March 2004

Environmental Technology Verification Report

SILVER LAKE RESEARCH CORP. WATERSAFE® PESTICIDE TEST

Prepared by
Battelle

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ETV ETV ETV

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Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

Silver Lake Research Corp. Watersafe® Pesticide Test

by

Patty White
Bo Liu
Amy Dindal
Zachary Willenberg
Karen Riggs

Battelle
Columbus, Ohio 43201

Notice

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the nation's air, water, and land resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, the EPA's Office of Research and Development provides data and science support that can be used to solve environmental problems and to build the scientific knowledge base needed to manage our ecological resources wisely, to understand how pollutants affect our health, and to prevent or reduce environmental risks.

The Environmental Technology Verification (ETV) Program has been established by the EPA to verify the performance characteristics of innovative environmental technologies across all media and to report this objective information to permitters, buyers, and users of the technology, thus substantially accelerating the entrance of new environmental technologies into the marketplace. Verification organizations oversee and report verification activities based on testing and quality assurance protocols developed with input from major stakeholders and customer groups associated with the technology area. ETV consists of seven environmental technology centers. Information about each of these centers can be found on the Internet at http://www.epa.gov/etv/.

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

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List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
DOC	dissolved organic carbon
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
GC/MS	gas chromatography/mass spectrometry
ID	identification
L	liter
LFB	laboratory-fortified blank
MCL	maximum contaminant level
mL	milliliter
µm	micrometer
ppb	parts per billion
PE	performance evaluation
PT	performance test
QA	quality assurance
QC	quality control
QMP	Quality Management Plan
RB	reagent blank
RPD	relative percent difference
SOP	standard operating procedure
STL	Severn Trent Laboratories
TSA	technical systems audit

Chapter 1
Background

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

ETV works in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and 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 EPA's National Exposure Research Laboratory and its verification organization partner, Battelle, operate the Advanced Monitoring Systems (AMS) Center under ETV. The AMS Center recently evaluated the performance of the Silver Lake Research Corp. Watersafe® Pesticide Test for measuring atrazine in water.

Chapter 2
Technology Description

The objective of the ETV AMS Center is to verify the performance characteristics of environmental monitoring technologies for air, water, and soil. This verification report provides results for verification testing of the Watersafe® Pesticide Test for measuring atrazine in water (Figure 2-1). Following is a description of the Watersafe® Pesticide Test, based on information provided by the vendor. The information provided below was not subjected to verification in this test.

The Watersafe® Pesticide Test (Model WS-289) is a one-step qualitative immunoassay for detecting the presence of unsafe levels of atrazine or simazine in water samples. The test procedure takes about 10 minutes and informs the user whether the levels of these common indicator pesticides exceed EPA maximum contaminant levels (MCLs) of 3 parts per billion (ppb) for atrazine and 4 ppb for simazine[1]. The Watersafe® Pesticide Test can be used on samples of surface water, groundwater, and treated or untreated drinking water. The test is designed for field use, requiring no instrumentation or other equipment, no power sources, and no refrigerated storage.

Figure 2-1. Silver Lake Research Corp. Watersafe® Pesticide Test

The Watersafe® Pesticide Test contains a test vial and a pipette for easy sample collection and handling. No mixing, measuring, or reagents are necessary. Exposing the test strip to a small sample of water triggers the binding of antibodies to atrazine or simazine molecules, resulting in a change in color intensity in the result window of the test strip. The test cannot differentiate between atrazine and simazine. If the bottom line on the test strip (next to the number 1, see Figure 2-1) is darker than the top line, then the sample result is negative. If the top line is darker than the bottom line, or the lines are equally dark, then the test result is positive. The test reaction is completely contained within the test strip.

The Watersafe® Pesticide Test comes in 1.5-inch x 2.9-inch x 8-inch packets, which are sold by the case. A case includes ten packets. Each test packet costs $5.99, and a case is $59.99.

Chapter 3
Test Design and Procedures

3.1 Introduction

This verification test was conducted according to procedures specified in the *Test/QA Plan for Verification of Test Kits for Detection of Atrazine in Water*[2]. A variety of sample matrices were tested: American Society for Testing and Materials (ASTM) Type I water[3], fresh pond water, brackish pond water, shallow (i.e., alluvial) groundwater, and chlorinated drinking water. These matrices are examples of water types that are typically monitored using the Watersafe® Pesticide Test; however, they do not represent all possible water types that could be tested.

Test kits specific for atrazine are typically cross-reactive for a variety of triazine analogues, some of which are degradation products of atrazine. The effect of two potentially cross-reactive atrazine degradation products (hydroxylatrazine and desethyl atrazine) on the performance of the Watersafe® Pesticide Test was verified in this test. The Watersafe® Pesticide Test was evaluated for the following parameters:

- Accuracy
- Precision
- Cross-reactivity of hydroxylatrazine and desethyl atrazine
- Matrix interference effects
- Occurrence of false positive and false negative results
- Other factors (ease of use, reliability, and sample throughput).

An analyst with five years of previous experience using immunoassay test kits performed all analyses to minimize error due to operator inexperience. A second person assisted the analyst during the test. The vendor opted to not provide training to the analyst on the use of the Watersafe® Pesticide Test prior to the initiation of the test. All testing was conducted at the Battelle laboratory in Duxbury, MA.

3.2 Test Design

The verification test involved challenging the Watersafe® Pesticide Test with samples of fresh pond water, brackish pond water, alluvial groundwater, and chlorinated drinking water. Natural and atrazine-fortified (i.e., unspiked and spiked) samples were analyzed using both the Watersafe® Pesticide Test and a laboratory reference method. ASTM Type I water samples

fortified with atrazine or an atrazine degradation product also were analyzed. Physico-chemical parameters (pH, temperature, salinity, conductivity, alkalinity, and dissolved organic carbon [DOC]) were measured in the environmental samples to provide supporting characterization data.

