. Environmental Protection Agency (EPA) through the Environmental Technology Verification (ETV) program. The purpose of ETV is to provide objective and quality assured performance data on environmental technologies, so that users, developers, regulators, and consultants can make informed decisions about purchasing and applying these technologies. The objective of this verification test of rapid toxicity technologies is to evaluate their ability to detect certain toxins that are particularly toxic to humans and their susceptibility to interfering chemicals in a controlled experimental matrix. Multiple water quality matrices or mixtures of contaminants and interfering compounds will not be evaluated because such is beyond the scope of this test and would more appropriately be evaluated on a case-by-case basis.

### **1.2 Test Description**

The verification test will be performed by Battelle, of Columbus, Ohio, which is managing the ETV Advanced Monitoring Systems (AMS) Center through a cooperative agreement with EPA. The scope of the AMS Center covers verification of monitoring technologies for contaminants and natural species in air, water, and soil. In performing the verification test, Battelle will follow the procedures specified in this test/QA plan, and will comply with the data quality requirements in the "Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center"<sup>1</sup>. Various contaminants will be added to drinking water at multiple concentration levels and analyzed by the participating technologies to assess their



ability to detect the toxicity of these contaminants in drinking water. After analysis of several concentrations of each contaminant, the lowest concentration of each contaminant that causes inhibition greater than that of the negative control will be reported. The precision of the results will be evaluated by making replicate measurements on each test sample and qualitative characteristics of each technology such as ease of use and field portability will be assessed through observations made by the test coordinator and operators throughout the verification test. The results from each technology will be reported individually. No direct comparison will be made between technologies, but each technology will undergo similar testing so it is convenient for end users to evaluate the ETV testing results.

### **1.3 Organization and Responsibility**

The verification test will be performed by Battelle with the participation of the interested vendors who will be having their technologies verified. The testing will occur at Battelle's Columbus and West Jefferson, Ohio laboratories and at a field location in the Columbus area. The organizational chart shown in Figure 1 shows the individuals from Battelle, the vendor companies, and the EPA who will have responsibilities in the verification test.

#### **1.3.1 Battelle**

Dr. Ryan James is the AMS Center Verification Test Coordinator. In this role, Dr. James will have overall responsibility for ensuring that the technical, schedule, and cost goals established for the verification test are met. More specifically, he will:

- Assemble a team of qualified technical staff to conduct the verification test.
- Direct the team in performing the verification test in accordance with the test/QA plan
- Ensure that all quality procedures specified in the test/QA plan and in the QMP are followed.
- Prepare the draft test/QA plan, verification reports, and verification statements.

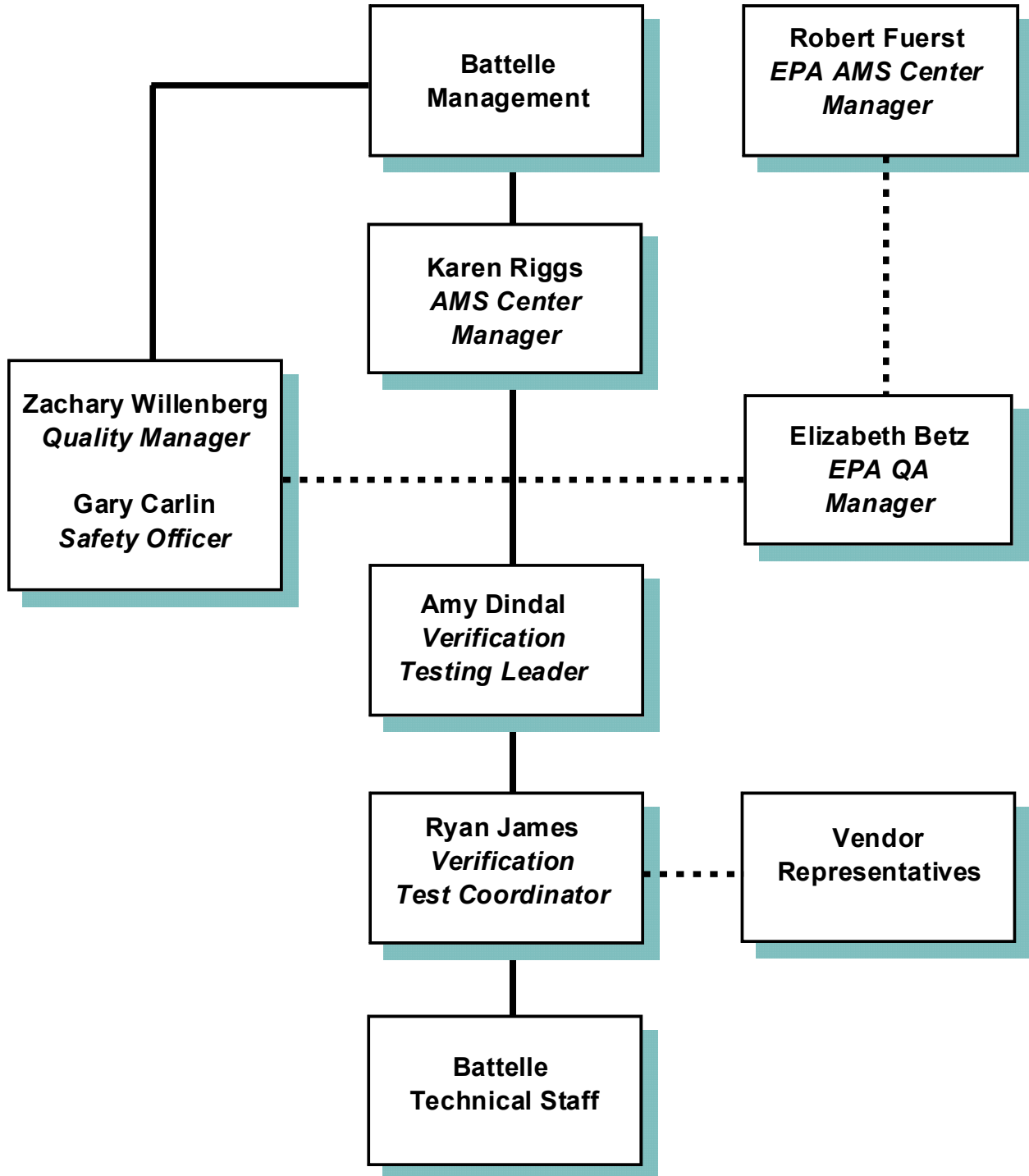


Figure 1. Organization Chart for the Verification Test

- Revise the draft test/QA plan, verification reports, and verification statements in response to reviewers' comments.
- Coordinate distribution of the final test/QA plan, verification reports, and verification statements.
- Respond to any issues raised in assessment reports and audits, including instituting corrective action as necessary.
- Serve as the primary point of contact for vendor representatives.
- Establish a budget for the verification test and monitor staff effort to ensure the budget is not exceeded.
- Ensure that confidentiality of vendor information is maintained.

