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Subject: Personnel Training and Qualification

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		02-17-20
Quality Assurance Officer	Jim Sumner		02-17-20

Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
12-01-00	0	Internal	Jim Sumner (ETS)	Original document
06-01-11	1	Internal	Jim Sumner (ETS)	• Corrected typographical errors. • Updated procedure for new training requirements. • Updated exhibits.
09-28-16	2	External (TVA) Internal	Rick Sherrard, Donald Snodgrass (TVA) Jim Sumner (ETS)	• Replaced Laboratory Supervisor responsibility: "Posts when revisions to SOPs have been made on the bulletin board." with "Conducts meetings to discuss procedural changes, SOP/QAP revisions and the implementation of new procedures." • Added Exhibit Q2.3: Example Practical Test.
10-01-17	3	Internal	Jim Sumner (ETS)	• Updated procedure to NELAP format.
02-17-20	4	External (TVA) Internal	Rick Sherrard (TVA) Jim Sumner (ETS)	• Updated plan to include the sequence of training performed. Clarified plan to state that training occurs when personnel are shown new tasks or procedures and whenever a task or procedure has been added or revised.

Scope and Application

The purpose of this procedure is to provide the requirements for training and qualifying personnel to ensure they can perform drinking water, wastewater and toxicity testing activities and to ensure compliance with project-specific instructions and standard operating procedures.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Subject: Personnel Training and Qualification

Definitions

Performance-Based Training: A systematic approach to training that is based on tasks and the related knowledge and skills required for competent job performance.

Procedural Training: Training that is required for the operation of specific equipment or processes necessary for testing activities.

Qualification (personnel): The characteristics or abilities gained through education, training, or experience, as measured against established requirements, such as standards or tests that qualify an individual to perform a required function.

Standard Operating Procedure: An approved and controlled procedure that will (1) provide enough, concise, and clear information to enable trained project personnel to effectively and efficiently perform the activity described, and (2) be used as a basis for training and indoctrination.

Trainee: The person responsible for attending and completing project training requirements.

Trainer: The person responsible for implementing and conducting personnel training according to Environmental Testing Solutions' procedures.

Training Documentation: Written verification of attendance and successful completion of a training course or requirement. Training documentation may take the form of attendance sheets, training logs, personnel training records, hands on training – task instructions, performance checklists, certificates, exams, or other verifiable documentation of training received.

Project-Specific Training Requirements

Environmental Testing Solutions incorporates three levels of training (Refer to Figure Q2.1: Sequence of Training).

Level One: This level of training includes Corporate Policies (such as Standard of Conduct, Ethics and Data Integrity, and General Safety).

Corporate Policy training is required of all personnel working for Environmental Testing Solutions for any length of time. This training occurs immediately after being hired and whenever a policy has been added or revised.

Level Two: This level of training is the most extensive and is required of all personnel involved in laboratory testing. Personnel are trained according to approved, controlled project Standard Operating Procedures (SOPs).

Personnel are required to read, understand, and demonstrate an acceptable level of proficiency in performing the tasks and procedures described in approved project SOPs and Task Instructions. This training occurs when personnel are shown new tasks or procedures and whenever a task or procedure has been added or revised.

Level Three: This level of training includes instructions, either verbal or written, concerning more specific or project dependent tasks or procedures or modifications of a task or procedure.

This training is required for Environmental Testing Solutions' personnel, as applicable.

Qualification Criteria for Approved Trainers

Following are the minimum criteria required of an Approved Trainer.

1. Perform tasks described in the SOP for a minimum of one year.
2. Demonstrate proficiency in performing tasks to the satisfaction of the Laboratory Supervisor.
3. Proficiency is documented in internal surveillances, initial demonstration of competency (IDC) / ongoing demonstration of competency (ODC) testing and written tests (Exhibit Q2.2).
4. Develop and write a SOP for a new procedure.

Determination of Technical Proficiency

The Laboratory Supervisor or approved trainer will observe the trainee's performance of project tasks and activities described in project SOPs. Proficiency for each SOP will be documented in the training log (Exhibit Q2.1). An acceptable level of proficiency must be demonstrated before assignment to independent work.

Assessment of Technical Competence and Compliance to Procedures

Technical competence and compliance to written procedures will be assessed through internal surveillances, analysis of blind certified samples, IDC/ODC testing (as described in QAP-Q5), written tests, performance evaluations and/or practical tests.

Subject: Personnel Training and Qualification

Responsibilities

Laboratory Supervisor

- Prepares, reviews, and issues revisions to project-specific instructions, practices, and procedures.
- Conducts and documents training on revised project-specific instructions, practices, and procedures.
- Conducts quality assurance training and orientation.
- Advises project personnel of applicable company and client requirements, practices, and procedures.
- Determines the criteria used to designate an approved trainer.
- Approves and designates trainers.
- Ensures that newly hired personnel are adequately trained before assignment to independent work.
- Evaluates technical expertise and personnel compliance with documented procedures and instructions by periodic surveillance, reviews, inspections, or blind tests.
- Conducts Corporate Policy training.
- Conducts meetings to discuss procedural changes, SOP/QAP revisions and the implementation of new procedures.

Approved Trainer

- Plans and schedules performance-based training.
- Conducts training on SOPs for which they are approved.
- Observes the trainee on their ability to satisfactorily perform project tasks and procedures described in SOPs.
- Determines the proficiency of the trainee based on satisfactory completion of the tasks outlined in SOPs.
- Documents completion of training and proficiency in performing project-specific procedures in accordance with the instructions in the training log.
- Determines trainee performance in IDC/ODC tests and written tests.

Subject: Personnel Training and Qualification

Trainee

- Attends all required programs and project specific training.
- Reads and demonstrates an acceptable level of proficiency in performing the tasks and activities described in SOPs before assignment to independent work.
- Documents that they have read and been trained on applicable SOPs in accordance with the instructions in the training log.
- Performs IDC/ODC testing and written/practical tests.