All samples were analyzed by the Watersafe® Pesticide Test and by gas chromatography/mass spectrometry (GC/MS) according to modified EPA Method 525.2[4]. Each sample was analyzed in triplicate using the test kit. Samples were given to the analyst blind and in random order.

The Watersafe® Pesticide Test and reference method results were used to assess accuracy. Replicate sample results were used to assess precision. Cross-reactivity of hydroxyatrazine and desethyl atrazine were assessed by evaluating the Watersafe® Pesticide Test results for samples that contained one of the degradation compounds, 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. Data analysis procedures are described in Section 5 of this report.

3.3 Test Samples

Test samples included quality control (QC) samples, performance test (PT) samples, and environmental water samples. Table 3-1 lists the number and type of each sample analyzed. Each type of test sample is described further below.

3.3.1 QC Samples

The only QC sample included in this test were reagent blank (RB) samples. The RB samples were prepared from ASTM Type I water and were exposed to identical sample analysis procedures as the test samples. These samples were used to help ensure that no sources of contamination were introduced in the sample handling and analysis procedures. At least 10% of the test samples were RB samples. The RB sample results were also used to test for false positives (Section 5.5).

3.3.2 PT Samples

PT sample types are listed in Table 3-1. The first type of PT sample consisted of ASTM Type I water spiked at five different atrazine concentration levels. The PT sample concentration range included the 3 ppb MCL for atrazine in drinking water[1]. Three replicates of each PT sample were analyzed using the Watersafe® Pesticide Test. One replicate of each PT sample was analyzed by the reference method to confirm the nominal spike concentration.

Table 3-1. Test Samples

Type of Sample	Description	Replicates	Reference Laboratory Analyses	Performance Factor[a]
Quality Control				
Reagent blanks (10%)	minimum 10% frequency	20	1	QC, false positive
Performance Test				
Performance test #1	0.1 ppb atrazine	3	1	Accuracy, precision, false positive/negative
Performance test #2	0.5 ppb atrazine	3	1	
Performance test #3	1 ppb atrazine	3	1	
Performance test #4	3 ppb atrazine	3	1	
Performance test #5	5 ppb atrazine	3	1	
Cross-reactivity test #1	3 ppb hydroxyatrazine	3	1	Cross-reactivity, false positive
Cross-reactivity test #2	3 ppb desethyl atrazine	3	1	
Environmental				
Fresh water	Fresh surface water, unspiked	3	1	Accuracy, precision, matrix effects, false positive/negative
Fresh water spike #1	Fresh surface water with 1 ppb atrazine spike	3	1	
Fresh water spike #2	Fresh surface water with 3 ppb atrazine spike	3	1	
Brackish water	Brackish water, unspiked	3	1	
Brackish water spike #1	Brackish water with 1 ppb atrazine spike	3	1	
Brackish water spike #2	Brackish water with 3 ppb atrazine spike	3	1	
Groundwater	Groundwater, unspiked	3	1	
Groundwater spike #1	Groundwater with 1 ppb atrazine spike	3	1	
Groundwater spike #2	Groundwater with 3 ppb atrazine spike	3	1	
Chlorinated drinking water	Chlorinated drinking water	3	1	
Chlorinated drinking water spike #1	Chlorinated drinking water with 1 ppb spike	3	1	
Chlorinated drinking water spike #2	Chlorinated drinking water with 3 ppb atrazine spike	3	1	
Performance Evaluation Sample		-	1	Reference method accuracy
Total		77	21	-

[a] Other performance factors that were evaluated qualitatively include ease of use and reliability.

The second type of PT sample was a cross-reactivity check sample. Two samples consisted of ASTM Type I water spiked with two different cross-reactive atrazine degradation products (hydroxyatrazine and desethyl atrazine) at a level of 3 ppb. Three replicates of each cross-reactivity check sample were analyzed using the Watersafe® Pesticide Test. One replicate was analyzed by the reference method to confirm the absence of atrazine in the samples.

All PT samples were prepared at Battelle using certified, commercially available standards. PT sample results were used to assess accuracy, precision, cross-reactivity, and occurrence of false positive and false negative results using the data analysis methods described in Section 5.

3.3.3 Environmental Samples

Environmental samples were collected from a variety of sources to evaluate the performance of the Watersafe® Pesticide Test with various sample matrices. Samples were collected from the following sources:

- Fresh surface water from a South Carolina pond
- Brackish surface water from a South Carolina pond
- Groundwater from an alluvial aquifer on the Missouri River
- Chlorinated drinking water from the Battelle Duxbury, MA, laboratory.

As shown in Table 3-1, each environmental water sample also was fortified with atrazine at two spike levels. The fortified samples were prepared at Battelle to increase the analyte concentration by the amount shown in Table 3-1. The spike solution was prepared in the laboratory from a certified, commercially available atrazine standard. Three replicates of each sample were analyzed. The data for the environmental samples were used to assess accuracy, precision, potential matrix effects, and occurrence of false positives and false negatives following the data analysis procedures described in Section 5.

3.4 Sample Collection

Environmental samples were collected within 14 days of the preparation of atrazine-fortified samples. The chlorinated drinking water from Battelle was collected directly from the tap into certified clean amber glass bottles. Fresh and brackish pond water samples were collected directly into certified clean amber glass bottles. The samples were collected near the shoreline by submerging the containers no more than one inch below the surface of the water. The groundwater sample was collected directly from a tap at the well head.

The sample identification (ID) information, date, name of person collecting the sample, sample location, time of collection, and sample temperature at the time of collection were recorded on a chain-of-custody form for all field samples. All environmental samples collected in the field were stored at 4°C and shipped to Battelle on the day of collection, following chain-of-custody procedures. Samples were stored in the dark at 4°C until test sample preparation (see Section 3.5).