Ms. Amy Dindal is a Verification Testing Leader for the AMS Center. As such, Ms. Dindal will provide technical guidance and oversee the various stages of verification testing. She will:

- Support Dr. James in preparing the test/QA plan and organizing the testing.
- Assist Dr. James in anticipating and resolving potential technical problems with the verification test.
- Review the draft test/QA plan.
- Review the draft verification reports and statements.

Ms. Karen Riggs is Battelle's manager for the AMS Center. As such, Ms. Riggs will:

- Review the draft test/QA plan.
- Review the draft verification reports and verification statements.
- Ensure that necessary Battelle resources, including staff and facilities, are committed to the verification test.

- Ensure that vendor confidentiality is maintained.
- Support Dr. James in responding to any issues raised in assessment reports and audits.
- Maintain communication with EPA's technical and quality managers.
- Facilitate a stop work order if Battelle or EPA QA staff discovers adverse findings that will compromise test results.

Battelle Technical Staff will conduct the testing of the technologies during the verification test and associated experimental activities. The responsibilities of these technical staff include:

- Assist in the preparation of samples.
- Analyze samples for the verification test as described in this test/QA plan.
- Make qualitative observations about the operation of the technologies.

Mr. Zachary Willenberg is Battelle's Quality Manager for the AMS Center. As such Mr. Willenberg will:

- Review the draft test/QA plan.
- Conduct quality review of the documentation exhibiting the capability of an outside laboratory to perform solution confirmation analyses.
- Conduct a technical systems audit during the verification test.
- Audit at least 10% of the verification data.
- Prepare and distribute an assessment report for each audit.
- Verify implementation of any necessary corrective action.
- Issue a stop work order if self audits indicate that data quality is being compromised; notify Battelle's AMS Center Manager if stop work order is issued.

- Provide a summary of the quality assurance/quality control (QA/QC) activities and results for the verification reports.
- Review the draft verification reports and statements.
- Have an overall responsibility for ensuring that the test/QA plan is followed.

Mr. Gary Carlin is Battelle's Environmental Health and Safety representative for the AMS Center. As such Mr. Carlin will:

- Review the safety issues related to handling the contaminants and provide input into the test/QA plan.
- Advise staff working with the contaminants on personal protective equipment and training needs.

### 1.3.2 Vendors

Vendor representatives will:

- Review the draft test/QA plan.
- Approve the test/QA plan.
- Provide one complete commercially-available rapid toxicity technology and associated equipment/materials required for operation throughout the duration of the verification test.
- Provide all needed consumables for number of test samples included in the verification test.
- As desired, instruct Battelle personnel on how to operate the technology prior to testing.
- Review their respective draft verification report and statement.

### 1.3.3 EPA

EPA's responsibilities in the AMS Center are based on the requirements stated in the "Environmental Technology Verification Program Quality Management Plan"<sup>2</sup>. The roles of the specific EPA staff are as follows:

Ms. Elizabeth Betz is EPA's Quality Assurance Manager. For the verification test, Ms. Betz will:

- Review the draft test/QA plan.
- Direct the performance, at the EPA's discretion, of external technical systems audit(s) during the verification test.
- Notify the Battelle AMS Center Manager to facilitate a stop work order if the external audit indicates that data quality is being compromised.
- Prepare and distribute an assessment report summarizing results of the external audit.
- Review draft verification reports and statements.

Mr. Robert Fuerst is EPA's manager for the AMS Center. As such, Mr. Fuerst will:

- Review the draft test/QA plan.
- Notify the Battelle AMS Center Manager to facilitate a stop work order if an external audit indicates that data quality is being compromised.
- Review the draft verification statements.
- Review the final verification reports.
- Coordinate submission of the verification reports and statements for final EPA approvals.

### 1.3.4 Analytical Laboratories Used for Stock Solution Confirmation

When methods are available, the contaminant concentration of the stock solutions will be confirmed. When the analyses are within Battelle's capabilities, Battelle will perform the

analyses, but if it is more cost effective, Battelle may collaborate with or establish a subcontract with a commercial laboratory to perform the analyses.

In order to be selected to perform the confirmatory analyses during the verification test, a commercial laboratory will need to provide Battelle documentation that demonstrates its competence to perform the needed analysis, such documentation may include copies of: their method/standard operating procedure, quality assurance manual, state government certifications/approvals for analysis of the appropriate contaminant, and/or staff training records, where available. If the prospective laboratory does not demonstrate their capability adequately, another laboratory will be selected and their competence verified in a similar manner.

## 2.0 VERIFICATION APPROACH

### 2.1 Scope of Testing

The verification test of rapid toxicity technologies will focus on a broad range of contaminants that have been prioritized by the ETV stakeholder process. These rapid toxicity technologies do not provide identification or concentration of specific contaminants, but serve as a rapid screening tool to determine if the water being tested is toxic. As part of this verification test, the rapid toxicity technologies will be subjected to various concentrations of contaminants representing several categories of chemicals such as industrial chemicals, pesticides, rodenticides, pharmaceuticals, nerve agents, and biological toxins. Each contaminant will be added to separate drinking water samples and analyzed by the rapid toxicity technologies. In addition to determining whether or not these technologies can detect the toxicity caused by each contaminant, the response of these technologies to interfering compounds likely to be present in clean drinking water, such as chemicals used for water treatment and byproducts of water treatment processes, will be evaluated. Table 1 shows the contaminants and the interfering compounds that will be evaluated during this verification test.