Laboratory Technician

- Attend all required program and project specific training.
- Maintain proficiency in and knowledge of applicable project-specific instructions and procedures.
- Keeps up to date in their training as SOPs and policies are updated and revised.

Required Records

Records of project procedural training are quality records and are maintained in accordance with QAP-Q4. Copies of training documentation are placed in the training log and maintained by the Laboratory Supervisor.

Figure Q2.1: Sequence of Training
Level I

All New Hires
Pass Drug Screening
DMV Driving Records Obtained
Provided a Composition Notebook to Take Notes

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Read and Sign off: Corporate Policies

Procedure Number	Subject	Lab Tech	Courier
POLICY-P1	Employee Handbook	X	X
POLICY-P2	Standard of Conduct Policy	X	X
POLICY-P3	Ethics and Data Integrity Policy	X	X
POLICY-P4	Employee Handwriting Sample	X	X
POLICY-P5	New Hire Documentation	X	X
POLICY-P6	General Safety Policy	X	X
POLICY-P7	Safety Video Training Checklist	X	
POLICY-P8	Laboratory Orientation Checklist (Laboratory Walk-Through)	X	X
POLICY-P9	Radiation Protection Policy	X	
POLICY-P10	Exit Interview Documentation		
POLICY-P11	Rules Governing Certified Laboratories Training Checklist	X	

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Read and Sign off: Quality Assurance Plan

Procedure Number	Subject	Lab Tech	Courier
QAP-Q1	Program	X	
QAP-Q2	Personnel Training and Qualification	X	
QAP-Q3	Quality Improvement	X	
QAP-Q4	Documents and Records	X	
QAP-Q5	Work Processes	X	
QAP-Q6	Design	X	
QAP-Q7	Procurement	X	
QAP-Q8	Inspection and Acceptance Testing	X	
QAP-Q9	Management Assessment	X	
QAP-Q10	Independent Assessment	X	
QAP-Q11	Classification, Control and Use of Products and Materials	X	
QAP-Q12	Statistical Analyses and Data Review and Verification	X	

Level II

↓

Hands-on Training, Read and Sign off: Task Instructions

Procedure Number	Subject	Lab Tech	Courier
SOP-T1	Data Entry	X	X
SOP-T2	Rounding Rules	X	
SOP-T3	Washing Dishes	X	
SOP-T4	Analytical Balances	X	
SOP-T5	Serological Pipette Use	X	
SOP-T6	Volumetric Pipette Use	X	
SOP-T7	Digital Variable Volume Pipette Use	X	
SOP-T8	Repeater Pipette Use	X	

↓ **Lab Techs Only**

Shadowing other Employees and Specific SOP Training

↓

Read and Sign-off on SOP

↓

Pass IDC or Reference Toxicant Test for the Analysis

↓

Perform Analysis Independently

↓

Ongoing Demonstration of Competency: ODC, Complete Audit Checklists, Written Testing or Practical Testing

↓

Approved Trainer After Demonstrated Competency and Knowledge of Specific Analysis

Exhibits

Exhibit Q2.1: Example Training Log
Exhibit Q2.2: Example Written Test
Exhibit Q2.2: Example Practical Test

Subject: Personnel Training and Qualification

Exhibit Q2.1: Example Training Log

Environmental Testing Solutions, Inc.

QAP Procedures

SECTION	**QAP-Q2**
REVISION NUMBER	**4**
EFFECTIVE DATE	**02-17-20**
PAGE	**1 OF 1**

Subject: Personnel Training and Qualification

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		02-17-20
Quality Assurance Officer	Jim Sumner		02-17-20

Employee Training Documentation

The employee will print, sign and date the trainee section for the referenced procedure after (1) the applicable procedure has been read and understood and (2) after training has been received by an approved trainer, laboratory supervisor or quality assurance officer. Failure to adhere or comply with laboratory procedures may be grounds for immediate termination of employment.

Trainee By signing below, the trainee has Read, Understood, and Will Comply with the referenced procedure.			**Trainer**		
Printed name	**Signature**	**Date**	**Printed name**	**Signature**	**Date**

Trainer Approval by Laboratory Supervisor or Quality Assurance Officer

The employee will print, sign and date the trainer section for the referenced procedure after the laboratory supervisor or quality assurance officer has determined the employee is proficient and experienced in performing the referenced procedure (as indicated in QAP Q2) and is able to effectively explain and demonstrate all requirements of the referenced procedure.

Trainer By signing below, the trainer will uphold all requirements and expectations of the laboratory supervisor in training employees.			**Laboratory Supervisor or Quality Assurance Officer**		
Printed name	**Signature**	**Date**	**Printed name**	**Signature**	**Date**

QAP Q2–Revision 4 – Exhibit Q2.1

Subject: Personnel Training and Qualification

Exhibit Q2.2: Example Written Test

Environmental Testing Solutions, Inc.

Written Test

SECTION	**TEST-C3**
REVISION NUMBER	**7**
EFFECTIVE DATE	**02-17-20**
PAGE	**1 OF 2**

Subject: pH

Analyst:

Printed name	Signature	Date

Written test:

1. What is the method(s) #?
2. What is the method edition?
3. What is the reporting limit?
4. What are the units for pH?
5. What do these units stand for?
6. What are the sample interferences?
7. What is the sample holding time?
8. Are duplicates required for this parameter?
9. If duplicates are required, what is the frequency?
10. What is the sample volume?
11. Do you ever change the sample volume?
12. What is the matrix of the sample(s)?
13. Does this method(s) allow for different types of matrices?
14. If yes, what are they?
15. If no, why?
16. If a different matrix is allowed, how do you measure the pH of that sample?
17. If a different matrix is allowed, how do you prepare the sample for measurement?
18. What standards are used to calibrate the meter?
19. What is the frequency of calibration?
20. What standards are used to verify the calibration?
21. What is the expiration date of the standards?
22. Do you rinse the probe during calibration?
23. What is the rinse water?
24. Are samples stirred while being measured?

