

September 2006

Environmental Technology Verification Report

AQUA SURVEY, INC.
NEURO-IQ TOX TEST KIT™

Prepared by
Battelle

Battelle
The Business of Innovation

Under a cooperative agreement with

 **EPA** U.S. Environmental Protection Agency

ETV ✓ ETV ✓ ETV ✓

September 2006

Environmental Technology Verification Report

ETV Advanced Monitoring Systems Center

**Aqua Survey, Inc.
Neuro-IQ Tox Test Kit™**

by
Stephanie Buehler
Raj Mangaraj
Amy Dindal
Zachary Willenberg
Karen Riggs

Battelle
Columbus, Ohio 43201

Notice

The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, has financially supported and collaborated in the extramural program described here. This document has been peer reviewed by the Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation by the EPA for use.

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 technology across all media and to report this objective information to permittees, 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 six 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 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>.

Acknowledgments

The authors wish to acknowledge the support of all those who helped plan and conduct the verification test, analyze the data, and prepare this report. Many thanks go to Battelle's Hazardous Materials Research Center for providing the facilities for and personnel capable of working with chemical warfare agents. We sincerely appreciate the contribution of drinking water samples from the Metropolitan Water District of Southern California (Paul Rochelle and Melinda Stalvey), the New York Department of Environmental Protection (Virginia Murray), and Orange County Utilities, Orlando, Florida (Theresa Slifko and Liza Robles). We would also like to thank Armah de la Cruz (U.S. EPA, National Exposure Research Laboratory), Ricardo DeLeon (Metropolitan Water District of Southern California), Yves Mikol (New York City Department of Environmental Protection), and Helen Schurz Rogers (Centers for Disease Control and Prevention National Center for Environmental Health) for their careful review of the test/QA plan and this verification report.

Contents

	<u>Page</u>
Notice.....	ii
Foreword.....	iii
Acknowledgments.....	iv
List of Abbreviations	vii
Chapter 1 Background	1
Chapter 2 Technology Description	2
Chapter 3 Test Design.....	3
3.1 Introduction.....	3
3.2 Test Samples	3
3.2.1 PT Samples.....	4
3.2.2 DW Samples.....	5
3.2.3 QC Samples.....	6
3.2.4 Operational Factors	6
3.3 Verification Schedule.....	7
3.4 Test Procedure	7
3.4.1 Test Sample Preparation and Storage.....	7
3.4.2 Test Sample Analysis Procedure.....	7
3.4.3 Drinking Water Characterization	8
Chapter 4 Quality Assurance/Quality Control.....	10
4.1 Sample Chain-of-Custody Procedures	10
4.2 QC Samples	10
4.3 Equipment/Calibration.....	12
4.4 Characterization of Stock Solutions.....	12
4.5 Audits.....	13
4.5.1 Performance Evaluation Audit	13
4.5.2 Technical Systems Audit.....	13
4.5.3 Audit of Data Quality	14
4.6 QA/QC Reporting.....	14
4.7 Data Review.....	14
Chapter 5 Statistical Methods and Reported Parameters.....	16
5.1 Accuracy	16
5.2 False Positive/False Negative Rates	16
5.3 Precision.....	17
5.4 Potential Matrix and Interferent Effects	17
5.5 Operational Factors.....	17
Chapter 6 Test Results	18
6.1 Accuracy	18
6.2 False Positive/False Negative Rates	20
6.3 Precision.....	26

6.4 Potential Matrix and Interferent Effects	26
6.4.1 Interferent PT Samples	26
6.4.2 DW Samples	27
6.5 Operational Factors	27
6.5.1 Technical Operators	27
6.5.2 Non-Technical Operator	28
Chapter 7 Performance Summary	30
Chapter 8 References	37

Figures

Figure 2-1. Neuro-IQ Tox Test Kit™	2
Figure 6-1. Side View of PPE Worn by Non-Technical Operator.	29
Figure 6-2. Testing of the Neuro-IQ Tox Test Kit™ with the Non-Technical Operator Wearing PPE.....	29

Tables

Table 3-1. Lethal Dose of Target Contaminants.....	4
Table 3-2. Performance Test Samples	5
Table 3-3. Drinking Water Samples	6
Table 3-4. ATEL Water Quality Characterization of Drinking Water Samples.....	9
Table 4-1. Reference Methods for Target Contaminants and Interferents	11
Table 4-2. Performance Evaluation Samples and Percent Difference	13
Table 4-3. Summary of Data Recording Process.....	15
Table 6-1. Contaminant-Only PT Sample Results.....	19
Table 6-2a. VX False Positive/Negative Results.....	21
Table 6-2b. GB False Positive/Negative Results.....	22
Table 6-2c. GD False Positive/Negative Results.....	23
Table 6-2d. Aldicarb False Positive/Negative Results	24
Table 6-2e. Dicrotophos False Positive/Negative Results.....	25
Table 7-1. VX Summary Table.....	31
Table 7-2. GB Summary Table.....	32
Table 7-3. GD Summary Table.....	33
Table 7-4. Aldicarb Summary Table	34
Table 7-5. Dicrotophos Summary Table.....	35

List of Abbreviations

AMS	Advanced Monitoring Systems
ASTM	American Society for Testing and Materials
ATEL	Aqua Tech Environmental Laboratories, Inc.
Ca	calcium
DI	deionized
DPD	diethyl-p-phenylene diamine
DW	drinking water
ECD	electron capture detection
EPA	U.S. Environmental Protection Agency
ETV	Environmental Technology Verification
GB	sarin
GC	gas chromatography
GD	soman
HAZWOPER	Hazardous Waste Operations and Emergency Response
HDPE	high density polyethylene
HMRC	Hazardous Materials Research Facility
ICP	inductively coupled plasma
kg	kilogram
L	liter
LC	liquid chromatography
LD ₅₀	lethal dose for half of test subjects
LOD	limit of detection
LRB	laboratory record book
MB	method blank
Mg	magnesium
mg/L	milligram per liter
mL	milliliter
MS	mass spectrometry
µg/L	microgram per liter
µMHO	micromho
NaOH	sodium hydroxide
NDR	negative differential resistance

ng	nanogram
NTU	nephelometric turbidity unit
OP	organophosphate
PE	performance evaluation
PPE	personal protective equipment
PT	performance test
QA	quality assurance
QC	quality control
QMP	quality management plan
SCBA	self-contained breathing apparatus
SM	standard method
SOP	standard operating procedure
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 permittees; 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 Aqua Survey, Inc., Neuro-IQ Tox Test Kit™ in detecting chemical agents, carbamate pesticides, and organophosphate (OP) pesticides in drinking water. Enzymatic test kits were identified as a priority technology category for verification through the AMS Center stakeholder process.

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 testing the Neuro-IQ Tox Test Kit™. Following is a description of the Neuro-IQ Tox Test Kit™, based on information provided by the vendor. The information provided below was not verified in this test.

The Neuro-IQ Tox Test Kit™ tests water supplies for the presence of contaminants in drinking water in sufficient concentrations to cause harm to humans. The Neuro-IQ-Tox Test Kit™ is acetylcholine/cholinesterase based and detects contaminants of interest by interrupting an enzymatic reaction. The presence or absence of contaminants at significant concentrations is predicted by adding two reagents to water samples and measuring the drop in pH after three minutes. This test is generally performed in replicates of up to four. If the pH of the test samples is higher (≥ 0.2 pH units) than the control water sample's three-minute pH reading, this indicates the possible presence of a significant threat contaminant concentration.



The test can be conducted by a technician with basic laboratory skills. Data are recorded on a scorecard provided with the kit.

Enough reagent is provided with the Neuro-IQ Tox Test Kit™ to assay up to 400 test water samples. The Neuro-IQ-Tox Test Kit™ retails for \$300.

Figure 2-1. Neuro-IQ Tox Test Kit™

Chapter 3 Test Design

3.1 Introduction

Enzymatic test kits, generally designed to be handheld and portable, detect the presence of chemical agents, carbamate pesticides, and/or OP pesticides by relying on the reaction of the cholinesterase enzyme. Under normal conditions, the enzyme reacts as expected with other chemicals present in the test kit. The activity of the enzyme is inhibited, however, by chemical agents, carbamate pesticides, and OP pesticides. The effects of this inhibition will then generally lead to a color change, indicating the presence or absence of these compounds.

The objective of this verification test was to evaluate the ability of the Neuro-IQ Tox Test Kit™ to detect chemical agents, carbamate pesticides, and OP pesticides in drinking water. This verification test assessed the performance of the Neuro-IQ Tox Test Kit™ relative to

- Accuracy
- False positive and negative rates
- Precision
- Potential matrix and interference effects
- Operational factors (operator observations, ease of use, and sample throughput).

