THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

U.S. Environmental Protection Agency

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**Atrazine Test Kit**
APPLICATION:	**ANALYSIS OF ATRAZINE IN WATER**
TECHNOLOGY NAME:	**Atrazine Tube Kit**
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The U.S. Environmental Protection Agency (EPA) has created 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 that consist of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers. The program evaluates the performance of innovative technologies by developing test plans that are responsive to the needs of stakeholders, conducting field or laboratory tests (as appropriate), collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and adequate quality are generated and that the results are defensible.

The 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 test kits for the analysis of atrazine in water. This verification statement provides a summary of the test results for the Beacon Analytical Systems, Inc. Atrazine Tube Kit.

VERIFICATION TEST DESCRIPTION

The Atrazine Tube Kit was verified in terms of its performance on the following parameters: accuracy, precision, linearity, method detection limit (MDL), cross-reactivity of hydroxylatrazine and desethyl atrazine, matrix interference effects, and rate of false positives/false negatives. Qualitative factors including ease of use, reliability, and sample throughput were also evaluated. All preparation and analyses were performed according to the manufacturer's recommended procedures. The verification test involved challenging the Atrazine Tube Kit with seven performance test (PT) samples and four types of environmental samples. The PT samples consisted of ASTM Type I

water samples fortified with atrazine or an atrazine degradation product. Five of the PT samples contained atrazine at concentrations ranging from 0.1 to 5 parts per billion (ppb), and two of the samples contained 3 ppb of a cross-reactive compound, but no atrazine. Four types of environmental samples also were analyzed: fresh pond water, brackish pond water, groundwater, and chlorinated drinking water. Environmental samples were filtered prior to test kit analysis. The background atrazine concentration in each environmental sample was less than 0.062 ppb. Each environmental sample was fortified in the laboratory at concentrations of 1 ppb and 3 ppb atrazine. All laboratory-fortified samples were prepared using certified, commercially available standards. All samples were analyzed by the Atrazine Tube Kit and by gas chromatography/mass spectrometry (GC/MS) according to modified EPA Method 525.2. Each sample was analyzed in triplicate using the test kit (seven replicates of the MDL sample were analyzed). Samples were given to the analyst blind and in random order.

The verification test was conducted in September 2003 at the Battelle laboratory in Duxbury, Massachusetts. Environmental samples were provided by the National Oceanic and Atmospheric Administration, National Ocean Service's Center for Coastal Environmental Health and Biomolecular Research Center at Charleston, and the University of Missouri - Rolla. Reference laboratory analyses were provided by the EPA's Office of Pesticide Programs, Environmental Chemistry Branch at the John C. Stennis Space Center. Test kit analyses were conducted by the Texas Commission on Environmental Quality.

The Atrazine Tube Kit and reference method results were used to assess accuracy and linearity. Replicate sample results were used to assess precision. Results for replicates of a low-level spiked sample were used to evaluate the MDL. Cross-reactivity of hydroxyatrazine and desethyl atrazine were assessed by evaluating the Atrazine Tube Kit results for samples that contained only one degradation compound, but not atrazine. Potential matrix effects were assessed by comparing accuracy and precision results for environmental samples (i.e., chlorinated drinking water, fresh surface water, brackish surface water, and groundwater) to those for ASTM Type I water samples. Performance parameters, such as ease of use and reliability, were based on documented observations of the analyst. Sample throughput was estimated based on the time required to analyze a sample set. QA oversight of verification testing was provided by Battelle and EPA. Battelle QA staff conducted a data quality audit of 10% of the test data, a performance evaluation audit, and a technical systems audit of the procedures used in this verification. This verification statement, the full report on which it is based, and the test/QA plan for this verification are all available at www.epa.gov/etv/centers/center1.html.