QAP Q2-Revision 4 – Exhibit Q2.2

Environmental Testing Solutions, Inc.

Written Test

SECTION	**TEST-C3**
REVISION NUMBER	**7**
EFFECTIVE DATE	**02-17-20**
PAGE	**2 OF 2**

Subject: pH

25. What is the required slope for the pH meter?
26. What is the LCS?
27. What is the acceptable range for the LCS?
28. Is there a temperature requirement for standards?
29. Is there a temperature requirement for samples?
30. How do you know if there is a problem with the probe?
31. How long does a pH probe usually last?
32. If you add filling solution to the pH probe, does this increase the probe life?
33. Does the pH probe have an automatic temperature compensator (ATC)?
34. If it does, what does that mean?
35. If it doesn't, what does that mean?
36. Are you required to record the date and time of the test?
37. Do you rinse between samples?
38. Are you required to verify the ATC?
39. Is the sample temperature required to be recorded?
40. What is the acceptable range for duplicates?
41. What is the acceptable range for SSW?
42. If the result is out of this range, what do you do?
43. What is the acceptable range for MHSW?
44. If the result is out of this range, what do you do?
45. What is the acceptable range for a toxicity effluent sample?
46. If the result is out of this range, what do you do?
47. What is the general trend for a minnow chronic test, in comparing the initial to the final pH result?
48. Why does this trend occur?
49. What is the general trend for a *Ceriodaphnia* chronic test, in comparing the initial to the final pH result?
50. Why does this trend occur?

Subject: Personnel Training and Qualification

Exhibit Q2.3: Example Practical Test

Environmental Testing Solutions, Inc.

Practical Test

SECTION	**TEST-AT18**
REVISION NUMBER	**6**
EFFECTIVE DATE	**03-01-20**
PAGE	**1 OF 2**

Subject: *Pimephales promelas* Acute Toxicity Test, EPA 2000.0

Analyst: _____

Reviewed performed by	Date	Reference Test # and/or Project #

Test Initiation:

Activity	Comments
Test/Sample preparation	
Glassware/plasticware prepared according to test type	
Number of replicates and test vessels correct based on test type	
Sample mixed thoroughly	
Aliquot of sample warmed to test temperature (25.0 ± 1.0°C)	
Sample diluted correctly and mixed	
Dilution water type correct and batch used recorded	
Volume prepared correct based on test type and number of replicates	
Volume of each replicate correct and measured	
Aliquot of each concentration/control poured off for chemical analyses, including full-strength sample	
Chemistry analyses demonstrated dilutions performed correctly and analyses met test acceptability criteria	
Test randomized and template used recorded	
Temperatures measured and recorded for each concentration/control and within acceptance limits (25.0 ± 1.0°C)	
Test organisms and initiation	
Larvae 1 to 14 days old	
Fed 2 to 5 hours prior to test initiation	
Temperature of larvae within acceptance limits (25.0 ± 1.0°C)	
Volume of transfer water poured off for pH analyses	
Larvae concentrated using mesh screen	
10 larvae loaded into each test cup, pipette tip cut to ensure larvae are not injured	
Larvae introduced into test solutions below water surface and allowed to swim from transfer pipette into solution to minimize dilution	
Unusual behavior of larvae recorded, if applicable	
Repeat count performed	
Visual inspection of larvae selected (health/appearance) acceptable	
Test placed into incubator and covered, location recorded	
Test initiated within 36-hours of sample collection	
All test initiation information recorded on benchsheet	

QAP Q2-Revision 4 – Exhibit Q2.3

Environmental Testing Solutions, Inc.

Practical Test

SECTION	TEST-AT18
REVISION NUMBER	6
EFFECTIVE DATE	03-01-20
PAGE	2 OF 2

Subject: *Pimephales promelas* Acute Toxicity Test, EPA 2000.0

24-hour Check:

Activity	Comments
Temperatures measured and recorded for each concentration/control and within acceptance limits (25.0 ± 1.0°C)	
Aliquot of each concentration/control poured off for chemical analyses	
Chemistry analyses met test acceptability criteria	
Number of living/dead organisms recorded at test initiation time ± 15 minutes	
Comments of larvae health recorded, if applicable	
Dead larvae removed and discarded	
Test placed into incubator and covered	
All 24-hour check information recorded on benchsheet	

Test Termination:

Activity	Comments
Temperatures measured and recorded for each concentration/control and within acceptance limits (25.0 ± 1.0°C)	
Aliquot of each concentration/control poured off for chemical analyses	
Chemistry analyses met test acceptability criteria	
Number of living/dead organisms recorded at test initiation time ± 15 minutes	
Comments of larvae health recorded, if applicable	
All 48-hour termination information recorded on benchsheet	
Test larvae discarded, test cups rinsed with tap water and recycled (if applicable)	

Test Acceptibilty:

Activity	Comments
Control survival ≥ 90%	
Reference toxicant test within control limits and performed within 7 days of compliance test (if applicable)	

Subject: Quality Improvement

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		07-01-21
Quality Assurance Officer	Jim Sumner		07-01-21

Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
12-01-00	0	Internal	Jim Sumner (ETS)	Original document
06-01-11	1	Internal	Jim Sumner (ETS)	• Corrected typographical errors.
06-20-12	2	External (TVA) Internal	William Rogers (TVA) Donald Snodgrass (TVA) Rick Sherrard (TVA) Jim Sumner (ETS)	• Provided clarification on unplanned and planned deviations and how they are documented.
09-28-16	3	External (TVA) Internal	Donald Snodgrass, Rick Sherrard (TVA) Jim Sumner (ETS)	• Provided clarification: "Annually refers to once within a calendar year. Semiannually refers to twice within a calendar year."
10-01-17	4	Internal	Jim Sumner (ETS)	• Updated procedure to NELAP format. • Light meter verification requirement changed.
07-01-21	5	Internal	Jim Sumner (ETS)	• Updated procedure and references to the approved analytical method identified in USEPA Method Update Rule, May 19, 2021.