3.2 Test Samples

This test evaluated the ability of the Neuro-IQ Tox Test Kit™ to detect VX, sarin (GB), and soman (GD) (chemical agents); aldicarb (carbamate pesticide); and dicotophos (OP pesticide) in performance test (PT) and drinking water (DW) samples. Quality Control (QC) samples were also included as part of the test matrix to ensure the integrity of the test. Contaminants were tested individually, and stock solutions of each contaminant were prepared separately in American Society for Testing and Materials (ASTM) Type II deionized (DI) water. Samples were prepared in the appropriate matrix using these stock solutions and analyzed on the same day. To minimize the loss of analytes to hydrolysis, contaminant stock solutions prepared in DI water were made on a daily basis. Chemical agent stock solutions were prepared twice daily, once in the morning and once in the afternoon. Aliquots of each stock solution were diluted to the appropriate concentration using volumetric glassware and volumetric or calibrated pipettes. In some cases, reference solutions were prepared in ASTM Type II DI water using the stock

solutions to prepare the test samples. In other cases, the actual stock solutions were submitted for concentration confirmation by the respective reference analysis (Table 4-1). Aqua Tech Environmental Laboratories, Inc. (ATEL) of Marion, OH performed the physiochemical characterization for each type of DW sample along with reference analyses of the interferent solutions. All other reference analyses were performed at Battelle.

3.2.1 PT Samples

PT samples were prepared separately in ASTM Type II DI water for each contaminant. The first type of PT samples consisted of ASTM Type II DI water spiked with the contaminant at five different concentrations: the lethal dose concentration given in Table 3-1 for each contaminant, along with dilutions at approximately 10, 100, 1,000, and 10,000 times less than the lethal dose. The contaminants were added individually to each spiked sample. The lethal dose of each contaminant was determined by calculating the concentration at which 250 milliliters (mL) of water is likely to cause the death of a 70-kilogram (kg) person based on human oral LD₅₀ (lethal dose for half of the test subjects) data.^(1,2) Human oral LD₅₀ data were not available for aldicarb, so rat oral LD₅₀ data were used instead.⁽³⁾ Each concentration level for the PT samples was analyzed in triplicate.

In addition to the contaminant-only PT samples described above, a second type of PT sample was a potential interferent sample. Three replicates of each interferent PT sample were analyzed to determine the susceptibility of the Neuro-IQ Tox Test Kit™ to these commonly found interferents in DW. One interferent PT sample contained calcium (Ca) and magnesium (Mg) from carbonates spiked into ASTM Type II DI water, and the other contained humic and fulvic acids isolated from the Elliot River (obtained from the International Humic Substances Society) spiked into ASTM Type II DI water. Each interferent mixture was prepared at two concentration levels: near the upper limit of what would be expected in drinking water (250 milligrams per liter (mg/L) total concentration for Ca and Mg, 5 mg/L total concentration for humic and fulvic acids) and at a mid-low range of what would be expected (50 mg/L total concentration for Ca and Mg, 1 mg/L total concentration for humic and fulvic acids). These spiked interferent levels were confirmed through analysis of aliquots by ATEL. Also, each contaminant was added to these samples, along with the potential interferent, at a concentration consistent with a 10x dilution of the lethal dose. The resulting samples were analyzed in triplicate. Table 3-2 lists the PT samples analyzed in this verification test for each contaminant.

Table 3-1. Lethal Dose of Target Contaminants

Contaminant (common name)	Oral Lethal Dose Concentration	Contaminant Class
VX	2.1 milligrams/liter (mg/L)	Chemical agent
GB (sarin)	20 mg/L	Chemical agent
GD (soman)	1.4 mg/L	Chemical agent
aldicarb	260 mg/L	Carbamate pesticide
dicrotophos	1400 mg/L	Organophosphate pesticide

Table 3-2. Performance Test Samples

Type of PT Sample	Sample Characteristics	Concentrations
Contaminant-only	Contaminants in DI water	VX: 2.1 to 0.00021 mg/L GB: 20 to 0.002 mg/L GD: 1.4 to 0.00014 mg/L aldicarb: 260 to 0.026 mg/L dicrotophos: 1400 to 0.14 mg/L
Interferent	Contaminants in 1 mg/L humic and fulvic acids	VX: 0.21 mg/L
	Contaminants in 5 mg/L humic and fulvic acids	GB: 2 mg/L GD: 0.14 mg/L
	Contaminants in 50 mg/L Ca and Mg	aldicarb: 26 mg/L
	Contaminants in 250 mg/L Ca and Mg	dicrotophos: 140 mg/L

3.2.2 DW Samples

Table 3-3 lists the DW samples analyzed for each contaminant in this test. DW samples were collected from four geographically distributed municipal sources (Ohio, New York, California, and Florida) to evaluate the performance of the Neuro-IQ Tox Test Kit™ with various DW matrices. These samples varied in their source, treatment, and disinfection process. All samples had undergone either chlorination or chloramination disinfection prior to receipt. Samples were collected from water utility systems with the following treatment and source characteristics:

- Chlorinated filtered surface water source
- Chlorinated unfiltered surface water source
- Chlorinated filtered groundwater source
- Chloraminated filtered surface water source

Approximately 175 liters (L) of each of the DW samples were collected in pre-cleaned, translucent, low-density polyethylene containers. After sample collection, an aliquot of each DW sample was sent to ATEL to determine the following water quality parameters: concentration of trihalomethanes, haloacetic acids, total organic halides, Ca and Mg, pH, conductivity, alkalinity, turbidity, organic carbon, and hardness. All DW samples were dechlorinated prior to their use with sodium thiosulfate pentahydrate to prevent the degradation of the target contaminants by chlorine. The dechlorination of the DW was qualitatively confirmed by adding a diethyl-p-phenylene diamine (DPD) tablet to an aliquot of DW. If the water did not turn pink, the dechlorination process was successful. If the water did turn pink, additional dechlorinating reagent was added and the dechlorination confirmation procedure repeated. Each DW sample was analyzed before addition of contaminant, as well as after fortification with each individual contaminant at a single concentration level (10x dilution of the lethal dose). Aliquots of each contaminant stock solution were diluted with DW samples to the appropriate concentration. Each sample was tested in triplicate.

Table 3-3. Drinking Water Samples

Drinking Water Sample Description			Contaminant Concentrations
Water Utility	Water Treatment	Source Type	
Columbus, Ohio (OH DW)	chlorinated filtered	surface	VX: 0.21 mg/L
New York City, New York (NY DW)	chlorinated unfiltered	surface	GB: 2.0 mg/L
Orlando, Florida (FL DW)	chlorinated filtered	ground	GD: 0.14 mg/L
Metropolitan Water District of Southern California (CA DW)	chloraminated filtered	surface	aldicarb: 26 mg/L
			dicrotophos: 140 mg/L

3.2.3 QC Samples

QC samples included method blank (MB) samples consisting of ASTM Type II DI water and control water samples, as indicated by the vendor. Control water samples were simply an unspiked version of the sample matrix being tested. For example, when the OH DW samples were tested, the control water was unspiked OH DW. All MB QC samples were exposed to sample preparation and analysis procedures identical to the test samples. Control water samples were prepared and used according to the protocol provided by the vendor. The MB samples were used to ensure that no sources of contamination were introduced in the sample handling and analysis procedures. At least 10% of the test samples (seven samples for each contaminant) were MB samples. For samples involving chemical agents, only five MB samples were run with each chemical agent. One control water sample was run with every set of three to four test samples of the same matrix. The test samples and MB samples were analyzed blindly by the operator in that the samples used for analysis were prepared by someone other than the operator and were marked with non-identifying numbers.

3.2.4 Operational Factors

3.2.4.1 Technical Operator

All of the test samples were analyzed by a technical operator who was trained by the vendor. Operational factors such as ease of use and sample throughput were evaluated based on observations recorded by the technical operator and the Verification Test Coordinator. Operational factors were noted during the laboratory portions of the verification test. These observations were summarized to describe the operational performance of the Neuro-IQ Tox Test Kit™ in this verification.

