

THE ENVIRONMENTAL TECHNOLOGY VERIFICATION
PROGRAM



ETV Joint Verification Statement

TECHNOLOGY TYPE: RAPID TOXICITY TESTING SYSTEM

APPLICATION: DETECTING TOXICITY IN DRINKING WATER

TECHNOLOGY NAME: BioTox™

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The U.S. Environmental Protection Agency (EPA) supports 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.

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 seven technology areas under ETV, is operated by Battelle in cooperation with EPA's National Exposure Research Laboratory. The AMS Center has recently evaluated the performance of rapid toxicity testing systems used to detect toxicity in drinking water. This verification statement provides a summary of the test results for the BioTox™ testing system.

VERIFICATION TEST DESCRIPTION

Rapid toxicity technologies use bacteria, enzymes, or small crustaceans that produce light or use oxygen at a steady rate in the absence of toxic contaminants. Toxic contaminants in drinking water are indicated by a change in the color or intensity of light or by a change in the rate of oxygen use. As part of this verification test, which took place between July 14 and August 22, 2003, various contaminants were added to separate drinking water samples and

analyzed by BioTox™. Response to interfering compounds in clean drinking water also was evaluated. Dechlorinated drinking water samples from Columbus, Ohio, (DDW) were fortified with contaminants at concentrations ranging from lethal levels to levels 1,000 times less than the lethal dose and analyzed. Endpoint and precision, toxicity threshold for each contaminant, false positive/negative responses, ease of use, and sample throughput were evaluated.

Inhibition results (endpoints) from four replicates of each contaminant at each concentration level were evaluated to assess the ability of the BioTox™ to detect toxicity at various concentrations of contaminants, as well as to measure the precision of the BioTox™ results. The response of BioTox™ to compounds used during the water treatment process (interfering compounds) was evaluated by analyzing separate aliquots of DDW fortified with each potential interferent at approximately 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, unspiked DDW was analyzed because Columbus, Ohio, uses chlorination as its disinfectant procedure. For the analysis of by-products of the chloramination process, a separate drinking water sample from St. Petersburg, Florida, which uses chloramination as its disinfection process, was obtained. The samples were analyzed after residual chlorine was removed using sodium thiosulfate. Sample throughput was measured based on the number of samples analyzed per hour. Ease of use and reliability were determined based on documented observations of the operators and the verification test coordinator.

Quality control samples included method blank samples, which consisted of American Society for Testing and Materials (ASTM) Type II deionized (DI) water; positive control samples fortified with zinc sulfate; and negative control samples, which consisted of the unspiked DDW. EPA QA staff also performed a technical systems audit while testing was being conducted.

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. EPA QA staff also performed a technical systems audit while testing was being conducted.

TECHNOLOGY DESCRIPTION

The following description of BioTox™ was provided by the vendor and was not subjected to verification in this test.

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

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

To determine whether a contaminant caused detectable inhibition, the inhibition exhibited by drinking water spiked with a contaminant was compared to the inhibition exhibited by the unspiked drinking water. Four replicates of each spiked sample were analyzed. A result was considered positive if the inhibition of the water

sample spiked with a contaminant plus or minus the standard deviation of four replicates did not include the inhibition of the unspiked drinking water.

The BioTox™ kit, which provides for 144 measurements, contains six vials of freeze-dried *V. fischeri* reagent, six vials of reagent diluent (12.5 milliliters [mL] each), and one 50-mL bottle of concentrated sample diluent. Reagent injection and data acquisition can be performed by a computer connected to the Triathler™ luminometer. The dimensions of the Triathler™ luminometer are 10 inches by 10 inches by 6 inches, and it weighs approximately 10 pounds. It only can be operated on 110-volt alternating current electricity. The BioTox™ kit costs \$128, the Triathler™ injector costs \$1,950, and the luminometer with liquid scintillation counter costs \$6,950.

VERIFICATION OF PERFORMANCE

Endpoint and Precision/Toxicity Threshold: The table below presents BioTox™ percent inhibition data and range of standard deviations for the contaminants and potential interferences that were tested. The toxicity thresholds also are shown for each contaminant tested.

| Parameter | Compound | Lethal Dose (LD) Conc. (mg/L) | Average Inhibitions at Concentrations Relative to the LD Concentration (%) | | | | Range of Standard Deviations (%) | Toxicity Thresh. (mg/L) |
|--------------------------------|--------------------------------|-------------------------------|--|-------|--------|----------|----------------------------------|-------------------------|
| | | | LD | LD/10 | LD/100 | LD/1,000 | | |
| Contaminants in DDW | Aldicarb | 280 | 3 | 0 | -1 | -10 | 3-12 | ND ^(a) |
| | Colchicine | 240 | -8 | -15 | 10 | -27 | 11-27 | ND |
| | Cyanide | 250 | 96 | 61 | 10 | -1 | 2-16 | 25 |
| | Dicrotophos | 1,400 | 2 | 5 | 2 | 6 | 4-10 | ND |
| | Thallium sulfate | 2,400 | 41 | 18 | 11 | -4 | 6-16 | 24 |
| | Botulinum toxin ^(b) | 0.30 | 5 | 2 | 10 | 2 | 5-8 | ND |
| | Ricin ^(c) | 15 | -5 | 3 | 2 | 0 | 6-10 | ND |
| | Soman | 0.068 ^(d) | 7 | -1 | 1 | 3 | 2-3 | ND |
| | VX | 0.22 | 8 | 3 | 5 | 2 | 2-9 | ND |
| Potential interferences in DDW | Interference | Conc. (mg/L) | Average Inhibitions at a Single Concentration (%) | | | | Standard Deviation (%) | |
| | Aluminum | 0.36 | 16 | | | | 12 | |
| | Copper | 0.65 | 96 | | | | 4 | |
| | Iron | 0.069 | 0 | | | | 2 | |
| | Manganese | 0.26 | 10 | | | | 9 | |
| | Zinc | 3.5 | 48 | | | | 10 | |

^(a) ND = Not detectable.

^(b) Lethal dose solution also contained 3 mg/L phosphate and 1 mg/L sodium chloride.

^(c) Lethal dose solution also contained 3 mg/L phosphate, 26 mg/L sodium chloride, and 2 mg/L sodium azide.

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

False Positive/Negative Responses: Slightly exaggerated inhibitions (false positive responses) may result if BioTox™ is used to analyze chloraminated water, which produced $13\% \pm 2\%$ inhibitions, with respect to ASTM Type II DI water. Inhibition greater than the negative control was not detected for lethal doses of aldicarb, colchicine, dicrotophos, botulinum toxin, ricin, soman, and VX; and, therefore, these results were considered false negative. Inhibition was $-49\% \pm 33\%$ for water from the system treated by chlorination, resulting in a risk of false negative responses when using ASTM Type II DI water as the control sample.