Scope and Application

To address the requirements and policies for reporting deviations, non-conformances and occurrences, project surveillances and the internal assessment system, root cause analyses, and corrective action planning and implementation.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Subject: Quality Improvement

Definitions

Corrective Action: Measures taken to rectify and/or prevent recurrence of a quality failure.

Deviation: A departure from specified requirements.

Non-conformance: A deficiency in characteristic, documentation, or procedures that renders the quality of an item or activity unacceptable or indeterminate. A nonconforming item or service has the potential to affect other programs.

Non-conformance Report: A document that identifies the nonconforming evaluated item(s), records the non-conformance(s), and corrective action to preclude a recurrence.

Occurrence: Reportable problems, concerns, failures, malfunctions, or deficiencies in equipment, processes and procedures. Also, any conditions or events that have or could have adverse or negative impact on safety, environment, health, quality, security, or operations.

Occurrence Report: A written evaluation of an event or condition that is prepared in sufficient detail to enable the reader to assess its significance, consequences, or implications and to evaluate the actions being proposed or employed to correct the condition or to avoid recurrences.

Quality: The totality of features and characteristics of an item or service that bear on its ability to satisfy given needs.

Quality Failure: An item or a service for which the quality is inadequate or is indeterminate.

Root Cause: A cause that, if corrected, would prevent recurrence of this and similar problems. It is a fundamental reason that an occurrence, performance problem, adverse trend, or finding exists, as determined through investigation and evaluation.

Root Cause Analysis: Methods used to identify direct, contributory, and root causes of significant conditions adverse to quality; environment, safety, and health; programmatic objectives; property concerns; performance problems; reportable occurrences; or adverse trends.

Surveillance: The act of monitoring or observing to verify whether an item or activity conforms to specified requirements.

Subject: Quality Improvement

Unplanned Deviations

Non-conformance

Those items not conforming to specified requirements that are identified prior to their use, acceptance, or intended purpose. Non-conforming items may come from outside vendors and suppliers. Non-conforming items will be identified by tagging, marking, or other appropriate methods and segregated, when practical, to prevent continued use. Non-conforming items will be documented on applicable benchsheets by the person identifying the non-conformance (e.g. pipette volume verification logsheet, balance log, etc.)

A non-conforming item may include a mechanical pipette that was identified as exceeding volume expectance limits or a thermometer that has greater than a 1.0°C correction factor. These items must be segregated and not used until they are calibrated and documented to be within acceptance limits.

A non-conformance may include using an instrument which has exceeded the calibration due date (e.g balance, light meter, certified weights, thermometer, etc.). Non-conformances will be documented on applicable test benchsheets by the person identifying the non-conformance and non-conformances may be documented in the final report submitted to the client.

Occurrences

Occurrences include any reportable problems, concerns, failures, malfunctions, or deficiencies in equipment, processes, procedures, or programs. Personnel will report occurrences to the Laboratory Supervisor. Occurrences do not include routine or preventive maintenance items, personnel concerns, or non-conformance attributed to sources external to Environmental Testing Solutions.

An occurrence is any condition or event that could:

- Have an adverse effect on the environment.
- Endanger the health and safety of workers.
- Affect the operations and intended purpose of facilities, or result in loss or damage of property.
- Equipment failure that compromises data quality or invalidates testing in progress.

Planned Deviations

Planned deviations from sampling and analysis plans and standard operating procedures will be documented in technical notebooks, on chain-of-custody forms, or as otherwise required by clients. This may include the preparation and issuance of deviation request forms. Deviations from test methods will be documented on applicable test benchsheets by the person initiating the deviation and included in the final report submitted to the client.

Planned deviations may include analyzing a sample which has exceeded holding time, collected without the proper preservation or received without proper chain of custody. In this example, the client is contacted and the client provides guidance on if the sample is to be analyzed (if another sample cannot be secured). In some instances, ETS is required to notify the appropriate state agency of the deviation. As indicated above, deviations are documented on applicable test benchsheets by the person initiating the deviation and included in the final report submitted to the client.

Project Surveillance and Self-Assessment Program

Project personnel should take a proactive approach to identify, within the project, activities requiring improvement, modification, and/or additional training. Meetings with project personnel are held to solicit suggestions for quality improvements and to prevent quality failures.

Project surveillance activities should address, but are not limited to, assessment of technical competence and proficiency, compliance to approved procedures, verification of data and statistical computations, and effectiveness of internal quality control. Surveillance activities performed are identified below.

Subject: Quality Improvement

Note: Annually refers to once within a calendar year. Semiannually refers to twice within a calendar year.

General Laboratory Activities:

Description of Activity	Minimum Frequency	Acceptance of Activity
Thermometers (SOP-G12)		
Calibration of NIST thermometer by a certified calibration company.	Annually	NIST thermometer must be within tolerance limits as determined by calibration company.
Calibration of digital thermometers and laboratory thermometers used in incubators against the NIST thermometers.	Quarterly	Thermometers are tagged identifying any correction factors. Thermometers with correction factors ≥ 1.0°C are taken out of service.
Calibration of laboratory thermometers against the NIST thermometers.	Annually	Thermometers are tagged identifying any correction factors. Thermometers with correction factors ≥ 1.0°C are taken out of service.
Balance and weights (SOP-G10)		
Calibration of balances by a certified calibration company.	Annually	Balances must be within tolerance limits as determined by calibration company.
Verification of balance calibration.	Before each use using at least 2 certified weights, which bracket the weight of items to be measured.	Measurements must be within control limits (± 5% of true value).
	Weekly using 7 weights.	Measurements must be within control limits (± 5% of true value).
Calibration of class "I" weights by a certified calibration company.	Every 5 years	Weights must be within tolerance limits as determined by calibration company.
Volume verification (SOP-G11)		
Verification and/or calibration of mechanical pipettes.	Every 6 months	Pipette volumes must be within established limits as identified by manufacturer.
Verification of cylinder volumes.	Annually	Graduated cylinders, which are washed in a dishwasher, must be verified and within 5% of the true value.
Illumination (SOP-G14)		
Light meter verified by comparison to a second meter.	Each use	Readings must be within ± 10% of one another.
Luminosity is verified on each shelf of all toxicity culture/test incubators and in the algae culture area.	Quarterly	Luminosity <u>should</u> be 50 – 100 ft-c in toxicity culture and test incubators. Luminosity <u>should</u> be 360 – 440 ft-c in algae culture area.