3.2.4.2 Non-Technical Operator

A subset of the samples was also tested by a non-technical operator using the Neuro-IQ Tox Test Kit™. The non-technical operator was someone with little to no laboratory experience who would be representative of a first responder. For this test, the non-technical operator was a State

of Ohio certified firefighter with Hazardous Waste Operations and Emergency Response (HAZWOPER) training. The non-technical operator was trained in the use of the Neuro-IQ Tox Test Kit™ by another Battelle staff person who was trained by the vendor. Because many of the contaminants being tested are highly toxic and unsafe to be handled outside of a special facility, MB samples and non-toxic control water samples were analyzed as part of the operational factors assessment. The control water samples were provided by the vendor or prepared and used according to the vendor's protocol as described in the previous section. Because no samples spiked with the contaminants of interest were used, only the operational aspects of the Neuro-IQ Tox Test Kit™ were evaluated with the non-technical operator. As the Neuro-IQ Tox Test Kit™ may be used by first-responders, its performance was evaluated under simulated first-response conditions by having the operator dressed in a Level B protective suit, neoprene latex gloves, boots, and a self-contained breathing apparatus (SCBA). The operator had prior experience working in personal protective equipment (PPE). One set of MB samples was also tested without the use of PPE. Ease of use from the perspective of the operator was documented both with and without the PPE.

3.3 Verification Schedule

The verification test of the Neuro-IQ Tox Test Kit™ took place from November 2005 through February 2006 at Battelle facilities in Columbus and West Jefferson, Ohio.

3.4 Test Procedure

3.4.1 Test Sample Preparation and Storage

All testing for this verification test was conducted within Battelle laboratories. Aldicarb and dicrotophos samples were tested at Battelle's Columbus laboratories, while VX, GB, and GD samples were tested at Battelle's Hazardous Materials Research Center (HMRC) facility in West Jefferson, OH. Appropriate safety guidelines associated with each laboratory were followed throughout the verification test. Samples were prepared fresh each day from stock solutions in either DI water, an interferent matrix, or a DW matrix. Sample solutions were prepared to the specified concentration based on the concentration of the stock solution, which was confirmed through reference analysis. Test solutions were prepared in 1L quantities such that appropriate aliquots (10 mL) of the sample preparation could be used for each test sample. Triplicate samples of 10 mL each were taken from the same sample preparation. Each sample was placed in its own container and labeled only with a sample identification number that was also recorded in a laboratory record book (LRB) along with details of the sample preparation.

3.4.2 Test Sample Analysis Procedure

The Neuro-IQ Tox Test Kit™ is intended to be used by a technician with basic laboratory skills. To test a water sample using the Neuro-IQ Tox Test Kit™, two different solutions had to be assayed: first a control water sample and then a test water sample. The control water sample is simply a sample of the same matrix being tested, only uncontaminated. According to the manufacturer, all chlorinated water samples should be dechlorinated prior to testing. This had

been done for all DW samples prior to testing (see Section 3.2.2). The steps for testing the control water and test water samples are described below.

First, the testing materials including the water samples and Reagents A and B (supplied with the test kit) were brought to room temperature. Then, the pH meter was calibrated using a 2-point calibration curve based on buffers provided with the test kit. Reagent B was prepared for use by adding 6 mL of DI water to the Reagent B vial and stirring. Reagent A was used as-is.

Next, the control water sample was tested. One control water sample was tested with each set of three to four samples. The test samples and the control water were of the same matrix. To test the control water sample, 40 μ L of Reagent A were added to 10 mL of the control water sample and the solution was then slowly stirred on a magnetic stir plate. After five minutes, 0.02M sodium hydroxide (NaOH) was added in 10 μ L increments until the pH of the solution was 8.30 ± 0.05 pH units. The pH did not need to stabilize, only reach the specified level. While still stirring on the magnetic stir plate, 200 μ L of Reagent B were added. Three minutes after adding Reagent B, the pH of the solution was recorded. The test water sample was tested following the same procedure as with the control water sample. The final pH for the test water sample, taken three minutes after adding Reagent B, was recorded for the sample.

To determine if a sample was positive or negative, the difference between the control water sample's final pH and the test water sample's final pH was calculated. If the difference was ≥ 0.2 pH units, then the test sample was considered positive. If the difference in pH was < 0.2 pH units, then the sample was considered negative or not detected. To allow for testing of all of the samples prescribed for this verification test, differences in pH were calculated on a sample by sample basis. In addition, three, not four, samples were tested with each control water sample since each type of sample need only to be tested in triplicate. These changes were recommended by the vendor.

3.4.3 Drinking Water Characterization

An aliquot of each DW sample, collected as described in Section 3.2.2, was sent to ATEL to determine the following water quality parameters: turbidity; concentration of dissolved and total organic carbon; conductivity; alkalinity; pH; concentration of Ca and Mg; hardness; and concentration of total organic halides, trihalomethanes, and haloacetic acids. Table 3-4 lists the characterization data from the four water sample types used in this verification test. Water samples were collected and water quality parameters were measured by ATEL in June 2005, while verification testing was tested with the DW between November 2005 and February 2006. The time delay between collection and testing was due to the fact that the water samples were collected for use during a separate ETV test conducted prior to this one. Because of this, an aliquot of each DW was tested by ATEL again in January 2006 to verify some of the parameters with the most potential to change over time. Note that dissolved organic carbon was not retested as this result was verified by the total organic carbon results, additionally the total organic halides and calcium and magnesium were not verified as there was no reason to expect a change in these parameters. The concentrations of most water quality parameters were similar; however, there was a decrease in levels of volatile compounds such as trihalomethanes and haloacetic acids over this time-period.

Table 3-4. ATEL Water Quality Characterization of Drinking Water Samples

Parameter	Unit	Method	Columbus, OH (OH DW)		New York City, NY (NY DW)		Orlando, FL (FL DW)		MWD ^(b) , CA (CA DW)	
			2005	2006	2005	2006	2005	2006	2005	2006
Turbidity	NTU ^(a)	EPA 180.1 ⁽⁴⁾	0.1	0.6	1.1	1.3	0.5	0.1	0.1	0.2
Dissolved Organic Carbon	mg/L	SM 5310 ⁽⁵⁾	2.1	NA	1.1	NA	1.6	NA	2.9	NA
Total Organic Carbon	mg/L	SM 5310 ⁽⁵⁾	2.1	2.3	1.6	4.1	1.7	2.1	2.5	2.7
Specific Conductivity	µMHO ^(c)	SM 2510 ⁽⁵⁾	572	602	84	78	322	325	807	812
Alkalinity	mg/L	SM 2320 ⁽⁵⁾	40	44	14	12	142	125	71	97
pH		EPA 150.1 ⁽⁶⁾	7.6	7.4	6.9	6.8	8.5	7.6	8.0	7.9
Calcium	mg/L	EPA 200.8 ⁽⁷⁾	33	NA	5.6	NA	8.8	NA	45	NA
Magnesium	mg/L	EPA 200.8 ⁽⁷⁾	7.7	NA	1.3	NA	43	NA	20	NA
Hardness	mg/L	EPA 130.2 ⁽⁸⁾	118	107	20	26	143	130	192	182
Total Organic Halides	µg/L	SM 5320 ⁽⁵⁾	220	NA	82	NA	300	NA	170	NA
Trihalomethanes	µg/L/ analyte	EPA 524.2 ⁽⁹⁾	74.9	16.6	39.0	23.1	56.4	41.8	39.2	24.1
Haloacetic Acids	µg/L/ analyte	EPA 552.2 ⁽¹⁰⁾	32.8	<6.0	39.0	<6.0	34.6	<6.0	17.4	<6.0

^(a) NTU = Nephelometric turbidity unit.

^(b) MWD = Metropolitan Water District of Southern California

^(c) µMHO = micromho

Chapter 4

Quality Assurance/Quality Control

QA/QC procedures were performed in accordance with the quality management plan (QMP) for the AMS Center⁽¹¹⁾ and the test/QA plan⁽¹²⁾ for this verification test.

QC procedures as noted in the reference methods or laboratory's operating procedures were followed in confirming analyses of stock or reference solutions of contaminants and interfering compounds and in characterizing the DW. The reference methods for this verification test are listed in Table 4-1. A summary of the QC samples and acceptance criteria associated with each method is presented in Table 7 in the test/QA plan.⁽¹²⁾

4.1 Sample Chain-of Custody Procedures

Sample custody was documented throughout collection, shipping, and analysis of the samples. Sample chain-of-custody procedures were in accordance with ASAT.I-009-DRAFT, *Standard Operating Procedure for Sample Chain of Custody*. The chain-of-custody forms summarized the samples collected and analyses requested and were signed by the person relinquishing samples once that person had verified that the custody forms were accurate. The original sample custody forms accompanied the samples; the shipper kept a copy. Upon receipt at the sample destination, sample custody forms were signed by the person receiving the samples once that person had verified that all samples identified on the custody forms were present in the shipping container.