3.5 Sample Preparation

All samples were assigned a unique sample ID at the time of preparation. The sample ID did not contain information about the nature of the sample. Prior to sample preparation, the fresh and brackish pond water samples were filtered with a 0.45-micrometer (µm) filter in the laboratory to remove gross particulate matter. After filtration, the following physico-chemical parameters were measured in each environmental water sample to characterize the sample matrix: pH, temperature, salinity, conductivity, and alkalinity. The physico-chemical parameters were measured in the laboratory instead of in the field to provide information about the sample matrix prior to analysis using the Watersafe® Pesticide Test. All instruments used to measure physico-chemical parameters were calibrated prior to use according to the applicable standard operating procedures (SOPs).[5] All measurements were recorded manually on data sheets designed specifically for this verification test. Instrument model, serial number, and calibration information were recorded on data sheets, and calibration records are maintained in the verification test files. An aliquot of each environmental sample was collected and shipped to Severn Trent Laboratories (STL) in Burlington, VT for DOC analysis according to Method 9060.[6] STL filtered all samples using a 0.45-micrometer (µm) filter immediately upon receipt and prior to DOC analysis.

The PT and fortified environmental samples were prepared from certified, commercially available standard solutions. The purchased standards were diluted to the appropriate concentration using pesticide-grade or equivalent solvent. All samples were stored in the dark at 4°C until use. No other preservatives were added to the samples because atrazine is stable in water for up to two years when samples are refrigerated.[7] The PT and fortified environmental samples were analyzed one day after sample preparation.

Each sample was split into 1-liter (L) and 40-milliliter (mL) aliquots. The 40-mL aliquot was retained for Watersafe® Pesticide Test analysis and stored in the dark at 4°C until use. Two 1-L aliquots were sent to the EPA's Office of Pesticide Programs Environmental Chemistry Laboratory at the John C. Stennis Space Center for reference analysis by modified EPA Method 525.2[4].

3.6 Sample Analysis

A technical staff member from the Texas Commission on Environmental Quality with previous experience in performing immunoassay analyses analyzed the complete set of samples using the Watersafe® Pesticide Test. The analyses were performed according to the instructions provided with the test kit.

Test kit results were recorded manually on data sheets designed specifically for this verification test. In addition to the test kit results, the data sheets included records of the time required for sample analysis and operator observations concerning the use of the test kit (e.g., ease of use, reliability).

3.7 Reference Analysis

The EPA reference method for atrazine was performed on a Hewlett-Packard 5971 GC/MS by EPA's Office of Pesticide Programs Environmental Chemistry Laboratory. The reference instrument was operated according to the recommended procedures in the instrument operating manual, and samples were analyzed according to modified EPA Method 525.2[(4)]. The modifications to the reference method were as follows: 1) hydrochloric acid was not used to preserve the samples, because atrazine is stable without acid preservation, and 2) the extraction solvents were changed from a mixture of ethyl acetate and methylene chloride to methylene chloride only. These modifications were adopted to improve the quantification of atrazine.

Samples were submitted to the reference laboratory blind, with the exception of the unspiked environmental samples, which were identified so that they could be used as laboratory matrix spike (MS) samples. Prior to reference analysis, the chlorinated water sample was treated with sodium sulfite according to Method 525.2[(3)] at the reference laboratory to remove the chlorine. The samples were stored in the dark in amber glass bottles at 4°C until extraction. The reference method sample extraction was performed from September 25 through October 2, 2003, and analysis was performed from September 25 through October 3, 2003. Results from the reference analysis were recorded electronically and compiled by the laboratory into a report format, including the sample ID and the analyte concentration for each sample.

3.8 Verification Schedule

The verification test took place over a four-week period. Table 3-2 shows the activities that were conducted, the corresponding dates, and the location.

Table 3-2. Verification Test Schedule

Date	Location	Activity
9/9/03	South Carolina	Collection of fresh and brackish pond water and shipment to Battelle laboratory
9/17/03	Missouri River	Collection of alluvial groundwater sample and shipment to Battelle laboratory
9/19/03	Battelle Laboratory	Environmental sample filtration
9/22/03	Battelle Laboratory	Collection of chlorinated drinking water sample
9/22/03	Battelle Laboratory	Environmental sample physico-chemical characterization, test sample preparation, shipment of reference samples and DOC samples to appropriate laboratories
9/23/03	Battelle Laboratory	Analysis of all samples using Watersafe ® Kit
9/25/03 – 10/03/03	EPA Environmental Chemistry Laboratory	Analysis of test samples using reference method
10/8/03	STL Burlington	Analysis of environmental water samples for DOC

Chapter 4
Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center[8] and the test/QA plan for this verification test[1]. QA/QC procedures and results are described below.

4.1 Laboratory QC for Reference Method

Laboratory QC for the reference method included analysis of laboratory RB, MS, analytical duplicate, and laboratory-fortified blank (LFB) samples. The instrument used for reference analyses was calibrated initially according to the procedures specified in the reference method. Instrument calibration was verified using an appropriate calibration check sample. All calibration check sample results were within 20% of the value of the standard.

Laboratory RB samples were analyzed to ensure that no sources of contamination were present. Four laboratory RB samples were analyzed with the test samples. Atrazine was not detected in any of the laboratory RB samples.

Laboratory MS samples were analyzed at a frequency of at least 5% to assess whether matrix effects potentially influenced the results of the reference analyses. The percent recovery (R) of the laboratory MS samples was calculated from Equation 1:

$$R = \frac{C_s - C}{s} \times 100 \quad (1)$$

where C_s is the analyzed concentration of the spiked sample, C is the analyzed concentration of the unspiked sample, and s is the concentration equivalent of the atrazine spike. If the percent recovery of a MS sample fell outside the range of 70 to 130%, then a matrix effect was suspected. MS sample results are presented in Table 4-1. All MS recoveries were within the acceptable range.