This test/QA plan specifically addresses verification testing of technologies that rapidly detect the toxicity of water samples. While there are many compounds that are toxic to the test organisms that are not toxic to humans, this verification test will focus on contaminants that are particularly toxic to humans. The technologies function by adding the water sample to a bacteria (*Vibrio fischeri*), enzyme (luciferase), or small crustacean (*Daphnia magna*) that either directly, or in combination with reagents, produce a background level of light production or rate of dissolved oxygen uptake (DOUR) in the absence of toxic contaminants. If toxic contaminants are present in the water, depending on the vendor, their toxicity is indicated by a change in color or intensity of the light production or by a decrease in the DOUR in proportion to the concentration level of the contaminant. This indication of toxicity will be generically referred to



as “inhibition” through the remainder of this test/QA plan. These technologies report the inhibition of each drinking water sample with respect to the inhibition of either ASTM Type II deionized (DI) water or non-toxic drinking water. The results are reported visually or via a digital display or electronic output signal. The technologies that are designed

**Table 1. Categories, Contaminants, and Water Treatment Interferences**

| Category                  | Contaminant            | Interfering Compounds   |
|---------------------------|------------------------|---|
| Industrial chemical       | cyanide                | manganese, iron, aluminum, copper sulfate, chloramination byproducts, chlorination byproducts, zinc sulfate |
| Carbamate pesticide       | aldicarb               |   |
| Organophosphate pesticide | dicrotophos            |   |
| Rodenticide               | thallium sulfate       |   |
| Pharmaceutical            | colchicine             |   |
| Nerve agents              | VX, soman              |   |
| Biological toxins         | ricin, botulinum toxin |   |

for use in a field location will be tested at a non-laboratory venue, and all of the technologies will be tested in a laboratory.

The verification of the rapid toxicity technologies will be done through the analysis of drinking water samples fortified with various concentrations of individual contaminants. ETV verifications usually include a comparison of the results generated by the technologies being verified with the results of analysis of the same samples using a standard reference method that measures the same endpoint, usually concentration. In the case of this verification test, the most common standard method for toxicity is the Whole Effluent Toxicity<sup>3</sup> (WET) method. Most of the rapid toxicity technologies employ organisms for the detection mechanism that are different from the organisms suggested for used in the WET method. The sensitivity of different organisms to contaminants will be different among different species. Therefore, this method

would not provide an appropriate benchmark for direct comparison with the results produced by all of the rapid toxicity technologies because of the lack of sameness of organism. It is well documented that the contaminants used during this verification test are toxic and the objective of the test is to determine each rapid toxicity technology's ability to detect this toxicity if the contaminant is present, not to compare their response with the WET method. In lieu of a traditional reference measurement of toxicity, the concentration of each contaminant or interfering compound fortified into the drinking water sample will be confirmed independently by standard confirmatory methods, when available. When a standard method is not available, QA oversight (balance calibration, audits, and purity tracking of the standards) of the solution preparation will ensure the accuracy of the sample concentration.

The rapid toxicity technologies provide a measure of toxicity, and will be evaluated by reporting:

- endpoint (% inhibition,  $EC_{50}$ ,  $EC_{30}$ , toxicity threshold) for all concentration levels, contaminants and potential interfering compounds
- precision
- false negative rate (frequency of inhibition similar to the negative control reported when a contaminant is present at toxic concentrations)
- false positive rate (frequency of detectable inhibition reported in unspiked drinking water samples)
- field portability
- ease of use
- throughput.

## 2.2 Experimental Design

The verification test will involve challenging the rapid toxicity technologies with drinking water samples fortified separately with the contaminants and interfering compounds listed in Table 1. Each technology will analyze samples containing the contaminants at concentration levels that would be lethal if ingested. Subsequent dilutions (dilution factors as per each vendor's protocol, otherwise tenfold) will be prepared and analyzed until there is no longer inhibition as measured by each technology. From these data, the lowest concentration at which the toxicity can be detected, or toxicity threshold, will be estimated for each technology with respect to each contaminant. A common endpoint such as the effective concentration causing 50% inhibition ( $EC_{50}$ ) will be reported for each technology along with data reflecting the rate of false positive and negative results and the precision of the rapid toxicity technologies. Inhibition results (endpoints) specific to each technology from four replicates of each contaminant at each concentration level will be evaluated in order to evaluate the precision of the technologies.

The response of the rapid toxicity technologies to compounds used during the water treatment process (see Table 1) will be evaluated as potential interfering compounds by analyzing separate drinking water samples fortified with the EPA's National Secondary Drinking Water Regulations (NSDWR)<sup>4</sup> level of each compound. For analysis of byproducts of the chlorination process, the unspiked drinking water sample will be analyzed because it will be from a water utility that uses chlorination as its disinfection process. For the analysis of byproducts of the chloramination process, a separate water sample will be obtained from a water system that uses chloramination as its disinfection process.

Sample throughput will be measured based on the number of samples analyzed per day. Performance parameters, such as ease of use and reliability, will be based on documented observations of the operators and test coordinator. Each technology will be used in a field

environment, as well as in a laboratory setting, to assess the impact of field conditions on performance.

## 2.3 Test Samples

Test samples to be used in this verification test will include drinking water (DW) and quality control (QC) samples. Tables estimating the number of samples to be analyzed are provided in the Appendix. The drinking water samples will be prepared from a single drinking water sample collected from a tap in a system that uses chlorination as the disinfectant procedure. The water will be dechlorinated (with sodium thiosulfate or as per vendor protocol) and fortified with various concentrations of contaminants and interferences. Individual solutions containing each contaminant and potential interfering compound separately will be prepared. Subsequent dilutions of the contaminant samples with dechlorinated drinking water or ASTM Type II DI water (as per vendor protocol) will be analyzed by the rapid toxicity technologies until there is no longer detectable inhibition. Mixtures of contaminants and interfering compounds will not be analyzed. The QC samples will include method blank samples and positive and negative control samples.