4.2 QC Samples

The QC measures for the reference methods included the analysis of a MB sample with the analyses of the reference or stock solution. MB samples were analyzed to ensure that no sources of contamination were present. If the analysis of an MB sample indicated a concentration above the minimum detection limit for the confirmatory instrument, contamination was suspected. Any contamination source(s) were corrected, and proper blank readings were achieved, before proceeding with the analyses. In general, a matrix spike or laboratory fortified spike sample was also analyzed. Average acceptable recoveries for these samples were between 70 and 150%. Samples outside of the acceptable range were generally flagged and rerun once the QC acceptance criteria had been met. QC samples were run with every batch of 1 to 20 samples. Specific QC samples and acceptance criteria associated with each method can be found in the appropriate reference (Table 4-1).

Table 4-1. Reference Methods for Target Contaminants and Interferents

Target Analyte/Interferent	Reference Method (Instrumentation)	Number of Observations	Expected Concentrations (mg/L)	Average Measured Concentration (mg/L) \pm SD	Recovery (%R) \pm SD
VX	Battelle Internally Developed Method (LC-MS)	10	2.1	2.1 \pm 0.1	101 \pm 5
GB (sarin)	HMRC-IV-118-05 ⁽¹³⁾ (GC-MS)	4	20.0	17.0 \pm 1.4	85 \pm 7
GD (soman)	HMRC-IV-118-05 ⁽¹³⁾ (GC-MS)	4	1.4	1.7 \pm 0.05	121 \pm 4
aldicarb	SOP for Analysis of Water Sample Extracts for Type 1 Analytes by Liquid Chromatography/Mass Spectrometry ⁽¹⁴⁾ (LC-MS)	2	26.0	34	123 \pm 7 ^(a)
		2	260	303	
dicotophos	SOP for Extracting and Preparing Water Samples for Analysis of Dicotophos, Mevinphos, and Dichlorovos ⁽¹⁵⁾ (GC-MS)	4	140	157 \pm 24	108 \pm 17 ^(a)
		1	1400	1326	
calcium (Ca)	EPA 200.8 ⁽⁷⁾ (ICP-MS)	1	125	140	112
magnesium (Mg)	EPA 200.8 ⁽⁷⁾ (ICP-MS)	1	125	130	104
Humic and fulvic acids	Standard Method 5310 ⁽⁵⁾ Combustion Infrared NDR	1	1.0	0.9	90

^(a) Average of two concentration levels.

QC samples as provided with the Neuro-IQ Tox Test Kit™ were also run per the vendor's instructions, and MB samples were run as part of the verification test (Section 3.2.3). Seven MB samples were run with each set of pesticide samples. Only five MB samples were run with each set of chemical agent samples. Of the 15 MB samples run across VX, GB, and GD samples, four positive responses were obtained, one with GB samples and three with GD samples. There was no indication of contamination despite the positive MB results on days when those samples were run. For ease of testing, at least seven sets of triplicate MB samples (21 total MB samples) were run for each pesticide, instead of seven total MB samples. All MB samples analyzed with aldicarb and dicotophos samples were negative.

4.3 Equipment/Calibration

The instruments used for the reference analyses were calibrated per the standard reference methods being used to make each measurement or the standard operating procedures (SOPs) of the analysis laboratory. Instruments used in the reference analyses for this test included gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), pH electrodes, inductively coupled plasma-mass spectrometry (ICP-MS), and gas chromatography with electron capture detector (GC-ECD). All calibrations were documented by Battelle in the project LRB. Calibration of mass spectrometers involved a 4- to 8-point calibration curve covering the range of concentrations of the reference solutions to be analyzed. Calibration of each reference instrument was performed as frequently as required by the reference method guidelines.

The vendor provided the Battelle technical operator with instructions on how to properly maintain components of the Neuro-IQ Tox Test Kit™ requiring calibration, namely the pH probe. The pH probe was calibrated at the beginning of each day of testing using at least a 2-point calibration curve based on buffer solutions provided by the vendor.

Pipettes used during solution preparation were maintained and calibrated as required by Battelle SOPs (i.e., minimum of every 6 months). Pipettes were checked and either recalibrated or replaced if they were dropped over the course of testing.

4.4 Characterization of Stock Solutions

During testing, aliquots of the stock solutions used for sample preparation were submitted for concentration confirmation via the respective methods. The results, along with the reference methods, are listed in Table 4-1. Averages and associated standard deviations are given in cases where more than two samples were tested. Recovery (%R) is calculated by the following equation:

$$\%R = \frac{C}{A} \times 100 \quad (1)$$

where C is the measured concentration (or average measured concentration if more than one sample was tested) and A is the expected concentration of the contaminant or interferent in solution. For aldicarb and dicrotophos, aliquots at two different concentration levels were confirmed through reference analysis. The %R, listed in Table 4-1, represents the average of the %R across both concentration levels for those compounds. Table 4-1 shows that %R values ranged from 85% to 123% across all analytes and interferents.

Contaminant stock solutions were prepared and tested individually. Interferent stock solutions contained multiple analytes in the same solution (e.g., calcium and magnesium or humic and fulvic acids together). Up to four aliquots of each stock solution were analyzed over the course of the verification test. In the case of VX, extra aliquots were analyzed and all were reported in Table 4-1. Aliquots were preserved or extracted on the day of preparation and stored as prescribed by the standard method.

4.5 Audits

4.5.1 Performance Evaluation Audit

The concentration of the standards used to prepare the samples fortified with contaminants and potential interfering compounds was confirmed by analyzing standards prepared in ASTM Type II DI water from two separate commercial vendors using the reference methods noted in Table 4-1. The standards from one vendor were used during the verification test, while the standards from the second vendor were used exclusively to confirm the accuracy of the standards from the first vendor.

Given the security requirements and lack of alternate sources for the chemical agents (VX, GB, and GD) used in this verification test, PE audits were not performed for these contaminants. PE audits were done for all remaining compounds when more than one source of the contaminant or potential interfering compounds was available. PE audits were performed only on compounds used to prepare test samples and not on any solutions supplied as part of the Neuro-IQ Tox Test Kit™. Agreement of the standards within 25% (percent difference) was required for the measurements to be considered acceptable. The percent difference (%D) between the measured concentration of the PE sample and the nominal concentration of that sample was calculated using the following equation:

$$\%D = \frac{M}{A} \times 100 \quad (2)$$

where M is the absolute value of the difference between the measured and the expected concentration, and A is the expected concentration. The results of the PE samples are given in Table 4-2. All %D values calculated were within the 25% acceptable tolerance.

Table 4-2. Performance Evaluation Samples and Percent Difference

Contaminant	Expected Concentration (ng/mL)	Measured Concentration (ng/mL)	Percent Difference (%)
aldicarb	50	57	14
dicrotophos	1000	1103	10
Ca	1000	890	11
Mg	1000	990	1

4.5.2 Technical Systems Audit

The Battelle Quality Manager conducted technical systems audits (TSAs) in November 2005 (11/01, 11/11, 11/16, 11/18), December 2005 (12/01, 12/29), and January 2006 (01/30) to ensure that the verification test was performed in accordance with the AMS Center QMP,⁽¹¹⁾ the test/QA plan,⁽¹²⁾ published reference methods, and any SOPs used by Battelle. As part of the audit, the Battelle Quality Manager reviewed the reference methods, compared actual test procedures to those specified or referenced in the test/QA plan, and reviewed data acquisition and handling

procedures. The Battelle Quality Manager also observed testing in progress and the reference method sample preparation and analysis, inspected documentation, and reviewed the LRBs used to record testing results. The Battelle Quality Manager also checked calibration certifications and conferred with Battelle staff. Observations and findings from this audit were documented and submitted to the Battelle Verification Test Coordinator for response. No major findings were reported from the audits. The records concerning the TSA are permanently stored with the Battelle Quality Manager.

4.5.3 Audit of Data Quality

At least 10% of the data acquired during the verification test was audited. The Battelle Quality Manager traced the data from initial acquisition, through reduction and statistical comparisons, to final reporting. All calculations performed on the data undergoing the audit were checked.

4.6 QA/QC Reporting

Each assessment and audit was documented in accordance with Section 3.3.4 of the AMS Center QMP.⁽¹¹⁾ Once the assessment report was prepared, the Battelle Verification Test Coordinator responded to each potential problem and implemented any necessary follow-up corrective action. The Battelle Quality Manager ensured that follow-up corrective action was taken. The results of the TSA were sent to the EPA.

4.7 Data Review

Records generated in the verification test were reviewed before they were used to calculate, evaluate, or report verification results. Table 4-3 summarizes the types of data recorded. The review was performed by a technical staff member involved in the verification test but not the staff member who 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.