Duplicates were analyzed to assess analytical precision. The relative percent difference (RPD) between the two duplicates was calculated from Equation 2.

$$RPD = \frac{|\ (C - C_D)\ |}{(C + C_D)/2} \times 100 \quad (2)$$

where C is the concentration of the sample analysis, and C_D is the concentration of the duplicate sample analysis. An LFB sample was analyzed in duplicate for this test. The duplicate concentrations were 0.97 ppb and 0.98 ppb atrazine. The RPD of 1% was within the acceptable limit of 30%.

Table 4-1. Reference Method Matrix Spike Sample Results

Sample ID	Sample Description	MS Sample Concentration (ppb)	Background Concentration (ppb)	Spike Concentration (ppb)	Percent Recovery
CAE-9	Fresh pond water	1.13	<0.25	1	113%
CAE-12	Brackish pond water	1.09	<0.25	1	109%
CAE-15	Groundwater	1.06	<0.25	1	106%

LFB samples were analyzed to determine whether the accuracy of the method was in control. The recovery of the LFB was calculated using Equation 1. LFB sample results are presented in Table 4-2. All atrazine recoveries were within the acceptable range of 70% to 130%.

Table 4-2. Reference Method Laboratory-Fortified Blank Sample Results

Sample ID	Analysis Date	LFB Sample Concentration (ppb)	Spike Concentration (ppb)	Percent Recovery
LFB A [(a)]	9/25/03	0.98	1	98%
LFB B	9/25/03	0.97	1	97%
LFB	9/29/03	0.95	1	95%
LFB	10/03/03	1.02	1	102%
LFB	10/03/03	0.99	1	99%

[(a)] LFB A and LFB B were analyzed in the same batch.

4.2 Audits

Three types of audits were performed during the verification test: a performance evaluation (PE) audit of the reference method, a technical systems audit (TSA) of the verification test performance, and a data quality audit. Audit procedures are described further below.

4.2.1 Performance Evaluation Audit

A PE audit was conducted to assess the quality of the reference measurements performed for the verification test. The PE audit involved challenging the reference instrument with an independent atrazine standard. For the PE audit, an independent, certified standard was obtained from a commercial supplier. The PE sample result had to be within the certified range to be considered acceptable. As shown in Table 4-3, the PE sample result was within the certified range.

Table 4-3. Reference Method Performance Evaluation Audit Results

Sample ID	Date of Analysis	Atrazine Concentration (ppb)	Certified Range (ppb)
PE sample Rep 1	9/24/03	10.49	5.5 - 14.5
PE sample Rep 2	9/24/03	11.66	5.5 - 14.5

4.2.2 Technical Systems Audit

Battelle Quality staff conducted a TSA from September 19 through 23, 2003 to ensure that the verification test was being conducted in accordance with the test/QA plan[(1)] and the AMS Center QMP.[(8)] As part of the TSA, test procedures were compared to those specified in the test/QA plan, data acquisition and handling procedures were reviewed, and the reference standards and method were reviewed. Observations and findings from the TSA were documented and submitted to the Battelle Verification Test Coordinator for response. None of the findings of the TSA required corrective action. TSA records are permanently stored with the Battelle Quality Manager.

4.2.3 Data Quality Audit

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

4.3 QA/QC Reporting

Each audit was documented in accordance with Sections 3.3.4 and 3.3.5 of the QMP for the ETV AMS Center[(8)]. Once the audit reports were prepared, the Battelle Verification Test Coordinator ensured that a response was provided for each adverse finding or potential problem. Minor deviations related to equipment calibration, use of Class A glassware for sample preparation, and chain-of-custody procedures were documented. These deviations did not negatively impact the quality of the test data. The results of the TSA were submitted to the EPA.

4.4 Data Review

Records generated in the verification test were reviewed before these records were used to calculate, evaluate, or report verification results. Table 4-4 summarizes the types of data that were recorded and reviewed. All data were recorded by Battelle or partner organization staff. Data were reviewed by a Battelle technical staff member involved in the verification test, but not the staff member that originally generated the record. The person performing the review added his/her initials and the date to a hard copy of the record being reviewed. Review of the data sheets was conducted throughout testing and no later than two weeks after data generation.

Table 4-4. Summary of Data Recording Process

Data Recorded	Responsible Party	Where Recorded	How often Recorded	Disposition of Data[(a)]
Dates and times of test events	Battelle and partner organization staff	ETV data sheets	Start/end of test	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Calibration information and results for physico-chemical parameters (temperature, salinity, etc.)	Battelle	ETV data sheets	Prior to sample preparation	Manually incorporated in data spreadsheets as necessary
Sample collection and preparation information, including chain-of-custody	Battelle and partner organization staff	ETV data sheets and chain-of-custody forms	At time of sample collection and preparation	Used to organize/check test results; manually incorporated in data spreadsheets as necessary
Test kit procedures and sample results	Battelle and partner organization staff	ETV data sheets	Throughout test duration	Manually incorporated in data spreadsheets
Reference method procedures and sample results	Partner organization staff	Data sheets or data acquisition system, as appropriate	Throughout sample analysis process	Transferred to spreadsheets
DOC analysis procedures and results	STL laboratory staff	Data sheets or data acquisition system, as appropriate	Throughout sample analysis process	Transferred to spreadsheets

[(a)] All activities subsequent to data recording were carried out by Battelle or partner organization staff.

Chapter 5
Data Analysis Methods

The data analysis methods used to evaluate the performance factors listed in Section 3.1 are presented in this chapter. Qualitative observations were also used to evaluate verification test data.