### 2.3.1 QC Samples

The QC samples will include method blank samples, which will consist of ASTM Type II DI water; positive control samples, which will consist of ASTM Type II DI water fortified with a contaminant and concentration selected by each vendor; and negative control samples, which will consist of the unspiked dechlorinated drinking water sample. The method blank samples will be used to help ensure that no sources of contamination are introduced in the sample handling and analysis procedures. The positive control samples will provide an indication to the operator whether or not the rapid toxicity technology is functioning properly. The vendor will provide the approximate inhibition endpoint that should result upon analysis of the positive

control by their technology. While there will not be required performance limits placed on the result of the positive control sample, if the result is not in the range of anticipated results specified by the vendor, it will indicate to the operator that the technology may have been operated incorrectly. The negative control sample will be used to set a background toxicity of the water sample. Any change in inhibition from the negative control sample can be considered to be due to the presence of a contaminant. At least one method blank and positive control sample will be analyzed for every 20 samples (more often if required by vendor's protocol) analyzed by each rapid toxicity technology. Negative control samples will be analyzed as often as required to accommodate the vendor's analytical protocol.

### **2.3.2 Drinking Water Fortified with Contaminants**

Approximately 50-L of a single drinking water sample, drinking water from a municipal supply that uses chlorination as its disinfectant procedure, will be collected in a high density polyethylene (HDPE) container as part of this verification test. The sample container will be pre-cleaned by the manufacturer and certified to be contaminant free. After sample collection, aliquots of the water sample will be analyzed for several water quality parameters such as: the concentration of trihalomethanes, haloacetic acids, total organic halides, copper, aluminum, iron, manganese, and zinc; turbidity; dissolved organic carbon content; pH; alkalinity; specific conductivity; and hardness. Prior to analysis, these aliquots will have been preserved as per the standard methods for these analyses. The remaining drinking water sample will be dechlorinated with approximately 1 mg of sodium thiosulfate pentahydrate for every liter of drinking water remaining. A scoop that holds approximately 1 mg will be used to measure the sodium thiosulfate pentahydrate into the water sample. It is acceptable for the vendors to use different dechlorination procedures as part of their protocol. All subsequent test samples will be prepared from this dechlorinated drinking water (DW) and stored in glass containers to avoid the leaching of chlorine from HDPE plastics.

A stock solution of each contaminant at the lethal dose concentration level will be prepared in the DW. Table A-1 in the Appendix lists the concentration level of the lethal dose sample of each contaminant and the number of samples to be analyzed. Those stock solutions may be diluted by factors of 10, 100, 1,000, and 10,000 to generate a dilution series for each technology to analyze<sup>a</sup>. All the technologies will analyze at least the lethal dose concentration and the first two dilutions, but the technologies will only continue on and analyze the rest of the dilutions as long as they are detecting inhibition with respect to the negative control sample. For example, if the 100-fold dilution sample produced no detectable inhibition, the technology would not be required to analyze the more dilute samples. However, if the 100-fold dilution sample produced detectable inhibition, the technology would be required to analyze additional dilutions until the inhibition is similar to the negative control sample, even if samples with dilution factors greater than 10,000 need to be prepared. If a technology detects inhibition at the lethal dose level, but not at the 10-fold dilution factor, additional dilutions should be performed in order to obtain detectable results at a minimum of two concentration levels. Each dilution set will be analyzed four times by each technology. The endpoints (% inhibition, EC<sub>50</sub>, etc.) reported by each technology will be recorded for each dilution sample or dilution series, whichever is appropriate for the specific endpoint. Each of the participating technologies will be tested as described above in Battelle laboratories.

Testing the operation of the rapid toxicity technologies in a field setting is a key component of the verification test. Evaluating the performance of each field portable technology while being used outside the laboratory without the availability of miscellaneous laboratory supplies is important to the buyers and users of these technologies. Technologies will only be evaluated in a field setting if the vendor states that the technology has that capability. At a non-laboratory field location, each field portable technology will analyze a single water sample (four

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<sup>a</sup>The scenario of multiple ten-fold dilutions is suggested for each vendor's technology to alleviate the practical difficulties of preparing different test solutions for each vendor. However, if the vendor's protocol includes dilutions (not necessarily ten-fold) to non-detectable levels, using their protocol would be acceptable as long as the appropriate endpoint data are obtained. Data forms will be provided to the operators to aid the documentation of the dilutions.

replicates) fortified with one of the contaminants (from Table 1) known only to the Verification Test Coordinator. The technologies will report its toxicity as prescribed by each vendor's protocol. The operators will follow the vendor's protocol exactly, as no guidance on concentration level or dilutions will be provided. All data obtained and dilutions performed while performing these analyses will be documented and the endpoints reported by each technology will be compared with those obtained in the laboratory setting.

### **2.3.3 Drinking Water Fortified with Interfering Compounds**

Individual aliquots of the drinking water sample described above (DW) will be fortified with one half the NSDWR guidance level of each potential interfering compound. Table A-2 in the appendix lists the interfering compounds along with the concentration at which they will be tested. Four replicates of each of these samples will be analyzed.

For the byproducts of the chlorination process, the unspiked drinking water sample (same as the negative control) will be analyzed in replicate since the water sample will be from a system disinfected with the chlorination process. For the byproducts of the chloramination process, a separate drinking water sample will be collected from a water utility that uses chloramination as the disinfecting process. This water sample will be characterized by measuring trihalomethanes, haloacetic acids, total organic halides, turbidity, dissolved organic carbon content, pH, alkalinity, specific conductivity, and hardness. Upon receipt, this sample will be dechlorinated as per vendor protocols to quench the chloramination process and, as for the other possible interfering compounds and chlorination byproducts, analyzed in replicates of four.

### **3.0 MATERIALS AND EQUIPMENT**

In general, this verification test relies on the materials and equipment provided by the vendors. Battelle will provide the following equipment and materials.

#### **3.1 Laboratory Supplies**

The following supplies will be needed for the preparation of the DW and QC samples:

- ASTM Type II DI water
- various laboratory supplies necessary for accurate preparation of the test samples and subsequent dilutions (ie. volumetric pipets, pipet bulbs, Eppendorf micro pipettes/pipette tips, volumetric flasks, disposable pipets, etc.)
- reference standards with a known level of purity for target analytes (NIST traceable or equivalent)
- glass sample containers
- sodium thiosulfate pentahydrate
- sodium hypochlorite
- n, n-diethyl-p-phenylenediamine (DPD) tablet
- personal protective equipment.