Table 4-3. Summary of Data Recording Process

Data to Be Recorded	Responsible Party	Where Recorded	How Often Recorded	Disposition of Data
Dates, times, and details of test events	Battelle	ETV laboratory record book or data recording forms	Start/end of test procedure, and at each change of a test parameter	Used to organize and check test results and manually incorporated into data spreadsheets as necessary
Sample preparation (dates, concentrations, etc.)	Battelle	ETV laboratory record books	When each solution was prepared	Used to confirm the concentration and integrity of the samples analyzed
Enzymatic test kit procedures and sample results	Battelle	ETV data sheets and laboratory record book	Throughout test duration	Manually incorporated into data spreadsheets for statistical analysis and comparisons
Reference method sample preparation	Battelle	ETV laboratory record book	Throughout sample preparation	Used to demonstrate validity of samples submitted for reference measurements
Reference method procedures, calibrations, QA, etc.	Battelle or subcontract laboratory	Laboratory record book or data recording forms	Throughout sampling and analysis processes	Retained as documentation of reference method performance
Reference method analysis results	Battelle or subcontract laboratory	Electronically from reference analytical method	Every sample analysis	Converted to spreadsheets for calculations

Chapter 5

Statistical Methods and Reported Parameters

The Neuro-IQ Tox Test Kit™ was evaluated for qualitative results (i.e., positive/negative responses to samples). All data analyses were based on these qualitative results. QC and MB samples were not included in any of the analyses.

5.1 Accuracy

Accuracy was assessed by evaluating how often the Neuro-IQ Tox Test Kit™ result is positive in the presence of a concentration above the limit of detection (LOD). Contaminant-only PT samples were used for this analysis. An overall percent agreement was determined by dividing the number of positive responses by the overall number of analyses of contaminant-only PT samples greater than the Neuro-IQ Tox Test Kit™'s LOD (see Equation 3). If the LOD was not known or available, then all analyzed contaminant-only PT samples greater than the concentration level where consistent negative results were obtained were used.

$$\text{Accuracy (\% Agreement)} = \frac{\# \text{ of positive contaminant only PT samples}}{\text{total \# of contaminant only PT samples}} \times 100 \quad (3)$$

5.2 False Positive/False Negative Rates

A false positive response was defined as a response indicating the presence of a contaminant when the PT interferent or DW sample was not spiked with contaminant. A false positive rate was reported as the number of false positive results out of the total number of unspiked samples (Equation 4).

A false negative response was defined as a response indicating the absence of a contaminant when the sample was spiked with a contaminant at a concentration greater than the Neuro-IQ Tox Test Kit™'s LOD as defined above. Spiked PT (contaminant and interferent) samples and spiked DW samples were included in the analysis. Contaminant-only PT samples above the Neuro-IQ Tox Test Kit™'s LOD or the level at which consistent negative responses are obtained (when the LOD was not known) were included in the analysis. A false negative rate was evaluated as the number of false negative results out of the total number of spiked samples for a particular contaminant (Equation 5).

$$\text{False Positive Rate} = \frac{\# \text{ of positive results}}{\text{total \# of unspiked samples}} \quad (4)$$

$$\text{False Negative Rate} = \frac{\# \text{ of negative results}}{\text{total \# of spiked samples}} \quad (5)$$

5.3 Precision

Precision measures the repeatability and reproducibility of the Neuro-IQ Tox Test Kit™'s responses. The precision of three replicates of each sample set was assessed. Responses were considered inconsistent if one or more of the three replicates differed from the response of the other samples in the replicate set. The precision for the Neuro-IQ Tox Test Kit™ was assessed by calculating the overall number of consistent responses for all the sample sets. The results are reported as the percentage of consistent responses out of all replicate sets (Equation 6).

$$\text{Precision (\% Consistent results)} = \frac{\# \text{ of consistent responses of replicate sets}}{\text{total \# of replicate sets}} \times 100 \quad (6)$$

5.4 Potential Matrix and Interferent Effects

The potential effect of the DW matrix on the Neuro-IQ Tox Test Kit™'s performance was evaluated qualitatively by comparing the results for the spiked and unspiked DW samples to those for the PT samples spiked with the contaminant at 10 times less than the lethal dose. Similarly, the potential effect of interferent PT samples was evaluated. The results indicating the correct or incorrect reporting of the presence of a contaminant were evaluated. The findings are reported and discussed in Section 6.4.

5.5 Operational Factors

Operational aspects of the Neuro-IQ Tox Test Kit™'s performance such as ease of use and sample throughput were evaluated through observations made during testing. Also addressed are the qualitative observations of the verification staff pertaining to the performance of the Neuro-IQ Tox Test Kit™ from both the technical and non-technical operators' perspective.

Chapter 6 Test Results

The Neuro-IQ Tox Test Kit™ did not produce distinctive “detected” or “not detected” responses. Instead, pH values were obtained for each sample. These pH values were then converted into qualitative results. This was done by comparing the pH value for a given sample to the pH value for the control water sample for that particular group of samples. The difference between the control water pH value and the sample pH value (both taken after Reagent B was added and three minutes had elapsed) was calculated. If the sample pH value was ≥ 0.2 pH units above the control water’s pH value, then the sample was concluded to be a positive hit, indicating the presence of the contaminant in the sample. If the test sample pH value was < 0.2 pH units above the control water’s pH value, then a non-detect was recorded for that sample. All of the results presented in this chapter were calculated using the qualitative responses determined for the Neuro-IQ Tox Test Kit™.

6.1 Accuracy

The accuracy results for the Neuro-IQ Tox Test Kit™ using the contaminant-only PT samples are discussed in this section. Table 6-1 presents the accuracy results for VX, GB, GD, aldicarb, and dicrotophos. The results for the lethal dose concentration of each contaminant are given in the table. Results are presented for all tested concentration levels; but, by definition, only those results above the kit’s LOD are included in the calculation. Because a LOD was not available for the Neuro-IQ Tox Test Kit™, only samples above the level for each contaminant where consistent negative responses were obtained were considered for accuracy calculations. For VX, GB, GD, and aldicarb, consistent negative responses were not obtained from any concentration level, so all contaminant-only PT samples were included in the accuracy calculations. For dicrotophos, consistent negative responses were found starting at a 1,000x dilution of the lethal dose (i.e., at 1.4 mg/L), thus only three sets of replicates were included in the accuracy calculations for that contaminant.

All concentration levels analyzed for VX generated 3 out of 3 positive responses for each set of replicates, resulting in 100% agreement for the overall accuracy. No other contaminant tested with the Neuro-IQ Tox Test Kit™ resulted in 100% overall accuracy. All but one concentration level for the GB samples resulted in 3 out of 3 positive responses. The GB lethal dose level (20 mg/L) samples generated 2 out of 3 positive responses, resulting in 93% agreement for overall accuracy. Similarly, only one level of GD contaminant-only PT samples did not have

Table 6-1. Contaminant-Only PT Sample Results

Contaminant	Concentration (mg/L)	Positive Results Out of Total Replicates	Accuracy
VX	2.1 ^(a)	3/3	100% (15/15)
	0.21	3/3	
	0.021	3/3	
	0.0021	3/3	
	0.00021	3/3	
GB	20 ^(a)	2/3	93% (14/15)
	2.0	3/3	
	0.20	3/3	
	0.020	3/3	
	0.0020	3/3	
GD	1.4 ^(a)	3/3	87% (13/15)
	0.14	3/3	
	0.014	3/3	
	0.0014	3/3	
	0.00014	1/3	
aldicarb	260 ^(a)	3/3	67% (10/15)
	26	3/3	
	2.6	3/3	
	0.26	0/3	
	0.026	1/3	
dicrotophos	1400 ^(a)	3/3	44% (4/9)
	140	0/3	
	14	1/3	
	1.4	0/3 ^(b)	
	0.014	0/3 ^(b)	

^(a) Lethal dose.

^(b) Not used in accuracy calculations because samples are at or below level of consistent negative response.

three positive results; the lowest tested concentration level for GD (0.00014 mg/L) generated only 1 out of 3 positive results. The resulting overall accuracy for GD was 87%. Aldicarb samples resulted in 67% overall accuracy. Samples at both 1,000x (0.26 mg/L) and 10,000x (0.026 mg/L) dilution of the lethal dose had less than three positive responses (0 out of 3 and 1 out of 3, respectively). Only those nine samples with dicrotophos concentrations of 14 to 1,400 mg/L were used in the assessment of accuracy, and of these levels only the lethal dose concentration generated 3 out of 3 positive responses, resulting in 44% overall accuracy.

6.2 False Positive/False Negative Rates

Contaminant-only PT samples, interferent PT samples, and DW samples were evaluated to determine false positive and false negative results for the Neuro-IQ Tox Test Kit™. A false positive response was defined as a positive result when the contaminant was not spiked into the sample. A false negative response was defined as a negative result when the sample was spiked with a contaminant at a concentration greater than the level where consistent negative responses were obtained (see Section 6.1). Tables 6-2a through 6-2e present the false positive and false negative responses for VX, GB, GD, aldicarb, and dicrotophos, respectively. The number of positive samples out of the total replicates analyzed is presented in each table.