5.1 Accuracy

Accuracy was assessed by determining whether the Watersafe ® Pesticide Test result agreed with the reference method result. A positive reference method result was considered to be greater than 3 ppb (±10%) atrazine.

5.2 Precision

Precision was assessed by determining whether the Watersafe ® Pesticide Test results for three replicates of the same sample were consistent.

5.3 Cross-Reactivity

The cross-reactivity of the Watersafe® Pesticide Test to two atrazine degradation products hydroxyatrazine and desethyl atrazine was assessed qualitatively by evaluating the test kit results for samples that contained only one of the degradation compounds, and no atrazine. The reference analysis results were used to confirm the absence of atrazine in the samples.

5.4 Matrix Interferences

The potential effect of the sample matrix on Watersafe® Pesticide Test performance was evaluated qualitatively by comparing the accuracy and precision results for the natural and atrazine-fortified environmental samples to those for the PT samples.

5.5 False Positive/False Negative Results

A false positive result was defined as a positive Watersafe® Pesticide Test result when the reference method analysis indicated that the atrazine concentration in the sample was below 3 ppb (±10%) atrazine. A false negative result was defined as a negative result when the reference method analysis indicated that the atrazine concentration in the sample was above 3 ppb (±10%) atrazine.

Chapter 6
Test Results

The results of the verification test of the Watersafe® Pesticide Test are presented in this section. Tables 6-1a and 6-1b present the sample results for the PT and environmental samples, respectively, including the test kit and reference method results.

Samples were given to the analyst blind and in random order, and were analyzed in batches of no more than ten samples each. As per vendor instructions, the only type of QC sample included in the test was RB samples. Watersafe® Pesticide Test results for all RB samples were negative. Results for each performance factor are presented below.

6.1 Accuracy

Accuracy results for the PT and environmental samples are presented in Tables 6-2a and 6-2b, respectively. The number of accurate results using the Watersafe® Pesticide Test was 18 out of 21 for the PT samples, and 31 out of 36 for the environmental samples. All samples with inaccurate results contained approximately 1 ppb atrazine.

6.2 Precision

Precision results for PT and environmental samples are presented in Tables 6-2a and 6-2b, respectively. Replicate sample results for the seven PT samples were consistent. Replicate sample results for the twelve environmental sample were consistent with the exception of three of the samples spiked at the 1 ppb atrazine level, where one replicate of each yielded an inconsistent result.

6.3 Cross-Reactivity

Results for PT samples fortified with 3 ppb hydroxyatrazine or 3 ppb desethyl atrazine are provided in Table 6-1a. The Watersafe® Pesticide Test results for these samples were negative.

Table 6-1a. Test Kit and Reference Method Results for PT Samples

Sample Description	Sample ID	Replicate	Test Kit Result	Reference Result (ppb atrazine)
0.1 ppb atrazine	CAE-2	1	N	0.09 [(a)]
0.1 ppb atrazine	CAE-2	2	N	
0.1 ppb atrazine	CAE-2	3	N	
0.5 ppb atrazine	CAE-3	1	N	0.54
0.5 ppb atrazine	CAE-3	2	N	
0.5 ppb atrazine	CAE-3	3	N	
1 ppb atrazine	CAE-4	1	P	1.20
1 ppb atrazine	CAE-4	2	P	
1 ppb atrazine	CAE-4	3	P	
3 ppb atrazine	CAE-5	1	P	3.71
3 ppb atrazine	CAE-5	2	P	
3 ppb atrazine	CAE-5	3	P	
5 ppb atrazine	CAE-6	1	P	5.61
5 ppb atrazine	CAE-6	2	P	
5 ppb atrazine	CAE-6	3	P	
3 ppb hydroxyatrazine	CAE-7	1	N	<0.074
3 ppb hydroxyatrazine	CAE-7	2	N	
3 ppb hydroxyatrazine	CAE-7	3	N	
3 ppb desethyl atrazine	CAE-8	1	N	<0.074
3 ppb desethyl atrazine	CAE-8	2	N	
3 ppb desethyl atrazine	CAE-8	3	N	

[(a)] Concentration above the reference method MDL of 0.074 ppb but below the 0.25 ppb limit of quantitation.
P = positive test result; >3 ppb atrazine.
N = negative test result; <3 ppb atrazine.

Table 6-1b. Test Kit and Reference Method Results for Environmental Samples

Sample Description	Sample ID	Replicate	Test Kit Result	Reference Result (ppb atrazine)
Fresh pond water	CAE-9	1	N	<0.074
Fresh pond water	CAE-9	2	N	
Fresh pond water	CAE-9	3	N	
Fresh pond water + 1 ppb atrazine	CAE-10	1	N	1.15
Fresh pond water + 1 ppb atrazine	CAE-10	2	N	
Fresh pond water + 1 ppb atrazine	CAE-10	3	N	
Fresh pond water + 3 ppb atrazine	CAE-11	1	P	3.53
Fresh pond water + 3 ppb atrazine	CAE-11	2	P	
Fresh pond water + 3 ppb atrazine	CAE-11	3	P	
Brackish pond water	CAE-12	1	N	<0.074
Brackish pond water	CAE-12	2	N	
Brackish pond water	CAE-12	3	N	
Brackish pond water + 1 ppb atrazine	CAE-13	1	N	1.18
Brackish pond water + 1 ppb atrazine	CAE-13	2	P	
Brackish pond water + 1 ppb atrazine	CAE-13	3	P	
Brackish pond water + 3 ppb atrazine	CAE-14	1	P	3.58
Brackish pond water + 3 ppb atrazine	CAE-14	2	P	
Brackish pond water + 3 ppb atrazine	CAE-14	3	P	
Groundwater	CAE-15	1	N	<0.074
Groundwater	CAE-15	2	N	
Groundwater	CAE-15	3	N	
Groundwater + 1 ppb atrazine	CAE-16	1	P	1.13
Groundwater + 1 ppb atrazine	CAE-16	2	N	
Groundwater + 1 ppb atrazine	CAE-16	3	N	
Groundwater + 3 ppb atrazine	CAE-17	1	P	3.3
Groundwater + 3 ppb atrazine	CAE-17	2	P	
Groundwater + 3 ppb atrazine	CAE-17	3	P	
Chlorinated drinking water	CAE-18	1	N	<0.074
Chlorinated drinking water	CAE-18	2	N	
Chlorinated drinking water	CAE-18	3	N	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	1	P	0.79
Chlorinated drinking water + 1 ppb atrazine	CAE-19	2	P	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	3	N	
Chlorinated drinking water + 3 ppb atrazine	CAE-20	1	P	2.73
Chlorinated drinking water + 3 ppb atrazine	CAE-20	2	P	
Chlorinated drinking water + 3 ppb atrazine	CAE-20	3	P	