TECHNOLOGY DESCRIPTION

The following description of the Atrazine Tube Kit is based on information provided by the vendor. This information was not verified in this test. The Atrazine Tube Kit is an immunological test for measuring atrazine residues in water. It uses polyclonal antibodies that bind both atrazine and an atrazine-enzyme conjugate. Atrazine in the sample competes with atrazine-enzyme conjugate for a limited number of antibody binding sites. Antibodies, which bind atrazine, are immobilized to the inside of the test tubes. The assay procedure involves (1) adding samples and calibrators containing known amounts of atrazine and an atrazine- enzyme conjugate to test tubes coated with anti-atrazine antibodies; (2) incubating the mixture for 20 minutes, and washing away unbound molecules; and (3) adding clear substrate solution to each tube. Bound atrazine-enzyme conjugate converts the substrate to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in each tube and each tube receives the same number of atrazine-enzyme conjugate molecules, a low concentration of atrazine in a sample allows the antibody to bind many atrazine-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of atrazine allows fewer atrazine-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution. Absorbance of test tubes at 450 nanometers is measured in a photometer specifically designed for 12- x 75-millimeter tubes. The calibration range of the test kit is 0.05 ppb to 5 ppb atrazine. The vendor-stated detection limit of the test kit is 0.05 ppb atrazine. The Atrazine Tube Kit contains two vacuum-packed foil bags, each containing 20 polystyrene test tubes coated with anti-atrazine antibodies and color-indicating dessicant; one vial each of three atrazine calibrators; and one vial each of negative control (0.0 ppb atrazine), assay control (exact value range printed on vial), atrazine-horseradish peroxidase enzyme conjugate, substrate, and stop solution. The Atrazine Tube Kit costs $200 per 40 tubes.

Other materials that are required but not provided with the Atrazine Tube Kit are the photometer, a watch or timer, wash bottle containing tap or deionized water, a 500 µL pipette with disposable tips, and a test tube rack that will retain the test tubes when inverted. These materials can be purchased separately from the vendor.

VERIFICATION OF PERFORMANCE

Quantitative performance results for all parameters except ease of use, reliability, and sample throughput are summarized in the following table:

Parameter	Performance Results	Comments
Accuracy (percent recovery) PT samples, 0.1 – 5 ppb atrazine Environmental samples: 1 ppb and 3 ppb atrazine-fortified, respectively: Fresh pond water Brackish pond water Groundwater Chlorinated drinking water	 82% - 133%; average 101% 86% and 75% 171% and 129% 94% and 83% 135% and 87%	 Background atrazine concentrations in all environmental samples were <0.062 ppb. 135% recovery in chlorinated drinking water + 1 ppb atrazine sample due to low reference method result (0.79 ppb).
Precision (relative standard deviation) PT samples, 0.1 – 5 ppb atrazine and cross-reactivity samples Environmental samples: 1 ppb and 3 ppb atrazine-fortified, respectively: Fresh pond water Brackish pond water Groundwater Chlorinated drinking water	 5.0% - 25.4%; average 15.9% 8.7% and 12.7% 16.4% and 12.0% 22.8% and 3.9% 9.8% and 14.5%	
Linearity Slope of regression equation y-intercept Correlation coefficient (r)	 0.81 0.24 0.9575	Results for PT samples from 0.1 ppb to 5 ppb atrazine used to assess linearity.
MDL	ND	Atrazine was not detected in the MDL sample (ASTM Type I water spiked with 0.1 ppb atrazine), so MDL could not be determined according to test/QA plan.
Cross-reactivity 3 ppb hydroxyatrazine 3 ppb desethyl atrazine	 Average result <0.05 ppb atrazine Average result 0.11 ppb atrazine	 Cross-reactivity samples did not contain atrazine.
Matrix interference effects	171% and 129% average recoveries for atrazine-fortified brackish pond water samples compared to 101% average recovery for PT samples	Positive bias observed in brackish pond water sample results.
False positive results	6 out of 38 results	Evaluated relative to 0.1 ppb atrazine. Three of the six false positive results associated with a sample containing an atrazine degradation product.
False negative results	None	Evaluated relative to 0.1 ppb atrazine. Three of these results associated with a sample containing an atrazine degradation product.

The Atrazine Tube Kit was relatively easy to use for an analyst with previous experience performing immunoassay analyses, although the narrow necks on the reagent bottles made it difficult to insert the pipette when the bottles were less than half full. An analyst with less experience may not achieve the same level of performance. Consistency in analytical technique was critical. The required volume for all reagents, standards, and samples for the Atrazine Tube Kit is 500 µL, thereby minimizing the chance of pipetting an incorrect volume. The wash step was accomplished more efficiently using tap water directly from a faucet rather than using a squeeze bottle. Although a single analyst can analyze samples with the Atrazine Tube Kit, the process was more efficient and less prone to error with a second person available to assist. The Atrazine Tube Kit is readily transportable and can be used in a mobile laboratory or indoor work space. The Atrazine Tube Kit operated without failure during the test. A batch of about 30 samples was analyzed in approximately one hour with the Atrazine Tube Kit.

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