For VX, GB, and GD, only one set of unspiked DW and PT interferent samples were run for all three chemical agents. Thus, the unspiked DW and PT-interferent sample results shown in Tables 6-2a through 6-2c are the same and from only one set of triplicate samples. For aldicarb and dicrotophos, sets of unspiked DW and PT interferent samples were run separately for each pesticide.

One false negative was found for VX; one of the three replicates for VX spiked 250 mg/L Ca and Mg was negative. However, 13 false positives were found: three positive responses for unspiked 1 mg/L humic and fulvic acids, one for unspiked 5 mg/L humic and fulvic acids, three for 50 mg/L Ca and Mg, as well as three positive responses for both unspiked OH and FL DW. These false positives were the same for GB and GD. GB also had one false negative response when only two of the three replicates at the lethal dose (20 mg/L) resulted in positive responses. For GD, 2 out of 39 samples were falsely negative. Both of these false negatives occurred at the lowest concentration contaminant-only PT sample (0.00014 mg/L).

Both aldicarb and dicrotophos had three false positive responses. In both cases they occurred in unspiked 5 mg/L humic and fulvic acid samples. Aldicarb also had eight false negative responses: three at 0.26 mg/L aldicarb in DI water, two at 0.026 mg/L aldicarb in DI water, and three in 250 mg/L Ca and Mg PT interferent samples spiked with aldicarb. For dicrotophos, seven false negative responses were found: three at 10x less than the lethal dose (140 mg/L dicrotophos in DI water), two at 100x less than the lethal dose (14 mg/L dicrotophos in DI water), and two in the 1 mg/L humic and fulvic acid samples spiked with the contaminant.

Table 6-2a. VX False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates ^(a)
Contaminant-only PT samples	DI water	2.1 ^(b)	3/3
	DI water	0.21	3/3
	DI water	0.021	3/3
	DI water	0.0021	3/3
	DI water	0.00021	3/3
Interferent PT samples ^(c)	1 mg/L humic and fulvic acids	Blank	3/3
	1 mg/L humic and fulvic acids	0.21	3/3
	5 mg/L humic and fulvic acids	Blank	1/3
	5 mg/L humic and fulvic acids	0.21	3/3
	50 mg/L Ca and Mg	Blank	3/3
	50 mg/L Ca and Mg	0.21	3/3
	250 mg/L Ca and Mg	Blank	0/3
	250 mg/L Ca and Mg	0.21	2/3
DW samples ^(c)	OH DW	Blank	3/3
	OH DW	0.21	3/3
	CA DW	Blank	0/3
	CA DW	0.21	3/3
	FL DW	Blank	3/3
	FL DW	0.21	3/3
	NY DW	Blank	0/3
	NY DW	0.21	3/3
False Positive Rate			13/24
False Negative Rate			1/39

^(a) Boxed results indicate false positive responses; shaded results indicate false negative responses.

^(b) Lethal dose.

^(c) Only one set of unspiked DW and PT interferent samples were run for VX, GB, and GD.

Table 6-2b. GB False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates ^(a)
Contaminant-only PT samples	DI water	20 ^(b)	2/3
	DI water	2.0	3/3
	DI water	0.20	3/3
	DI water	0.020	3/3
	DI water	0.0020	3/3
Interferent PT samples ^(c)	1 mg/L humic and fulvic acids	Blank	3/3
	1 mg/L humic and fulvic acids	2.0	3/3
	5 mg/L humic and fulvic acids	Blank	1/3
	5 mg/L humic and fulvic acids	2.0	3/3
	50 mg/L Ca and Mg	Blank	3/3
	50 mg/L Ca and Mg	2.0	3/3
	250 mg/L Ca and Mg	Blank	0/3
	250 mg/L Ca and Mg	2.0	3/3
DW samples ^(c)	OH DW	Blank	3/3
	OH DW	2.0	3/3
	CA DW	Blank	0/3
	CA DW	2.0	3/3
	FL DW	Blank	3/3
	FL DW	2.0	3/3
	NY DW	Blank	0/3
	NY DW	2.0	3/3
False Positive Rate			13/24
False Negative Rate			1/39

^(a) Boxed results indicate false positive responses; shaded results indicate false negative responses.

^(b) Lethal dose.

^(c) Only one set of unspiked DW and PT interferent samples were run for VX, GB, and GD.

Table 6-2c. GD False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates ^(a)
Contaminant-only PT samples	DI water	1.4 ^(b)	3/3
	DI water	0.14	3/3
	DI water	0.014	3/3
	DI water	0.0014	3/3
	DI water	0.00014	1/3
Interferent PT samples ^(c)	1 mg/L humic and fulvic acids	Blank	3/3
	1 mg/L humic and fulvic acids	0.14	3/3
	5 mg/L humic and fulvic acids	Blank	1/3
	5 mg/L humic and fulvic acids	0.14	3/3
	50 mg/L Ca and Mg	Blank	3/3
	50 mg/L Ca and Mg	0.14	3/3
	250 mg/L Ca and Mg	Blank	0/3
	250 mg/L Ca and Mg	0.14	3/3
DW samples ^(c)	OH DW	Blank	3/3
	OH DW	0.14	3/3
	CA DW	Blank	0/3
	CA DW	0.14	3/3
	FL DW	Blank	3/3
	FL DW	0.14	3/3
	NY DW	Blank	0/3
	NY DW	0.14	3/3
False Positive Rate			13/24
False Negative Rate			2/39

^(a) Boxed results indicate false positive responses; shaded results indicate false negative responses.

^(b) Lethal dose.

^(c) Only one set of unspiked DW and PT interferent samples were run for VX, GB, and GD.

Table 6-2d. Aldicarb False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates ^(a)
Contaminant-only PT samples	DI water	260 ^(b)	3/3
	DI water	26	3/3
	DI water	2.6	3/3
	DI water	0.26	0/3
	DI water	0.026	1/3
Interferent PT samples	1 mg/L humic and fulvic acids	Blank	0/3
	1 mg/L humic and fulvic acids	26	3/3
	5 mg/L humic and fulvic acids	Blank	3/3
	5 mg/L humic and fulvic acids	26	3/3
	50 mg/L Ca and Mg	Blank	0/3
	50 mg/L Ca and Mg	26	3/3
	250 mg/L Ca and Mg	Blank	0/3
	250 mg/L Ca and Mg	26	0/3
DW samples	OH DW	Blank	0/3
	OH DW	26	3/3
	CA DW	Blank	0/3
	CA DW	26	3/3
	FL DW	Blank	0/3
	FL DW	26	3/3
	NY DW	Blank	0/3
	NY DW	26	3/3
False Positive Rate			3/24
False Negative Rate			8/39

^(a) Boxed results indicate false positive responses; shaded results indicate false negative responses.

^(b) Lethal dose.

Table 6-2e. Dicrotophos False Positive/Negative Results

Sample Type	Matrix	Concentration (mg/L)	Positive Results Out of Total Replicates ^(a)
Contaminant-only PT samples	DI water	1400 ^(b)	3/3
	DI water	140	0/3
	DI water	14	1/3
Interferent PT samples	1 mg/L humic and fulvic acids	Blank	0/3
	1 mg/L humic and fulvic acids	140	1/3
	5 mg/L humic and fulvic acids	Blank	3/3
	5 mg/L humic and fulvic acids	140	3/3
	50 mg/L Ca and Mg	Blank	0/3
	50 mg/L Ca and Mg	140	3/3
	250 mg/L Ca and Mg	Blank	0/3
	250 mg/L Ca and Mg	140	3/3
DW samples	OH DW	Blank	0/3
	OH DW	140	3/3
	CA DW	Blank	0/3
	CA DW	140	3/3
	FL DW	Blank	0/3
	FL DW	140	3/3
	NY DW	Blank	0/3
	NY DW	140	3/3
False Positive Rate			3/24
False Negative Rate			7/33

^(a) Boxed results indicate false positive responses; shaded results indicate false negative responses.

^(b) Lethal dose.

6.3 Precision

The performance of the Neuro-IQ Tox Test Kit™ in measuring VX within sets of three replicate samples was generally consistent. Only two sets of replicates were inconsistent: unspiked 5 mg/L humic and fulvic acids and spiked 250 mg/L Ca and Mg. One positive and two negative responses were found for the unspiked humic and fulvic acid replicates while two positive and one negative responses were found for the Ca and Mg replicates. Thus, two of the 21 sets of replicates that were analyzed was determined to be inconsistent, indicating that 90% of the sample sets showed consistent results among the replicates.