P = positive test result; >3 ppb atrazine.
N = negative test result; <3 ppb atrazine.

Table 6-2a. Accuracy and Precision Results for PT Samples

Sample Description	Sample ID	Replicate	Test Kit Result	Reference Result (ppb atrazine)	Test Kit Result Accurate? [a]	Test Kit Results Consistent?
0.1 ppb atrazine	CAE-2	1	N	0.09	Y	Y
0.1 ppb atrazine	CAE-2	2	N		Y	
0.1 ppb atrazine	CAE-2	3	N		Y	
0.5 ppb atrazine	CAE-3	1	N	0.54	Y	Y
0.5 ppb atrazine	CAE-3	2	N		Y	
0.5 ppb atrazine	CAE-3	3	N		Y	
1 ppb atrazine	CAE-4	1	P	1.20	N	Y
1 ppb atrazine	CAE-4	2	P		N	
1 ppb atrazine	CAE-4	3	P		N	
3 ppb atrazine	CAE-5	1	P	3.71	Y	Y
3 ppb atrazine	CAE-5	2	P		Y	
3 ppb atrazine	CAE-5	3	P		Y	
5 ppb atrazine	CAE-6	1	P	5.61	Y	Y
5 ppb atrazine	CAE-6	2	P		Y	
5 ppb atrazine	CAE-6	3	P		Y	
3 ppb hydroxyatrazine	CAE-7	1	N	<0.074	Y	Y
3 ppb hydroxyatrazine	CAE-7	2	N		Y	
3 ppb hydroxyatrazine	CAE-7	3	N		Y	
3 ppb desethyl atrazine	CAE-8	1	N	<0.074	Y	Y
3 ppb desethyl atrazine	CAE-8	2	N		Y	
3 ppb desethyl atrazine	CAE-8	3	N		Y	
				Total number	21	7
				Number accurate/consistent results	18	7

[a] Relative to 3 ppb +10% atrazine threshold value.

Table 6-2b. Accuracy and Precision Results for Environmental Samples

Sample Description	Sample ID	Replicate	Test Result	Reference Result (ppb atrazine)	Result Accurate? [a]	Results Consistent?
Filtered fresh pond water	CAE-9	1	N	<0.074	Y	Y
Filtered fresh pond water	CAE-9	2	N		Y	
Filtered fresh pond water	CAE-9	3	N		Y	
Filtered fresh pond water + 1 ppb atrazine	CAE-10	1	N	1.15	Y	Y
Filtered fresh pond water + 1 ppb atrazine	CAE-10	2	N		Y	
Filtered fresh pond water + 1 ppb atrazine	CAE-10	3	N		Y	
Filtered fresh pond water + 3 ppb atrazine	CAE-11	1	P	3.53	Y	Y
Filtered fresh pond water + 3 ppb atrazine	CAE-11	2	P		Y	
Filtered fresh pond water + 3 ppb atrazine	CAE-11	3	P		Y	
Filtered brackish pond water	CAE-12	1	N	<0.074	Y	Y
Filtered brackish pond water	CAE-12	2	N		Y	
Filtered brackish pond water	CAE-12	3	N		Y	
Filtered brackish pond water + 1 ppb atrazine	CAE-13	1	N	1.18	Y	N
Filtered brackish pond water + 1 ppb atrazine	CAE-13	2	P		N	
Filtered brackish pond water + 1 ppb atrazine	CAE-13	3	P		N	
Filtered brackish pond water + 3 ppb atrazine	CAE-14	1	P	3.58	Y	Y
Filtered brackish pond water + 3 ppb atrazine	CAE-14	2	P		Y	
Filtered brackish pond water + 3 ppb atrazine	CAE-14	3	P		Y	
Groundwater	CAE-15	1	N	<0.074	Y	Y
Groundwater	CAE-15	2	N		Y	
Groundwater	CAE-15	3	N		Y	
Groundwater + 1 ppb atrazine	CAE-16	1	P	1.13	N	N
Groundwater + 1 ppb atrazine	CAE-16	2	N		Y	
Groundwater + 1 ppb atrazine	CAE-16	3	N		Y	

[a] Relative to 3 ppb +10% atrazine threshold value.

Table 6-2b. Accuracy and Precision Results for Environmental Samples, continued

Sample Description	Sample ID	Replicate	Test Result	Reference Result (ppb atrazine)	Result Accurate? [a]	Results Consistent? [b]
Groundwater + 3 ppb atrazine	CAE-17	1	P	3.3	Y	Y
Groundwater + 3 ppb atrazine	CAE-17	2	P		Y	
Groundwater + 3 ppb atrazine	CAE-17	3	P		Y	
Chlorinated drinking water	CAE-18	1	N	<0.074	Y	Y
Chlorinated drinking water	CAE-18	2	N		Y	
Chlorinated drinking water	CAE-18	3	N		Y	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	1	P	0.79	N	N
Chlorinated drinking water + 1 ppb atrazine	CAE-19	2	P		N	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	3	N		Y	
Chlorinated drinking water + 3 ppb atrazine	CAE-20	1	P	2.73	Y	Y
Chlorinated drinking water + 3 ppb atrazine	CAE-20	2	P		Y	
Chlorinated drinking water + 3 ppb atrazine	CAE-20	3	P		Y	
				Total number	36	12
				Number accurate/consistent results	31	9

[a] Relative to 3 ppb +10% atrazine threshold value.