#### **3.2 Field Supplies**



For the analysis of samples in the field setting, Battelle will provide only the water sample for analysis, the DW sample to be used by the operators for any necessary dilutions, and the QC samples. The operators will depend on only supplies provided by the vendor to analyze the samples.

### 3.3 Special Facilities

Four of the contaminants to be evaluated in this verification test (VX, soman, ricin, and botulinum toxin) can only be handled in laboratories that are specially designed and certified for the use of chemical and biological agents. Battelle's Medical Research and Evaluation Facility (MREF), which is a Department of Defense laboratory-scale facility conducting research with chemical and biological agents will be used for verification testing of the specified contaminants. The MREF is licensed to ship, receive, and handle select agents, as defined by the Centers for Disease Control and Prevention. The facility maintains state-of-the-art equipment and professional and technical staffing expertise to safely conduct testing and evaluation of hazardous chemical and biological materials.

The MREF and its personnel have the demonstrated capability for storing and safely handling VX, soman, ricin, and botulinum toxin. Battelle's agent stocks will be analyzed prior to testing to verify the purity of the agent used to make the test samples. Only chemical agents (CA) with purity greater than 85 percent will be used in this program. Handling of CA at the MREF are detailed in the following standard operating procedures (SOP): MREF SOP I-002 Storage, Dilution, and Transfer of GA, GB, GD, GF, TGD, VX, HD, HL, HN and L when CA Concentration/Quantity is Greater than Research Dilute Solution (RDS), MREF SOP I-003 Receipt, Transfer, Storage, and Use of Research Dilute Solution (RDS), and MREF SOP I-003 Disposal of Chemical Agent. These SOPs can be reviewed at the MREF facility, but copies cannot be made for external review. Biological agent use will be according to the CDC Select

Agents Program (32 CFR 626 and 627)<sup>5,6</sup> administered through the Biological Defense Research Program and the Battelle MREF Facility Safety Plan.

### **3.4 Technology Operators**

Given the agent facility restrictions, vendors will not be able to operate their technologies during this verification test. Each rapid toxicity technology will be tested independently while being operated by a Battelle staff member. All operators will have a bachelor's level degree in the sciences or equivalent work experience. The vendor will provide training to the operators by means of a visit to Battelle or a conference call prior to the start of the verification test and then will be asked to sign a consent form stating the names of the Battelle staff they have trained. Each operator will manipulate the water samples and reagents to generate solutions that can be analyzed by the rapid toxicity technologies. More than one operator may be used by Battelle, but operators will be restricted to only operating technologies on which they have been trained. Battelle staff from the MREF will operate the technologies while analyzing samples fortified with VX, soman, ricin, and botulinum toxin.

## 4.0 PROCEDURES

### 4.1 Test Sample Preparation and Storage

The drinking water sample will be collected as described in Section 2.3.2 and because free chlorine will degrade many of the contaminants and interferences during storage, the sample will be immediately dechlorinated with sodium thiosulfate (or other dechlorination reagent as per vendor's protocol). The dechlorination of the drinking water will be qualitatively confirmed by adding a DPD tablet to an aliquot of the DW. If the water does not turn pink, the dechlorination process will be determined to be successful, if it does turn pink an additional 0.5 mg of sodium thiosulfate pentahydrate (or other dechlorinating reagent as per vendor's protocol) will be added to the sample for every liter of water and the dechlorination confirmation procedure will be repeated. Once dechlorination is confirmed, all the DW samples and the negative control QC samples will be made from this sample, while the method blank and positive control QC samples will be prepared from ASTM Type II DI water. The positive control samples will be diluted to appropriate concentrations using ASTM Type II DI water in Class A volumetric glassware. All QC samples will be prepared prior to the start of the testing and stored at room temperature for a maximum of 60 days. The DW samples will be prepared within 7 days of testing and stored in the dark at room temperature. Once dechlorinated and fortified with the contaminants, the DW samples will only be preserved chemically if absolutely necessary, as this verification test is designed to evaluate the ability of rapid toxicity technologies to measure toxicity in drinking water. Therefore, minimal adaptation of the water sample from between the time it comes out of the tap to the analysis time is preferred. Sample treatment as required by the vendors (such as pH adjustment) may need to be performed in order for the technologies to operate properly.

The concentration of the contaminant and interfering compound stock solutions will be verified with standard confirmatory methods, when available. Four aliquots of each stock

solution will be analyzed. Aliquots to be analyzed by confirmatory methods will be preserved as prescribed by the standard method to be used.

## 4.2 Sample Identification

Aliquots to be analyzed by each technology will be drawn from the QC or drinking water samples and placed in uniquely identified sample containers. The sample containers will be identified by a unique identification (ID) number. A master log of the samples and sample ID numbers for each technology will be kept by Battelle. The ID number, date, person collecting, sample location, and time of collection will be recorded on a chain-of-custody form for all field samples.

## 4.3 Sample Analysis

### 4.3.1 Drinking Water Characterization

Table 2 lists the methods to be used to characterize the drinking water sample used as the water source throughout this verification test.

**Table 2. Drinking Water Characterization Standard Methods**

| Chemical              | Method                 |
|-----------------------|------------------------|
| Turbidity             | EPA 180.1 <sup>7</sup> |
| Organic Carbon        | SM 5310 <sup>8</sup>   |
| Specific Conductivity | SM 2510 <sup>9</sup>   |
| Alkalinity            | SM 2320 <sup>9</sup>   |
| pH                    | EPA 150.1 <sup>9</sup> |
| Hardness              | EPA 130.2 <sup>9</sup> |

### 4.3.2 Stock Solution Confirmatory Methodologies

Table 3 lists the methods to be used to verify the concentration of the stock solutions of the contaminants and metal interfering compounds analyzed during this verification test.