General Laboratory Activities: **(continued)**

Description of Activity	Minimum Frequency	Acceptance of Activity
Detergent residue check (SOP-G1)		
0.04% Bromothymol Blue indicator used to check for soap residue on clean glassware and plasticware.	Each batch	No residue, indicator does not turn blue (indicating no alkaline residue, pH < 7.6)
Autoclave sterility (SOP-B1)		
Autoclave tape used to verify sterility	Each batch	Tape must turn color, indicating that the items have been sterilized.
Spore ampules used to verify sterility	Monthly	Spore ampule must not turn color, indicating that the autoclave is effectively sterilizing.
Timer verification	Quarterly	Sterilization cycle verified with a timer.
Time calibration (SOP-G16)		
Clocks used to record and document time throughout the laboratory calibrated to the atomic clock	Quarterly	Calibrated to the atomic clock.

Drinking Water Analyses:

Description of Activity	Minimum Frequency	Acceptance of Activity
Test performance through blind standards		
Single-blind QC check samples (QCS) are analyzed.	Once during 1st and 3rd quarters	Results must be within the performance acceptance limits specified for the type of test performed.
Colilert (SOP-B6, B10)		
Sample bottle sterility check.	Each lot	No contamination identified in 2% of containers.
Auto-fluorescence	Each lot	No fluorescence in any of the quality control samples.
Positive and negative control samples are used to determine acceptable performance of colilert media.	Each lot	Acceptable results in all positive and negative control samples.
E. coli **Quanti-Tray (SOP-B10)**		
Crystal violet stain is used to ensure that the quanti-trays are sealed properly by the sealer.	Monthly	No leaking occurs between wells.
Sterile water sterility check.	Each lot	No contamination identified.

Subject: Quality Improvement

Wastewater Analyses:

Description of Activity	Minimum Frequency	Acceptance of Activity
Test performance through blind standards		
Single-blind QC check samples (QCS) are analyzed.	Annually (before September each year)	Results must be within the performance acceptance limits specified for the type of test performed.
Fecal Coliform by Membrane Filtration and Tube Fermentation (SOP-B7, B8, B9)		
Additional QC requirements are provided in the test SOP.		
Sample bottle sterility check.	Each lot	No contamination identified in 2% of containers.
Dilution water sterility check.	Each batch	No contamination identified.
Filter inhibition of bacteria check.	Each lot	Comparison of 5 plates using new filters to 5 plates using old filters. Number of colonies must not be statistically significant ($\alpha = 0.05$) and/or differ by > 10%.
Media inhibition of bacteria check.	Each lot	Comparison of 5 plates using new media to 5 plates using old media. Number of colonies must not be statistically significant ($\alpha = 0.05$) and/or differ by > 10%.
Solids, Total Suspended and Totals Dissolved (SOP-C9, C10)		
Multiple weigh procedures are performed on each sample.	Each sample	Values must not differ by more than 0.5 mg.
Spectrophotometer (COD SOP-C19, Sulfate SOP-C20)		
Calibration curve using five points.	Annually (each new lot of standards)	Correlation coefficient must be between 0.995 to 1.0.
Salinity (SOP-C5)		
Automatic temperature compensation verification.	Annually (each new probe)	LCS must be within ± 10% of the true value at 2, 25 and 35°C.

Subject: Quality Improvement

Toxicity Testing:

Description of Activity	Minimum Frequency	Acceptance of Activity
Test performance through blind standards		
Single-blind QC check samples (QCS) are analyzed.	Annually	Test endpoints must be within the performance acceptance limits specified for the type of test performed.
Test performance through reference toxicants		
Reference toxicants (NaCl, KCl, and Cu) are used to assess the sensitivity of test organisms and credibility of test system.	At a minimum quarterly	Individual test endpoint must be within ± 2 SD of mean using 20 data points.
Taxonomy of test organisms		
Selenastrum capricornutum (SOP-AT2)	Each batch	Must be correct genus and species.
Ceriodaphnia dubia (SOP-AT15)	Quarterly	Must be correct genus and species.
Pimephales promelas (SOP-AT22)	Annually (individuals raised from embryo annually)	Must be correct genus and species.
Americamysis bahia (SOP-AT45)	Annually	Must be correct genus and species.
Menidia beryllina (SOP-AT51)	Annually (individuals raised from larvae annually)	Must be correct genus and species.
Cyprinodon variegatus (SOP-AT57)	Annually (individuals raised from larvae annually)	Must be correct genus and species.
Growth endpoint		
Multiple weigh procedures are performed on *Pimephales promelas*.	Annually	*Pimephales promelas* are used as a surrogate for all species were growth estimates are evaluated. Values must not be statistically significant (α = 0.05) and/or differ by > 5.0%.
Reproduction endpoint		
Multiple count procedures are performed on *Ceriodaphnia dubia* reproduction.	Annually	Values must not be statistically significant (α = 0.05) and/or differ by > 5.0%.
Test organism transfer volume		
Pimephales promelas	Annually	*Pimephales promelas* are used as a surrogate for all vertebrate/mysid species. 10 – 15 mL for 10 organisms transferred by medicine cup < 1.00 mL for 10 organisms transferred by pipette (allowed to swim from pipette)
Ceriodaphnia dubia	Annually	< 0.25 mL for 5 organisms transferred by pipette