6.4 Matrix Interferences

Matrix characteristics for the four environmental water sample types (fresh pond water, brackish pond water, alluvial groundwater, and chlorinated drinking water) are provided in Table 6-3. Reference method results indicate that atrazine was not present in any of the natural (unspiked) environmental samples above the MDL of 0.074 ppb (Table 6-1b). The Watersafe® Pesticide Test did not yield a positive result for any of the unspiked environmental samples. Although the test kit results were positive for some of the 1 ppb atrazine-fortified environmental samples, the 1 ppb atrazine-fortified PT sample also yielded positive results. Therefore, the matrices that were tested did not appear to interfere with the performance of the test kit.

6.5 False Positive/False Negative Results

Table 6-4 presents the analysis of false positive and false negative results obtained from the Watersafe® Pesticide Test. RB, PT and environmental samples were included in this evaluation. As shown in Table 6-4, 56 samples had atrazine concentrations below 3 ppb $\pm$10% as measured by the reference method. For these samples, the Watersafe® Pesticide Test results were falsely positive for eight samples. All eight samples had atrazine concentrations near 1 ppb. Eighteen samples had atrazine concentrations above 3 ppb $\pm$10% as measured by the reference method. All of the test kit results for these samples were positive, resulting in no false negative results.

6.6 Other Factors

During the test, the analyst recorded observations regarding ease of use, reliability, and sample throughput. The Watersafe® Pesticide Test was very easy to use. The instructions were simple and easy to follow, and all the necessary items were included in the test packet. In some cases, it was difficult for the analyst to discern differences in the color and intensity of the lines on the test strips. Good lighting and eyesight were essential. The analyst found that test strips were easier to read and interpret when placed on a light-colored background such as a white piece of paper. The analyst and an assistant agreed on the interpretation of all test strips; therefore, the results were considered reliable and no samples were reanalyzed.

The Watersafe® Pesticide Test is well-suited for field use because it requires no instrumentation or other equipment, power sources, or refrigerated storage. The test packets are small and easily transportable.

During the test, each batch of ten samples was analyzed concurrently with the Watersafe® Pesticide Test in 30 minutes. A single sample can be analyzed in under 15 minutes.

Table 6-3. Physico-chemical Characterization of Environmental Sample Matrices

Sample Type	Temp. at time of sample collection (°C)	Temp. at time of sample preparation (°C)	pH (pH units)	Conductivity (µS)	Salinity (ppt)	Alkalinity (meq/L)	DOC[(a)] (mg/L)
Fresh pond water	25.6	18.8	7.8	1753	0	4.800	17.9
Brackish pond water	26.2	18.0	7.9	19,250	10	3.147	16.7
Alluvial groundwater	18.1	18.5	7.6	755	0	4.041	5.1
Chlorinated drinking water	-	19.2	6.5	163	0	0.6885	2.9

[(a)] Samples were filtered at STL with 0.45 µm filter immediately upon receipt at STL. Filter blank DOC concentration was 2 mg/L.

Table 6-4. Occurrence of False Positives and False Negatives

Sample Description	Sample ID	Replicate	Test Result	Reference Result (ppb atrazine)	False Positive	False Negative
Reagent blank	CAE-1	1	N	<0.074	N	
Reagent blank	CAE-1	2	N		N	
Reagent blank	CAE-1	3	N		N	
Reagent blank	CAE-1	4	N		N	
Reagent blank	CAE-1	5	N		N	
Reagent blank	CAE-1	6	N		N	
Reagent blank	CAE-1	7	N		N	
Reagent blank	CAE-1	8	N		N	
Reagent blank	CAE-1	9	N		N	
Reagent blank	CAE-1	10	N		N	
Reagent blank	CAE-1	11	N		N	
Reagent blank	CAE-1	12	N		N	
Reagent blank	CAE-1	13	N		N	
Reagent blank	CAE-1	14	N		N	

Table 6-4. Occurrence of False Positives and False Negatives, continued

Sample Description	Sample ID	Replicate	Test Result	Reference Result (ppb atrazine)	False Positive	False Negative
Reagent blank	CAE-1	15	N	<0.074	N	
Reagent blank	CAE-1	16	N		N	
Reagent blank	CAE-1	17	N		N	
Reagent blank	CAE-1	18	N		N	
Reagent blank	CAE-1	19	N		N	
Reagent blank	CAE-1	20	N		N	
0.5 ppb atrazine	CAE-3	1	N	0.54	N	
0.5 ppb atrazine	CAE-3	2	N		N	
0.5 ppb atrazine	CAE-3	3	N		N	
1 ppb atrazine	CAE-4	1	P	1.20	Y	
1 ppb atrazine	CAE-4	2	P		Y	
1 ppb atrazine	CAE-4	3	P		Y	
3 ppb atrazine	CAE-5	1	P	3.71		N
3 ppb atrazine	CAE-5	2	P			N
3 ppb atrazine	CAE-5	3	P			N
5 ppb atrazine	CAE-6	1	P	5.61		N
5 ppb atrazine	CAE-6	2	P			N
5 ppb atrazine	CAE-6	3	P			N
3 ppb hydroxyatrazine	CAE-7	1	N	<0.074	N	
3 ppb hydroxyatrazine	CAE-7	2	N		N	
3 ppb hydroxyatrazine	CAE-7	3	N		N	
3 ppb desethyl atrazine	CAE-8	1	N	<0.074	N	
3 ppb desethyl atrazine	CAE-8	2	N		N	
3 ppb desethyl atrazine	CAE-8	3	N		N	