**Table 3. Contaminant and Interfering Compounds Stock Solution Confirmatory Methods**

| Chemical   | Method   |
|--|--|
| Cyanide  | EPA 335.1 <sup>10</sup>  |
| Aldicarb   | EPA 531.1 <sup>11</sup>  |
| Dicrotophos  | EPA SW-846 (8141A) <sup>12</sup>   |
| Thallium, Manganese <sup>a</sup> , Iron <sup>a</sup> , Aluminum <sup>a</sup> , Copper <sup>a</sup> , and Zinc <sup>a</sup> | EPA 200.8 <sup>13</sup>  |
| Total Organic Halides  | SM 5320B <sup>12</sup>   |
| Trihalomethanes  | EPA 524.2 <sup>14</sup>  |
| Haloacetic Acids   | EPA 552.2 <sup>15</sup>  |
| Colchicine   | No standard method available. QA audits and balance calibration will assure accurate solutions.                                    |
| VX, Soman, Botulinum Toxin, Ricin  | MREF facility will perform purity analyses on chemical and biological agent materials using internal standard operating procedures |

<sup>a</sup>Potential interfering compounds

### 4.3.3 Technologies Undergoing Verification

Each vendor will be required to provide one complete unit of their rapid toxicity technology and associated equipment and consumables required for the duration of the verification test. Each of the technologies being verified will be used to analyze the full set of samples. As has been described, the sample set will include replicates of each of the QC and drinking water samples. The analyses will be performed according to the vendor's recommended

procedures as described in the user's instructions or manual, or during training provided to the Battelle staff. Similarly, calibration and maintenance of the technologies will be performed as specified by the vendor.

Results from the technologies being verified will be recorded manually by the operator on appropriate data sheets. In addition to the analytical results, the data sheets will include records of the time required for sample analysis and operator observations concerning the use of the technologies (i.e., frequency of calibration, ease of use, maintenance, etc.).

#### **4.4 Schedule**

The verification test described here will take place throughout July and August 2003 at Battelle's laboratories in and near Columbus, Ohio and at a nearby field location. It will be necessary for participating vendors to provide their technologies to Battelle by a specified date so testing staff may become familiar with operating the units before testing begins. Vendor staff should provide training in operating the technologies either in person or by teleconference. Rapid toxicity technologies and associated equipment (but not consumables) will be returned to the vendors at the completion of report writing. Technologies will be decontaminated by washing with an aqueous solution of bleach before being returned to vendor.

## 5.0 DATA HANDLING AND REPORTING

### 5.1 Data Acquisition and Review

A variety of data will be acquired and recorded electronically or manually by Battelle staff in this verification test. Operation, maintenance, and results from the rapid toxicity technologies and sampling procedures, will generally be documented on data sheets or in laboratory record books. Results from the confirmatory method instruments used will be compiled in electronic format. Table 4 summarizes the types of data to be recorded.

**Table 4. Summary of Data Recording Process for the Verification Test**

| Data to be Recorded   | Responsible Party                 | Where Recorded   | How Often Recorded   | Disposition of Data <sup>(a)</sup>  |
|---|-----------------------------------|--|--|---|
| 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, concentrations, preservation)                          | Battelle                          | Laboratory record books  | When each solution is prepared                             | Used to confirm the concentration and integrity of the samples analyzed                       |
| Test parameters (contaminant concentrations, location, etc.)                      | Battelle                          | Laboratory record books  | When set or changed, or as needed to document stability.   | Used to organize/check test results, manually incorporated in data spreadsheets as necessary. |
| Stock solution confirmatory method 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 are carried out by Battelle.

Records received by or generated by any Battelle staff during the verification test will be reviewed by a more senior Battelle staff member within two weeks of data collection, before these records are used to calculate, evaluate, or report verification results. These records may include electronic records; laboratory record books; field sampling records; or equipment calibration records. The review will be documented by the person performing the review by adding his/her initials and date to a hard copy of the record being reviewed. This hard copy will then be returned to the Battelle staff member who received, generated, or will be storing the record. In addition, data calculations performed by Battelle will be spot-checked by a more senior Battelle technical staff member to ensure that calculations are performed correctly. Calculations to be checked include confirmation analysis results and statistical calculations described in this test/QA plan.

The data obtained from this verification test will be compiled and reported independently for each rapid toxicity technology being verified. No intercomparison of the results from one vendor's technology to another's will be made.

## **5.2 Data Evaluation**

### **5.2.1 Endpoints**

Each of the technologies produce their own unique endpoints that are all derived from the inhibition data gathered when analyzing various concentrations of contaminants in drinking water samples. Some of these endpoints include  $EC_{50}$ , effective concentration causing 30% inhibition ( $EC_{30}$ ), and toxicity thresholds (estimate of lowest detectable concentration). For each technology, these data will be documented and presented with respect to each contaminant and concentration level. Where possible, the data from each technology will be converted to common endpoints to ease comparison among the verification reports.



### 5.2.2 Precision

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

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

where n is the number of replicate samples,  $I_k$  is the inhibition measured for the  $k^{\text{th}}$  sample, and  $\bar{I}$  is the average inhibition of the replicate samples. The rapid toxicity technology precision at each concentration level will be reported in terms of the relative standard deviation (RSD), e.g.,

$$RSD = \left| \frac{S}{\bar{C}} \right| \times 100 \quad (4)$$

Also, the relative standard deviation (RSD) of the results for endpoints such as  $EC_{50}$  determined from analysis of replicate dilution series will be calculated and used as a measure of rapid toxicity technology precision with respect to each contaminant.

### 5.2.3 False Positive/Negative Responses

Results will be considered false positive only when an unspiked drinking water sample, administered blindly to the operators, produces inhibition greater than that of the negative control.

Results will be considered false negative only when a rapid toxicity technology is exposed to a lethal concentration of some contaminant in the drinking water sample and the rapid toxicity technology does not indicate inhibition greater than the negative control. The rate of false positives/false negatives will be expressed as a percentage of total samples analyzed for each contaminant.

### 5.2.4 Field Portability

The results obtained from the measurements made on drinking water samples in the laboratory and field setting will be compiled independently for each rapid toxicity technology and compared to assess the accuracy of the measurements under the different analysis conditions. Means and standard deviations of the endpoints generated in both locations will be used to make the comparison. Also, qualitative observations of each technology's performance in a non-laboratory setting will be 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 will be documented and discussed in the report.

#### **5.2.5 Other Performance Factors**

Ease of use will be qualitatively assessed throughout the verification test through observations of the operators and Verification Test Coordinator. Clarity of the vendors instruction manual, user-friendliness of any needed software, overall convenience of the technologies and accessories/consumables, and any unique qualitative observations are among the observations that will be documented and discussed in the verification report.