Toxicity Testing: **(continued)**

Description of Activity	Minimum Frequency	Acceptance of Activity
Artemia **nauplii (SOP-AT16)**		
Samples are submitted to a certified laboratory for inorganic and organic analyses.	Each lot	Analyses must be comparable to previous *Artemia* lots.
Verification of the number of A*rtemia* nauplii fed to organisms in chronic toxicity tests.	Each lot	350 – 500 nauplii per 50 µL (1 drop)
Verification of nutritional quality.	Each lot	Side-by-side reference toxicant tests are performed with the new and old *Artemia* lots in *Pimephales promelas* chronic toxicity tests. Organism health and sensitivity using the new lot must be within established laboratory limits.
Consumables		
Medicine and Solo cups	Each lot	*Ceriodaphnia dubia* survival and reproduction or minnow survival and growth must not be significantly reduced ($\alpha = 0.05$).
Cubitainers	Each lot	*Ceriodaphnia dubia* survival and reproduction or minnow survival and growth must not be significantly reduced ($\alpha = 0.05$).
Yeast, Wheat Grass, Trout Chow (YWT) mixture (SOP-AT6)		
Samples are submitted to a certified laboratory for inorganic and organic analyses.	Each lot	Analyses must be comparable to previous YWT lots.
Verification of total solids.	Each batch	1.7 – 1.9 g/L
Verification of nutritional quality.	Each lot	Side-by-side reference toxicant tests are performed with the new and old YWT lots in *Ceriodaphnia dubia* chronic toxicity tests. Organism health and sensitivity using the new lot must be within established laboratory limits.
	Each batch	*Ceriodaphnia dubia* survival and reproduction is evaluated in cultures prior to being used in toxicity tests.
Selenastrum capricornutum **(SOP-AT2)**		
Verification of cell count.	Each batch	$3.0 – 3.5 \times 10^7$ cells/mL (tests and cultures) 1.71×10^7 cells/mL (North Carolina tests)
Verification that *Selenastrum* is not contaminated.	Each batch	No contamination identified.

Toxicity Testing: **(continued)**

Description of Activity	Minimum Frequency	Acceptance of Activity
Synthetic water (SOP-AT1)		
Moderately hard synthetic water (MHSW) submitted to a certified laboratory for inorganic and organic analyses.	Annually	Analyses must be below method specific limits (< 1.0 µg/L Al, As, Cr, Co, Cu, Fe, Pb, Ni, Zn; < 0.1 µg/L Cd, Hg, Ag; < 0.05 µg/L total organochloride pesticides plus PCBs) or current USEPA's National Recommended Water Quality Criteria, where available.
Salt synthetic water (Salt SW) submitted to a certified laboratory for inorganic analyses	Annually	Analyses must be below method specific limits (< 1.0 µg/L Al, As, Cr, Co, Cu, Fe, Pb, Ni, Zn; < 0.1 µg/L Cd, Hg, Ag) or current USEPA's National Recommended Water Quality Criteria, where available.

Root Cause Analysis

An integral part of quality improvement includes a root cause analysis that aids in the identification, analysis, correction, and prevention of recurrence of problems. Not all problems are of equal severity or significance; therefore, the effort to solve problems should be graded in proportion to their significance. The Laboratory Supervisor determines the risk or potential risk posed by the problem.

The root cause will be determined for audit and/or surveillance findings identified by external surveillances or audit activities, and for occurrences and non-conformance. For unsatisfactory observations identified during internal assessment activities, the root cause will be determined, if appropriate.

Subject: Quality Improvement

Corrective Action Planning and Implementation

Findings identified during external surveillances or audit activities may require the development and implementation of a correction action plan.

Unsatisfactory (negative) observations incurred from internal assessment activities will require resolution and/or corrective action to prevent recurrence. These actions may include, but are not limited to, the following.

- Quick-fix where the error or problem was corrected during or immediately following the assessment.
- Discuss the negative observation and the requirements or procedures deviated from with the person(s) responsible. This may involve a written warning or other disciplinary actions taken with the person(s) responsible.
- Discuss the negative observation and the requirements or procedures deviated from with the person(s) responsible and conduct a follow-up surveillance to ensure the problem or deficiency has been corrected.
- Conduct retraining and re-evaluation of technical proficiency.

Responsibilities

Laboratory Supervisor

- Notifies the Laboratory Director (or ensures notification) of occurrences and provides preliminary information on the event or condition.
- Initiates, as appropriate, actions to control the event or condition that resulted in an occurrence.
- Identifies project-specific surveillance activities in the QA plan and determines the frequency and acceptance criteria of the activity.
- Conducts project surveillances.
- Documents surveillances, observations, and corrective actions (as applicable) on a Surveillance and Corrective Action Report forms (Exhibit Q3.1).
- Determines the root cause of problems identified and documents the cause as applicable.
- Develops corrective action plans and implements quality-related corrective actions generated by surveillances, audits, recommendations of the QA Officer, independent assessments, occurrences and/or non-conformance reports.
- Evaluates the effect of non-conformances and notifies the Laboratory Director and QA Officer of non-conformances and their effects upon the work in the laboratory.
- Reviews project QA and technical procedures for appropriateness and adequacy every two years.

Subject: Quality Improvement

QA Officer

- Assists or provides input to the Laboratory Supervisor in developing surveillance and assessment activities for specific projects.
- Assumes responsibility for activities identified for the Laboratory Supervisor, as directed by the Laboratory Director.
- Notifies the Laboratory Director of any significant quality failures, accomplishments, and closure of corrective actions.

Laboratory Technician

- Conducts project surveillances, as requested.
- Document results and observations resulting from surveillance activities and takes corrective action, as necessary.
- Notifies the QA Officer or Laboratory Supervisor of quality deficiencies.
- Notifies the Laboratory Supervisor of occurrences and provides preliminary information on the event or condition.
- Initiates, as appropriate, actions to control the event or condition that resulted in an occurrence.