The Neuro-IQ Tox Test Kit™ results for GB and GD were also consistent in 19 out of 21 sets of replicates, indicating that 90% of the sample sets showed consistent results for these two contaminants. For GB, samples at the lethal dose of the chemical agent as well as samples in unspiked 5 mg/L humic and fulvic acids were inconsistent. For GD, inconsistencies were found in PT samples at 10,000x less than the lethal dose (i.e., at 0.00014 mg/L) and in unspiked 5 mg/L humic and fulvic acid samples.

Results for samples spiked and not spiked with aldicarb were consistent 95% of the time with results being the same in 20 out of 21 sample sets. Only the PT sample at 10,000x less than the lethal dose (i.e., at 0.026 mg/L) were inconsistent. Of the 21 sample sets, 19 showed consistent results for dicrotophos samples, resulting in 90% precision. Replicates at both the 14 mg/L (in DI water) concentration level and the 1 mg/L humic and fulvic acids spiked with the pesticide were inconsistent.

6.4 Potential Matrix and Interferent Effects

6.4.1 Interferent PT Samples

The Neuro-IQ Tox Test Kit™ was able to consistently detect VX, GB, and GD at 10x less than the lethal dose in DI water (see Tables 6-2a – c, respectively). Across all three chemical agents at 10x less than the lethal dose spiked into interferent PT samples, the Neuro-IQ Tox test produced positive responses for all sample replicates except in one instance. The one exception was that for interferent samples spiked with VX in 250 mg/L Ca and Mg, only two out of three positive responses were achieved. Only one set of unspiked interferent PT samples provided all negative responses, that for 250 mg/L Ca and Mg. All other unspiked interferent samples had one or more positive responses. These results seem to indicate that the Neuro-IQ Tox Test Kit™ may have some sensitivity to the interferents used in this test.

For both aldicarb and dicrotophos samples (see Tables 6-2d and e), unspiked 5 mg/L humic and fulvic acid samples had positive responses for all three replicates, further confirming the potential sensitivity of the Neuro-IQ Tox Test Kit™ to this interferent. In interferent PT samples spiked with aldicarb, results were as expected except for 250 mg/L Ca and Mg replicate. For these spiked samples, no positive responses were found. Aldicarb spiked into DI water at 10x less than the lethal dose was consistently detected by the Neuro-IQ Tox Test Kit™. Similarly, for 1 mg/L humic and fulvic acids spiked with dicrotophos, only one positive response was

generated. Dicrotophos spiked at 10x less than the lethal dose in DI water was not detected in any of the contaminant-only PT sample replicates. Dicrotophos was however detected in the next lowest contaminant-only PT sample concentration level, and at 10x less than the lethal dose in all other spiked interferent samples.

6.4.2 DW Samples

OH and FL unspiked DW samples were positive for all three replicates when tested as part of the chemical agent's sample set. These results indicate that there could be potential confounding compounds in these DW samples to which the Neuro-IQ Tox Test Kit™ is sensitive. No false positives or negatives were found for DW samples tested as part of the pesticides' sample set.

6.5 Operational Factors

6.5.1 Technical Operators

The Neuro-IQ Tox Test Kit™ was operated by one Battelle technician throughout testing with the pesticides and by a different Battelle technician throughout testing with chemical agents. The technicians were trained by the vendor in the operation of the test kit. Training was conducted at Battelle for one half day by the vendor. Both technicians had extensive laboratory experience.

The combination of the stir plate and the pH meter made the Neuro-IQ Tox Test Kit™ cumbersome to use. Multiple problems were encountered with the test kit operation. At one point, the pH probe supplied with the kit did not work properly, and the vendor had to supply another probe for testing to continue. After Reagent B was added and three minutes had passed, the pH was often still fluctuating, making it hard to determine the actual pH at that point in time. Since the instructions indicate that taking the pH after exactly three minutes is critical, such an issue was troublesome. Reaching a stable pH of 8.30 after adding NaOH was also generally difficult. However, per the vendor's direction, the pH did not have to stabilize at that level in order to move on with the test.

Two reagents were used to test a water sample with the Neuro-IQ Tox Test Kit™. Reagent A is stored frozen and must come to room temperature before it can be used. Reagent B had to be reconstituted with DI water before use. Individual vials of each reagent were provided with the kit to make daily testing easier.

Because of the Neuro-IQ Tox Test Kit™'s design, only one sample could be analyzed at a time. It took the operators different lengths of time to complete testing for one group of three replicates. For one operator, it took, on average, 75 minutes (± 17 minutes) to test a set of three replicate water samples using the Neuro-IQ Tox Test Kit™. It took the other operator 52 ± 7 minutes to test one set of replicates. Overall, it took an average of 64 minutes (± 18 minutes) to complete testing on a set of three samples using the Neuro-IQ Tox Test Kit™. The operators were able to analyze between three and six sets of samples per day.

6.5.2 Non-Technical Operator

Unspiked DI water samples were tested on the Neuro-IQ Tox Test Kit™ by a non-technical operator both in and not in PPE (see Section 3.2.4). The SCBA apparatus, including the mask, was worn throughout the entire testing procedure when PPE was to be worn. However, the operator ran the air from the SCBA only part of the time during testing to conserve the tank. The pH meter was operated using batteries and a portable (battery-operated) magnetic stir plate was used for this portion of the test. Figure 6-1 shows the full PPE as worn for this verification test. Figure 6-2 shows the testing of the Neuro-IQ Tox Test Kit™ with the non-technical operator wearing PPE. With the PPE on, two negative and one positive response were obtained. Without the PPE, three negative responses were recorded.

During the initial test of the Neuro-IQ Tox Test Kit™ with PPE, the operator exceeded the intended pH (8.3) when adding NaOH. Adjusting the pH to 8.3 as the kit directions indicate proved to be slightly difficult and was easy for the operator to overshoot. The test was restarted so that the proper pH could be obtained. Reagent A was hard to handle with the gloves on, and the magnetic stir plate was difficult to adjust while in full PPE.

The Neuro-IQ Tox Test Kit™ instructions indicate that it is to be used by a “technician with basic laboratory skills.” Most first responders do not have any laboratory skills. The pipettes needed for the test were cumbersome, confusing, and difficult to use for a non-technical operator. The 50-mL beakers used for each sample were small and the level of the liquid in them was shallow, making it difficult, particularly while in PPE, to place the pH probe and magnetic stirrer to obtain proper readings. This setup required patience and time from the operator and could be problematic in the field, especially for a first-responder when time is critical. Testing of three MB samples while in PPE took 52 minutes, while testing of three MB samples without PPE took 40 minutes. Consequently, having the PPE on did slow the operator down a bit as it took 12 more minutes to conduct the test with PPE than without. During the portability testing, a table-top surface was used, making the setup of the Neuro-IQ Tox Test Kit™ a bit easier. If no such surface were available in the field, the test kit would be very difficult for the operator to set up and use. As noted earlier in this report, a control water sample is needed for the Neuro-IQ Tox Test Kit™ protocol. This means that water that is the same matrix as the test sample but not contaminated would have to be obtained to use this kit. This could be problematic in the field. Overall, the Neuro-IQ Tox Test Kit™ would be hard for a first-responder with no experience and no laboratory skills to use if the operator is donned in the level of PPE used in this verification test.



Figure 6-1. Side View of PPE Worn by the Non-Technical Operator



Figure 6-2. Testing of the Neuro-IQ Tox Test Kit™ with the Non-Technical Operator Wearing PPE

Chapter 7

Performance Summary

The Neuro-IQ Tox Test Kit™ results for this verification test for samples containing VX, GB, GD, aldicarb, and dicotophos are presented in Tables 7-1 through 7-5. The results for each contaminant are presented in a separate table. Qualitative responses for each set of sample replicates as well as accuracy, false negatives and positives, and precision are presented in each table. A summary of the other performance factors associated with the Neuro-IQ Tox Test Kit™ is presented at the end of this chapter. These performance factors apply across all contaminants.

Table 7-1. VX Summary Table

Parameter		Matrix	VX Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	2.1 mg/L ^(a)	3/3
			0.21 mg/L	3/3
			0.021 mg/L	3/3
			0.0021 mg/L	3/3
			0.00021 mg/L	3/3
	Interferent PT Samples	Humic and Fulvic Acids	0.21 mg/L	6/6
Ca and Mg		0.21 mg/L	5/6	
	DW Samples	DW	0.21 mg/L	12/12
Accuracy		100% (15 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Thirteen false positive responses were obtained. Seven positive responses were found across unspiked 1 mg/L and 5 mg/L humic and fulvic acids as well as unspiked 50 mg/L Ca and Mg samples. All six replicates for unspiked OH and FL DW yielded positive results.		
False Negatives		One false negative result was obtained for spiked PT and DW samples. One replicate of the spiked 250 mg/L Ca and Mg samples returned a negative result.		
Precision		90% (20 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

^(a) Lethal dose.

