THE ENVIRONMENTAL TECHNOLOGY VERIFICATION PROGRAM

ETV Joint Verification Statement

TECHNOLOGY TYPE:	**Atrazine Test Kit**
APPLICATION:	**ANALYSIS OF ATRAZINE IN WATER**
TECHNOLOGY NAME:	**Atrazine ELISA 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 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 Abraxis LLC Atrazine ELISA Kit for measuring atrazine.

VERIFICATION TEST DESCRIPTION

The Atrazine ELISA 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 ELISA 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 PT 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 ELISA 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 ELISA 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 ELISA 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 ELISA Kit is based on information provided by the vendor. This information was not verified in this test. The Atrazine ELISA Kit applies the principle of enzyme-linked immunosorbent assay (ELISA) to determine atrazine in water samples. The Atrazine ELISA Kit uses a colorimetric procedure to detect atrazine. A sample and an enzyme conjugate are added to a disposable test tube, followed by atrazine antibodies attached covalently to paramagnetic particles. Any atrazine that may be in the sample competes with the atrazine enzyme label conjugate for a finite number of antibody binding sites. At the end of a 15-minute incubation period, a magnetic field is applied; and atrazine and labeled-atrazine bind to the antibodies on the paramagnetic particles in proportion to their original concentration. Unbound reagents are decanted. After decanting, the particles are washed with a washing solution. A substrate is added and enzymatically converted from a colorless to a blue solution until terminated by acidification. The atrazine concentration is determined by measuring the absorbance of the sample solution with a photometer and comparing it to the absorbance of the standards. The calibration range of the test kit is 0.1 ppb to 5 ppb atrazine. The vendor-stated detection limit of the test kit is 0.05 ppb atrazine.

The Atrazine ELISA Kit contains a vial of atrazine antibody (rabbit anti-atrazine covalently bound to paramagnetic particles suspended in a buffered solution with preservative and stabilizers), a vial of horseradish peroxidase-labeled atrazine analog diluted in a buffered solution with preservative and stabilizers, three vials of atrazine standard concentrations with preservative and stabilizers, a vial of concentrated atrazine (3 ± 0.6 parts per billion [ppb]) with preservative and stabilizers, a vial of an atrazine-free solution with preservative and stabilizers for use as a zero standard, a vial of a hydrogen peroxide and 3,3',5,5'-tetramethylbenzidine solution in an organic base, a vial of diluted acid, a vial of preserved deionized water,

and five bags of 22 polystyrene tubes. The Atrazine ELISA Kit is 14 by 6-¼ by 3-½ inches. Final results and calibration curves are printed out on the photometric analyzer or sent directly to a lab computer. List price is $350 for a 100-test kit. Other materials that are required but are not provided with the Atrazine ELISA Kit are pipettes, a vortex mixer, a magnetic separation system, and a photometer capable of readings at 450 nanometers (nm). These materials can be purchased separately or rented.

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	102% to 127%; average 120%	
Environmental samples: 1 ppb and 3 ppb atrazine-fortified, respectively:		
Fresh pond water	130% and 102%	Background atrazine concentrations in all environmental samples were <0.062 ppb.
Brackish pond water	110% and 107%	
Groundwater	111% and 100%	
Chlorinated drinking water	140% and 122%	
Precision (relative standard deviation)		
PT samples, 0.1 – 5 ppb atrazine and cross-reactivity samples	6.9% to 24.1%; average 13%	
Environmental samples: 1 ppb and 3 ppb atrazine-fortified, respectively:		
Fresh pond water	3.5% and 10.6%	
Brackish pond water	15.2% and 7.1%	
Groundwater	7.7% and 8.3%	
Chlorinated drinking water	3.7% and 11.1%	
Linearity		
Slope of regression equation	1.23	Results for PT samples from 0.1 ppb to 5 ppb atrazine used to assess linearity.
y-intercept	-0.025	
Correlation coefficient (r)	0.9937	
MDL	0.06 ppb atrazine	Based on analysis of 0.1 ppb atrazine spiked into ASTM Type I water sample (seven replicates).
Cross-reactivity		
3 ppb hydroxyatrazine	Average result 0.06 ppb atrazine	Cross-reactivity samples did not contain atrazine.
3 ppb desethyl atrazine	Average result 0.25 ppb atrazine	
Matrix interference effects	No apparent interferences from matrices tested	
False positive results	4 out of 38 results	Evaluated relative to 0.1 ppb atrazine (lowest calibration standard). Three of the four false positive results associated with a sample containing an atrazine degradation product.
False negative results	None	Evaluated relative to 0.1 ppb atrazine (lowest calibration standard). Three of these results associated with a sample containing an atrazine degradation product.

During the test, the analyst recorded observations regarding ease of use, reliability, and sample throughput. The Atrazine ELISA Kit was easy to use by an analyst with previous experience in performing immunoassay analyses. An analyst with less experience may not achieve the same level of performance. Consistent analytical technique was the most important parameter, particularly with respect to addition of reagents. Although a single analyst can analyze samples with the Atrazine ELISA Kit, the process was more efficient and less prone to error with a second person available to assist. The Atrazine ELISA Kit is readily transportable and can be used in a mobile laboratory or indoor work space. The Atrazine ELISA Kit operated without failure during the test. A batch of about 50 samples was analyzed with the Atrazine ELISA Kit in approximately 1½ hours.

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