Sample throughput will be evaluated quantitatively based on the number of samples that can be analyzed per hour.

### **5.3 Reporting**

The data obtained in the verification test will be compiled separately for each vendor's technology, and the data evaluations will be applied to each technology's data set without reference to any other. At no time will data from different vendor's rapid toxicity technology be intercompared or ranked. Following completion of the data evaluations, a draft verification report will be prepared for each vendor's rapid toxicity technology, stating the verification test procedures and documenting the performance observed. The draft verification reports will each be submitted to the respective vendors for review and comment. Battelle will consider the

comments provided by each vendor when revising the verification reports, but does not guarantee that revisions made to the final verification reports will reflect those comments. A written response to all substantive vendor comments not included in the body of the report will be prepared and submitted to the vendor. After vendor review, the revised reports will be submitted to EPA and AMS Center stakeholders for peer review. The reports will then be revised again to address the peer review comments and submitted for final EPA approval.

A verification statement for each technology will be prepared in parallel with the verification reports. The verification statement is a two- to three-page summary of the technology, the test procedures, and the test results. Upon approval by EPA, each verification statement will be signed by a senior manager of Battelle and by an EPA laboratory director. Battelle will reserve the right to post the final verification reports and statements on the ETV website (<http://www.epa.gov/etv>). Original signed verification statements will be provided to the respective vendors for use in marketing their products.

## **6.0 QUALITY ASSURANCE /QUALITY CONTROL**

The QA/QC activities associated with this verification test will focus primarily on sample preparation and handling, and data recording and analysis. An independent audit covering each of these areas will be performed by the Battelle Quality Manager to ensure the quality of the verification test.

### **6.1 QC of Stock Solution Confirmatory Method**

When confirmation analyses of the stock solutions of both contaminant and potential interfering compounds are performed, both method blank and negative control samples will also be performed. Method blank samples will be analyzed to ensure that no sources of contamination are present. If the analysis of a method blank sample indicates a concentration above the MDL for the confirmatory instrument, contamination will be suspected. Any contamination source(s) will be corrected, and proper blank readings will be achieved, before proceeding with the verification test. The negative control sample will indicate the background level of contaminant or potential interfering compound in the DW sample. If this concentration is more than 15% of the fortified concentration of contaminant or potential interfering compound, a correction for the recovery of the confirmation analyses will be performed to account for the amount present in the background.

### **6.2 QC of Drinking Water QC Samples**

The method blank samples analyzed with the DW samples are expected to produce very little or no inhibition. If the inhibition is different from what the vendor suggests for a method blank sample, the analysis will be repeated once. For this re-analysis, a new method blank

sample will be prepared and clean vials/containers and fresh reagents will be used. If similar results persist, the vendor will be notified, but the verification test will proceed.

While there will not be required performance limits placed on the result of the positive control sample, if the result is not in the range of anticipated results specified by the vendor, it will indicate to the operator that the technology may have been operated incorrectly and the analysis of that sample will be repeated once using clean vials/containers and fresh reagents. If the unexpected result persists, the vendor will be notified, but the verification test will proceed.

## **6.3 Audits**

### **6.3.1 Technical Systems Audit**

The Battelle Quality Manager will conduct a technical systems audit at least once during the course of the verification test. The purpose of this audit is to ensure that the verification test is being performed in accordance with this test/QA plan and the AMS Center QMP<sup>1</sup>, and that all procedures described in this test/QA plan are being followed. This audit will review the contaminant standard and stock solution confirmatory methods used, compare actual test procedures to those specified in this test/QA plan, and review data acquisition and handling procedures. An independent technical systems audit may also be performed by EPA Quality Management staff during the verification test, at EPA's discretion.

Before using an outside laboratory to perform stock solution confirmation analyses, the Battelle Quality Manager will conduct an audit of the laboratory's quality documents. If there are areas of concern with the quality documents, the commercial laboratory will be notified, and if they are willing to adapt their procedures, the laboratory will still be used. If not, another laboratory will be selected.

### **6.3.2 Performance Evaluation (PE) Audits**

The concentration of the standards used to prepare the DW samples fortified with contaminants and potential interfering compounds will be confirmed by analyzing standards prepared in Type II DI Water from two separate commercial vendors using the confirmatory methods. The standards from one vendor will be used during the verification test, while the standards from the second vendor will be used exclusively to confirm the accuracy of the displayed concentration of the first vendor. Agreement of the standards within 25% is required for the measurements to be considered as acceptable. Failure to achieve this agreement will trigger a repeat of the performance evaluation comparison. Failure in the second comparison requires obtaining another set of standards, and repeating the performance audit.

Given the security requirements and lack of confirmatory methodology for some of the contaminants in this verification test, PE audits will not be performed for all of the contaminants. They will be done when more than one source of the contaminant or potential interfering compounds are available and there are available confirmatory methods.

### **6.3.3 Audit of Data Quality**

At least 10% percent of the data generated during the verification test will be audited during the verification test. Battelle's Quality Manager will trace 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 will be checked.

## **6.4 QA/QC Reporting**

Each assessment and audit will be documented in accordance with Section 3.3.4 of the QMP for the AMS Center. The results of the technical systems audit will be sent to the EPA. Assessment reports will include the following:

- Identification of any adverse findings or potential problems.
- Response to adverse findings or potential problems.
- Recommendations for resolving problems.
- Confirmation that solutions have been implemented and are effective.
- Citation of any noteworthy practices that may be of use to others.

### **6.5 Corrective Action**

The Battelle or EPA Quality Managers during the course of any assessment or audit will identify to the technical staff performing experimental activities any immediate corrective action that should be taken. If serious quality problems exist, the Battelle Quality Manager is authorized to stop work. Once the assessment report has been prepared, the Verification Test Coordinator will ensure that a response is provided for each adverse finding or potential problem, and will implement any necessary follow-up corrective action. The Battelle Quality Manager will ensure that follow-up corrective action has been taken.

## **7.0 HEALTH AND SAFETY**

### **7.1 Handling of Contaminants**

#### **7.1.1 Stock Solution / DW Sample Preparation**

All handling of solid and highly concentrated aqueous solutions of contaminants and possible interferences will be done inside of a laboratory hood with hood sash set to the lowest height that still allows for safe manipulation of materials. The following guidelines should be adhered to:

- Personal protective equipment shall include safety glasses with side shields, a laboratory coat and nitrile lab gloves. Gloves shall be immediately changed if they become contaminated. (The same gloves can be used for sodium hydroxide)
- All contaminated waste shall be handled as hazardous waste and sent out through Battelle Waste Operations.