Table 6-4. Occurrence of False Positives and False Negatives, continued

Sample Description	Sample ID	Replicate	Test Result	Reference Result (ppb atrazine)	False Positive	False Negative
Fresh pond water	CAE-9	1	N	<0.074	N	
Fresh pond water	CAE-9	2	N		N	
Fresh pond water	CAE-9	3	N		N	
Fresh pond water + 1 ppb atrazine	CAE-10	1	N	1.15	N	
Fresh pond water + 1 ppb atrazine	CAE-10	2	N		N	
Fresh pond water + 1 ppb atrazine	CAE-10	3	N		N	
Fresh pond water + 3 ppb atrazine	CAE-11	1	P	3.53		N
Fresh pond water + 3 ppb atrazine	CAE-11	2	P			N
Fresh pond water + 3 ppb atrazine	CAE-11	3	P			N
Brackish pond water	CAE-12	1	N	<0.074	N	
Brackish pond water	CAE-12	2	N		N	
Brackish pond water	CAE-12	3	N		N	
Brackish pond water + 1 ppb atrazine	CAE-13	1	N	1.18	N	
Brackish pond water + 1 ppb atrazine	CAE-13	2	P		Y	
Brackish pond water + 1 ppb atrazine	CAE-13	3	P		Y	
Brackish pond water + 3 ppb atrazine	CAE-14	1	P	3.58		N
Brackish pond water + 3 ppb atrazine	CAE-14	2	P			N
Brackish pond water + 3 ppb atrazine	CAE-14	3	P			N
Groundwater	CAE-15	1	N	<0.074	N	
Groundwater	CAE-15	2	N		N	
Groundwater	CAE-15	3	N		N	
Groundwater + 1 ppb atrazine	CAE-16	1	P	1.13	Y	
Groundwater + 1 ppb atrazine	CAE-16	2	N		N	
Groundwater + 1 ppb atrazine	CAE-16	3	N		N	

Table 6-4. Occurrence of False Positives and False Negatives, continued

Sample Description	Sample ID	Replicate	Test Result	Reference Result (ppb atrazine)	False Positive	False Negative
Groundwater + 3 ppb atrazine	CAE-17	1	P	3.3		N
Groundwater + 3 ppb atrazine	CAE-17	2	P			N
Groundwater + 3 ppb atrazine	CAE-17	3	P			N
Chlorinated drinking water	CAE-18	1	N	<0.074	N	
Chlorinated drinking water	CAE-18	2	N		N	
Chlorinated drinking water	CAE-18	3	N		N	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	1	P	0.79	Y	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	2	P		Y	
Chlorinated drinking water + 1 ppb atrazine	CAE-19	3	N		N	
Chlorinated drinking water + 3 ppb atrazine	CAE-20	1	P	2.73 [(a)]		N
Chlorinated drinking water + 3 ppb atrazine	CAE-20	2	P			N
Chlorinated drinking water + 3 ppb atrazine	CAE-20	3	P			N
				Total sample number	56	18
				Number false positives or negatives	8	0

[(a)] Within 3 ppb ± 10%

Chapter 7
Performance Summary

The Watersafe® Pesticide Test was evaluated for the following parameters:

- Accuracy
- Precision
- Cross-reactivity of hydroxylatrazine and desethyl atrazine
- Matrix interference effects
- Occurrence of false positive and false negative results
- Other factors (ease of use, reliability, and sample throughput).

Performance results are summarized in Table 7-1. During the test, the analyst recorded observations regarding ease of use, reliability, and sample throughput. The Watersafe® Pesticide Test was easy to use, with simple instructions. All materials were provided in the small test packets. In some cases, the intensity and color of the lines on the test strips were difficult to discern and interpret. These difficulties were minimized by ensuring adequate lighting and placing the test strips on a light-colored background. The Watersafe® Pesticide Test operated without failure throughout the test.

The Watersafe® Pesticide Test is well-suited for field use because it is small and easily transported, and requires no additional equipment, power, or special handling. A single sample can be analyzed in less than 15 minutes; a batch of ten samples can be analyzed in about 30 minutes.

Table 7-1. Performance Summary for Watersafe® Pesticide Test

Parameter	Performance Results	Comments
Accuracy (number of accurate results out of total number of tests)		
PT samples, 0.1 – 5 ppb atrazine and cross-reactivity samples	18 out of 21	Samples with inaccurate results contained ~1 ppb atrazine.
Environmental samples:		
Fresh pond water	9 out of 9	Samples with inaccurate results contained ~1 ppb atrazine.
Brackish pond water	7 out of 9	
Groundwater	8 out of 9	
Chlorinated drinking water	7 out of 9	
Precision (number of consistent sets of replicate sample results out of total number of sets)		
PT samples, 0.1 – 5 ppb atrazine and cross-reactivity samples	7 out of 7	
Environmental samples:		
Fresh pond water	3 out of 3	Samples with inconsistent replicate results contained ~1 ppb atrazine.
Brackish pond water	2 out of 3	
Groundwater	2 out of 3	
Chlorinated drinking water	2 out of 3	
Cross-reactivity		
3 ppb hydroxyatrazine	Negative	Cross-reactivity samples did not contain atrazine.
3 ppb desethyl atrazine	Negative	
Matrix interference effects	No apparent matrix effects	
False positive results	8 false positive results out of 56 tests	Evaluated relative to 3 ppb test threshold level. False positive results were for samples with ~1 ppb atrazine.
False negative results	None	Evaluated relative to 3 ppb test threshold level.

Chapter 8
References

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