Required Records

Internal assessments and surveillances will be documented on Surveillance and Corrective Action Report (SCAR) forms and will be maintained in accordance with QAP-Q4 (Exhibit Q3.1). North Carolina (NC) wastewater compliance samples that do not meet acceptance criteria upon receipt must be documented on Sample Conditions upon Receipt (SCUR) forms and faxed to the regional NC Environmental Quality (NC DEQ) office (Exhibit Q3.2). SCUR forms will be maintained in the client file in accordance with QAP-Q4.

Records required for documenting the identification, validation, implementation, completion, verification, and closure of corrective actions resulting from external audits or surveillances will be maintained.

Exhibits

Exhibit Q3.1: Surveillance and Corrective Action Report (SCAR).
Exhibit Q3.2: Sample Conditions Upon Receipt (SCUR) Form.

Exhibit Q3.1: Surveillance and Corrective Action Report (SCAR).

Surveillance and Corrective Action Report (SCAR)

Date:		Surveillance performed by:	
Logbook:		Analysis performed by:	
		Date:	

Type of corrective action needed:

☐	Date missing	☐	Not calculated correctly
☐	Time missing	☐	LCS not calculated
☐	Sample identification missing	☐	Duplicate not calculated
☐	Data "write over"	☐	Blank missing
☐	Cross-out was not initialed	☐	QC out of range (footnote)
☐	All blank lines are not crossed through and initialed		
☐	Other: _____		

Correction(s) performed by:	
Date of correction(s):	

Explanation of corrective action: _____

_____

Corrective action taken to prevent reoccurrence: _____

_____

QAP Q3-Revision 4–Exhibit Q3.1

Exhibit Q3.2: Sample Conditions Upon Receipt (SCUR) Form.

Environmental Testing Solutions, Inc.

SAMPLE CONDITION UPON RECEIPT (SCUR)
DEVIATION

The sample(s) identified below deviated from required preservation, hold time, sampling protocol or sample documentation.

Attention: Mr. Jason Smith (NC DEQ, Laboratory Certification Branch)

Date: _____

Received from:

Name _____

Sample collector _____

Phone number _____

Deviation:

Sample date _____

Analysis _____

Deviation

☐ The sample(s) was not received on ice (0 to 6.0°C).

☐ The sample(s) was not received within holding time.

☐ The sample(s) was not collected and/or preserved correctly (e.g. head space in volatiles, improper container, or preservation/dechlorination) _____

_____

☐ The chain-of-custody did not have all the appropriate information (e.g. collector's name, date collected, time collected, sample identification, number of containers for each analysis).

☐ Other: _____

_____

Action taken:

☐ Sample(s) accepted and analyzed per client request.

☐ SCUR and COC e-mailed to NC DEQ, Asheville Regional Office – Laboratory Certification Branch.

North Carolina Certificate Number: Wastewater: 600

SOP Q3-Revision 4 – Exhibit Q3.2

Subject: Documents and Records

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		02-17-20
Quality Assurance Officer	Jim Sumner		02-17-20

Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
12-01-00	0	Internal	Jim Sumner (ETS)	Original document
09-01-09	1	External (TVA, Environmental Standard, Inc.) Internal	William Rogers (TVA) Cynthia Russell (TVA) Rick Sherrard (TVA) Rock Vitale (Environmental Standards, Inc.) Jim Sumner (ETS)	• Clarified documentation retention policy.
06-01-11	2	Internal	Jim Sumner (ETS)	• Added exhibits.
09-28-16	3	External (TVA) Internal	Rick Sherrard, Donald Snodgrass (TVA) Jim Sumner (ETS)	• Added: "Four external drives, which provide additional copies of the central server files, are rotated at least monthly. The rotation is documented on the External Server Backup Log (Exhibit Q4.3)." • Added: Exhibit Q4.3: External Backup Server Log.
10-01-17	4	Internal	Jim Sumner (ETS)	• Updated procedure to NELAP format. • Document Retention section revised to include current system of providing backups for data storage. External drives are no longer utilized and Exhibit Q4.3 was removed.
02-17-20	5	External (TVA) Internal	Rick Sherrard (TVA) Jim Sumner (ETS)	• Updated documentation retention policy. Records are maintained for a minimum of 5 years. Any records, which exceed this time period, may be destroyed and discarded at the discretion of the laboratory director (unless there are contractual commitments that state otherwise). • Clarified the length of time documents are stored in the laboratory safe.

Scope and Application

The purpose of this procedure is to identify controlled documents developed, issued, and maintained by the Environmental Testing Solutions. This procedure also identifies the requirements to ensure that documents are prepared, reviewed for adequacy, approved for release, and distributed to authorized personnel.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Subject: Documents and Records

Definitions

Controlled Documents: Any document for which distribution and status are to be kept current by the issuer in order to assure that authorized holders or users of the document have available the most up-to-date version for their actions.

Controlled Procedure: A procedure for which distribution is limited and regulated.

Document Control: The system of practices and procedures followed to ensure that documents which specify quality requirements or prescribe activities affecting quality are reviewed for adequacy, approved for release by authorized personnel, and distributed to and used at the location where the prescribed activity is performed.

Non-record Material: Informational material excluded from the definition of records. This includes material maintained solely for reference, extra copies of documents kept only for convenience of reference, stocks of publications, and of processed documents. Examples of reference type materials include books, periodicals, pamphlets, catalogs, reports, and similar bookcase-type material collected and maintained only for the convenience of reference. Also included are reading files, chronological files, and other extra copy files.

Quality Assurance (QA) Record: A completed document that furnishes evidence of the quality of items and/or activities affecting quality.

Record Copy: The official copy of a record retained for legal, operational, or historical purposes.

Records: Information created by or for Environmental Testing Solutions preserved or appropriate for preservation as evidence of the organization, functions, policies, decisions, procedures, operations, or other activities. Information may also be preserved or appropriate for preservation because of the information value of the data, regardless of physical form or characteristics. It includes, but is not limited to, all paper, film and electronic documents, reports, correspondence, notebooks, diaries, personal calendars, appointment books, telephone directories, notes, and memoranda used, generated or received by Environmental Testing Solutions' directors, employees, consultants, and subcontractors.