#### **7.1.2 Field Handling**

Field handling of the contaminant and interfering compound solutions will be accomplished by taking the following precautions:

- All containers shall be stored and transported in double containment.
- Safety goggles, nitrile gloves with long cuffs, and a chemical resistant disposable lab coat shall be worn when handling chemicals. Gloves shall be immediately changed if they become contaminated.



### **7.1.3 VX, Soman, Ricin, and Botulinum Toxin**

These contaminants will be handled following the safety procedures required at the MREF facility as listed in Section 3.3.

## 8.0 REFERENCES

1. Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center, U.S. EPA Environmental Technology Verification Program, prepared by Battelle, Columbus, Ohio, Version 4.0 December 2002
2. "Environmental Technology Verification Program Quality Management Plan" (QMP), December 2002, EPA/600/R-03/021
3. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, U.S. EPA Document, 5<sup>th</sup> Ed., EPA-821-R-02-012 October 2002.
4. Secondary Drinking Water Regulations: Guidance for Nuisance Chemicals, U.S. EPA Document, EPA-810-K-92-001, July 1992.
5. Code of Federal Regulations, Title 32, Chapter 5, Part 626, Biological Defense Safety Program.
6. Code of Federal Regulations, Title 32, Chapter 5, Part 626, Biological Defense Safety Program, Technical Safety Requirements.
7. EPA Method 180.1, "Turbidity (Nephelometric)", Methods for the Determination of Inorganic Substances in Environmental Samples EPA-600-R-93-100 1993.
8. American Public Health Association, et al. Standard Methods for the Examination of Water and Wastewater. 19<sup>th</sup> Edition, 1997. Washington, DC.
9. EPA Methods for Chemical Analysis of Water and Wastes EPA-600-4-79-020.
10. EPA Method 335.1, Cyanides Amenable to Chlorination, 1974, in "Methods for Chemical Analysis of Water and Wastes, " EPA-600-4-79-020, March 1983.
11. EPA Method 531.1, Measurement of n-Methylcarbamoyloximes and n-methylcarbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization, Revision 3.1, 1995.
12. SW846 Method 8141A, "Organophosphorous Compounds by Gas Chromatography:Capillary Column Technique", Revision 1, September 1994.
13. EPA Method 200.8, "Determination of Trace Elements in Waters and Wastes by Inductively-Coupled Plasma Mass Spectrometry", Revision 5.4, 1994.

14. EPA Method 524.2, "Purgeable Organic Compounds by Capillary Column GC/Mass Spectrometry", Methods for the Determination of Organic Compounds in Drinking Water-Supplement III EPA/600/R-95-131.
15. EPA Method 552.2, "Haloacetic Acids and Dalapon by Liquid-Liquid Extraction, Derivatization and GC with Electron Capture Detector", Methods for the Determination of Organic Compounds in Drinking Water-Supplement III EPA/600/R-95-131.

**APPENDIX**

**SUMMARY OF SAMPLES FOR**  
**RAPID TOXICITY TECHNOLOGIES**

**Table A-1. Summary of Contaminant Test Samples**

| Type of Sample  | Sample Characteristics   | Lethal Dose Concentration        | No. of Samples <sup>a</sup> |
|-----------------|--|----------------------------------|-----------------------------|
| Quality Control | Method Blank   | NA                               | At least 5% of all samples  |
|                 | Positive Control (contaminant and concentration determined by each vendor) | NA                               | At least 5% of all samples  |
|                 | Negative Control (unfortified drinking water)                              | NA                               | One per dilution series     |
|                 | Unfortified drinking water   | NA                               | At least 5% of all samples  |
| Drinking Water  | Cyanide  | 250 mg/L                         | 12-20                       |
|                 | Aldicarb   | 140 mg/L                         | 12-20                       |
|                 | Dicrotophos  | 1.4 g/L                          | 12-20                       |
|                 | Thallium Sulfate   | 2.8 g/L                          | 12-20                       |
|                 | Colchicine   | 240 mg/L                         | 12-20                       |
|                 | VX   | 0.2 mg/L                         | 12-20                       |
|                 | Soman  | 0.30 mg/L                        | 12-20                       |
|                 | Botulinum Toxin  | 0.3 mg/L                         | 12-20                       |
| Ricin           | 15 mg/L  | 12-20                            |                             |
| Field Location  | One drinking water sample fortified with any one of the above contaminants | One of the above concentrations. | 12-20                       |

<sup>a</sup> Each lethal dose concentration will be analyzed along with 2 to 4 dilutions. Four replicates of each dilution series will be analyzed. Therefore, the number of samples listed in the table is an approximate range.

**Table A-2. Summary of the Interfering Compound Test Samples**

| Type of Sample  | Sample Characteristics   | Concentration          | No. of Samples               |
|-----------------|--|------------------------|------------------------------|
| Quality Control | Method Blank   | NA                     | At least 5% of all samples   |
|                 | Positive Control (contaminant and concentration determined by each vendor) | NA                     | At least 5% of all samples   |
|                 | Negative Control (unfortified drinking water)                              | NA                     | One per interfering compound |
|                 | Unfortified drinking water   | NA                     | At least 5% of all samples   |
| Drinking Water  | Manganese  | 0.25 mg/L              | 4                            |
|                 | Iron   | 0.15 mg/L <sup>a</sup> | 4                            |
|                 | Aluminum   | 0.5 mg/L <sup>a</sup>  | 4                            |
|                 | Copper   | 0.6 mg/L <sup>a</sup>  | 4                            |
|                 | Zinc   | 2.5 mg/L <sup>a</sup>  | 4                            |
|                 | Chloramination byproducts  | NA <sup>b</sup>        | 4                            |
|                 | Chlorination byproducts  | NA                     | 4                            |

<sup>a</sup> National Secondary Drinking Water Regulations that set non-mandatory water quality standards

<sup>b</sup> A separate water sample will be collected from a system using chloramination as its disinfection process for these analyses.