Table 7-2. GB Summary Table

Parameter		Matrix	GB Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	20 mg/L ^(a)	2/3
			2.0 mg/L	3/3
			0.20 mg/L	3/3
			0.020 mg/L	3/3
			0.0020 mg/L	3/3
	Interferent PT Samples	Humic and Fulvic Acids	2.0 mg/L	6/6
		Ca and Mg	2.0 mg/L	6/6
DW Samples	DW	2.0 mg/L	12/12	
Accuracy	93% (14 out of 15) of the contaminant-only PT samples were positive.			
False Positives	Thirteen false positive responses were obtained. Seven positive responses were found across unspiked 1 mg/L and 5 mg/L humic and fulvic acids as well as unspiked 50 mg/L Ca and Mg samples. All six replicates for unspiked OH and FL DW yielded positive results.			
False Negatives	One false negative result was obtained for spiked PT and DW samples. One replicate of the spiked DI water samples at the lethal dose returned a negative result.			
Precision	90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.			

^(a) Lethal dose.

Table 7-3. GD Summary Table

Parameter		Matrix	GD Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	1.4 mg/L ^(a)	3/3
			0.14 mg/L	3/3
			0.014 mg/L	3/3
			0.0014 mg/L	3/3
			0.00014 mg/L	1/3
	Interferent PT Samples	Humic and Fulvic Acids	0.14 mg/L	6/6
Ca and Mg		0.14 mg/L	6/6	
	DW Samples	DW	0.14 mg/L	12/12
Accuracy		87% (13 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Thirteen false positive responses were obtained. Seven positive responses were found across unspiked 1 mg/L and 5 mg/L humic and fulvic acids as well as unspiked 50 mg/L Ca and Mg samples. All six replicates for unspiked OH and FL DW yielded positive results.		
False Negatives		Two false negative results were obtained for spiked PT and DW samples. Two replicates of the spiked DI water samples at 10,000x less than the lethal dose (0.00014 mg/L) returned a negative result.		
Precision		90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

^(a) Lethal dose.

Table 7-4. Aldicarb Summary Table

Parameter		Matrix	Aldicarb Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	260 mg/L ^(a)	3/3
			26 mg/L	3/3
			2.6 mg/L	3/3
			0.26 mg/L	0/3
			0.026 mg/L	1/3
	Interferent PT Samples	Humic and Fulvic Acids	26 mg/L	6/6
Ca and Mg		26 mg/L	3/6	
	DW Samples	DW	26 mg/L	12/12
Accuracy		67% (10 out of 15) of the contaminant-only PT samples were positive.		
False Positives		Three false positive responses were obtained. Positive responses were found for all replicates of the unspiked 5 mg/L humic and fulvic acids samples.		
False Negatives		Eight false negative results were obtained for spiked PT and DW samples. Five samples of the spiked DI water samples returned a negative result. All three replicates of the spiked 250 mg/L Ca and Mg samples yielded negative results.		
Precision		95% (20 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

^(a) Lethal dose.

Table 7-5. Dicrotophos Summary Table

Parameter		Matrix	Dicrotophos Concentration	Number Detected/Number of Samples
Qualitative Results	Contaminant-Only PT Samples	DI Water	1400 mg/L ^(a)	3/3
			140 mg/L	0/3
			14 mg/L	1/3
			1.4 mg/L	0/3 ^(b)
			0.14 mg/L	0/3 ^(b)
	Interferent PT Samples	Humic and Fulvic Acids	140 mg/L	4/6
	Ca and Mg	140 mg/L	6/6	
	DW Samples	DW	140 mg/L	12/12
Accuracy		44% (4 out of 9) of the contaminant-only PT samples above the level of consistent negative responses were positive.		
False Positives		Three false positive responses were obtained. Positive responses were found for all replicates of the unspiked 5 mg/L humic and fulvic acids samples.		
False Negatives		Seven false negative results were obtained for spiked PT and DW samples. Five samples of the spiked DI water samples returned a negative result. Two replicates of the spiked 1 mg/L fulvic and humic acid samples yielded negative results.		
Precision		90% (19 out of 21) of the sample sets showed consistent results among the individual replicates within that set.		

^(a) Lethal dose.

^(b) Not used in accuracy calculations because samples are at or below level of consistent negative response.

Operational Factors:

Technical Operators

The Neuro-IQ Tox Test Kit™ was operated by one Battelle technician throughout testing with the pesticides and by a different Battelle technician throughout testing with chemical agents. Both technicians had extensive laboratory experience. Multiple problems were encountered with the test kit operation, including a faulty pH probe and unstable pH readings after adding Reagent B and when trying to reach a pH of 8.30. Two reagents are used to test a water sample with the Neuro-IQ Tox Test Kit™. Reagent A is frozen and must come to room temperature before it can be used. Reagent B has to be reconstituted with DI water before use. Individual vials of each reagent were provided with the kit to make daily testing easier. Between the two operators, it took an average of 64 ± 18 minutes to complete testing on a set of three samples using the Neuro-IQ Tox Test Kit™. The operators were able to analyze between three and six sets of samples a day.

Non-Technical Operator

Unspiked DI water samples were tested on the Neuro-IQ Tox Test Kit™ by a non-technical operator both with and without PPE. Adjusting the pH to 8.30 was not easy for the operator to accomplish and many times that pH was exceeded. Reagent A was hard to handle with the gloves on, and the magnetic stir plate was difficult to adjust while in full PPE. The pipettes needed for the test were cumbersome, confusing, and difficult to use for a non-technical operator. The 50-mL beakers used for each sample were small, and the level of the liquid in them was shallow, making it difficult, particularly while in PPE, to correctly place the pH probe and magnetic stirrer. Testing of three MB samples while in PPE took 52 minutes, while testing of three MB samples without PPE took 40 minutes. The test kit would be very difficult for the operator to set up and use if no table-top surface was available in the field. A control water sample, or a water sample that is the same matrix as the test sample but not contaminated, is needed for the Neuro-IQ Tox Test Kit™ protocol. Obtaining such a sample could be problematic in the field. Overall, the Neuro-IQ Tox Test Kit™ would be hard for a first-responder with no experience with the kit and no laboratory skills to use if the operator is donned in the level of PPE used in this verification test.

Chapter 8

References

1. U.S. Army Center for Health Promotion and Preventative Medicine, USACHPPM Technical Guide 230, *Chemical Exposure Guidelines for Deployed Military Personnel*, January 2002.
2. Gosselin et al., *Clinical Toxicology of Commercial Products*. 5th edition, Baltimore, MD, 1984.
3. World Health Organization, *The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification: 2004, 2005*.
4. EPA-600-R-93/100. EPA Method 180.1. *Turbidity (Nephelometric), Methods for the Determination of Inorganic Substances in Environmental Samples*. 1993.
5. American Public Health Association, et al. *Standard Methods for Examination of Water and Wastewater*. 19th Edition. 1997. Washington D.C.
6. EPA 600/4-79/020 Method 150.1. *pH, Electrometric Method*. 1982.
7. EPA 600/R-94/111 Method 200.8. *Determination of Trace Metals by Inductively Coupled Plasma - Mass Spectrometry*. 1994.
8. EPA 600/4-79/020 Method 130.2. *Hardness, Total (mg/L as CaCO₃) Titrimetric, EDTA*. 1982.
9. EPA 600/R-95/131. EPA Method 524.2. *Purgeable Organic Compounds by Capillary Column GC/Mass Spectrometry. Methods for Determination of Organic Compounds in Drinking Water, Supplement III*. 1995.
10. EPA 600/R-95/131. 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*. 1995.
11. *Quality Management Plan (QMP) for the ETV Advanced Monitoring Systems Center*, Version 5.0, U.S. EPA Environmental Technology Verification Program, Battelle, Columbus, Ohio, March 2004.

-
12. *Test/QA Plan for Verification of Enzymatic Test Kits*, Battelle, Columbus, Ohio, September 2005.
 13. Battelle, SOP HMRC-IV-118-05: *Standard Operating Procedure for the Determination of CA in Wastewater*.
 14. Battelle, *Standard Operating Procedure for Analysis of Water Extracts for Type I Analytes by Liquid Chromatography/Mass Spectrometry*, Version 1, January 2004.
 15. Battelle, *Standard Operating Procedure for Extracting and Preparing Water Samples for Analysis of Dicrotophos, Mevinphos, and Dichlorovos*, Version 3, March 2005.