Subject: Documents and Records

Document Control

Document control is achieved by:

- identifying documents to be controlled and their specific distribution, and
- identifying personnel responsible for preparing, reviewing, approving, and issuing documents.

Controlled Documents

At a minimum, the following documents prepared by the Environmental Testing Solutions will be controlled:

- Corporate Policies
- Quality Assurance Plan
- Standard Operating Procedures
- Task Instructions
- Training Logs
- Project Plans
- Various Laboratory Logbooks, including Bench Sheets

Controlled documents will be designated by "*Confidential*". Copies of controlled documents not bearing "*Confidential*" are deemed uncontrolled or information only copies and do not necessarily represent the latest version. For laboratory logbooks, individual bench sheets reference the SOP, revision number and exhibit number.

Maintenance of Controlled Documents

Controlled documents will be maintained by the Laboratory Director. Copies for release or distribution are made available to employees.

Auditors and visitors that could obtain access to controlled documents must sign in and out of the laboratory using the Visitor Log (Exhibit Q4.1).

Record Control

Records that provide evidence of quality are identified in Table Q4.1. Quality records not included in this section are identified in other quality assurance procedures in this Quality Assurance Plan or in Standard Operating Procedures.

Records will be legible, accurate, and appropriately complete for the work accomplished.

Subject: Documents and Records

Records

Table Q4.1. Environmental Testing Solutions' quality records.

Type of Record	Location of Record Copy
Corporate Policies	Laboratory
Quality Assurance Plan	Laboratory
Standard Operating Procedures	Laboratory
Task Instructions	Laboratory
Project Plans	Project Files
Client Specifications	Laboratory Supervisor
Procedural Training Documentation	Laboratory Supervisor
Project Surveillance and Corrective Action Reports	QA Officer
Notebooks, Logbooks, and Data Collection Forms	Laboratory Safe (Fireproof) for < 2-years Attic for > 2-years
Chain-of-Custody Forms	Project Files
Maintenance and Calibration Records	Laboratory Safe (Fireproof) for < 2-years Attic for > 2-years

Note: While in use, the record copy will be maintained in the Laboratory. Completed notebooks, logbooks, and/or data collection forms (as appropriate) will be transferred to the Laboratory Safe for a minimum of 2-years (as space allows) and assigned identification numbers at that time for final storage (Exhibit Q4.2).

Document Retention

Records are maintained for a minimum of 5 years. Any records, which exceed this time period, may be destroyed and discarded at the discretion of the laboratory director (unless there are contractual commitments that state otherwise). Due to the type of testing performed, ETS would not be able to separate the original data from logbooks for a specific client (Toxicity testing logbooks are maintained for each type of test performed and contain multiple clients within the same logbook. In addition, multiple logbooks provide documentation associated with an individual test.). ETS maintains scanned copies of the individual bench sheets associated with each test performed for a client for a minimum of 5 years. Clients that request to obtain this scanned data must provide a written request to have this data submitted to them at cost, where they may maintain indefinite storage.

Client files and reports are stored on the ETS server. The monitoring and backups for these files are maintained by an outside data management company. Three layers of backup are utilized to provide the secure and safe storage of data.

1. All data from workstations are redirected to the ETS local server. This data has a local, file backup with versioning that is stored in the folder structure on ETS server itself. The data is secured behind the local password structure and is locked down with NTFS security.
   - This layer is retained from 1 to 10 days depending on performance of the server and serves for quick file recovery.
2. The ETS server backs up to a locally stored backup appliance with an image-based backup. This backup has the same security configured as the first layer and adds an additional layer of data encryption. These backups require an encryption key which is safely stored in the data management company's remote management system. This layer retains 21 days of backups, which includes at least 3 full backups spread 7 days apart.
3. Off-site backup is also performed, which includes a system-state backup, password structure backup and a file level backup. This data is processed on the ETS server and encrypted before being sent across the wire to the data management company's data center (located in Asheville, NC). Once the data is sent to the data center, the encrypted backup is replicated to a second data center (located in Atlanta, GA) for redundancy. This layer maintains the same backup retention as layer 2.

Responsibilities

Laboratory Supervisor

- Identifies documents to be controlled.
- Prepares and/or revises controlled documents.
- Reviews and approves controlled documents.
- Authorizes the release of controlled documents.
- Ensures records are maintained in accordance with described procedures and requirements.
- Ensures that completed notebooks, logbooks, and/or data collection forms (as appropriate) are transferred to laboratory safe for final storage and assigned identification numbers at that time.

QA Officer

- Serves as the distribution control point for the identification and distribution of controlled copies of quality assurance and standard operating procedures.
- Submits the confidential copy of appropriate controlled documents to the designated location.
- Removes and maintains electronic copies of superseded or voided documents.

Exhibits

Exhibit Q4.1: Visitor Log.
Exhibit Q4.2: Logbook Tracking and Archive.

Subject: Documents and Records

Exhibit Q4.1: Visitor Log.

Environmental Testing Solutions, Inc.

Page _____

Visitor Log

Date	Time in	Time out	Visitor printed name	Visitor signature	Reason for visit

QAP Q4-Revision 5–Exhibit Q4.1

Subject: Documents and Records

Exhibit Q4.2: Logbook Tracking and Archive.

Page _____

CHEMISTRY (C)
Logbook Tracking and Archive

Logbook tracking number	Test type	Test dates	Archived to safe		Moved to attic for long-term storage		Destroyed	
			Date	Initials	Date	Initials	Date	Initials
C-208								
C-209								
C-210								
C-211								
C-212								
C-213								
C-214								
C-215								
C-216								
C-217								
C-218								
C-219								
C-220								
C-221								
C-222								
C-223								
C-224								
C-225								
C-226								
C-227								
C-228								

QAP Q4-Revision 5–Exhibit Q4.2

Subject: Work Processes

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		07-01-21
Quality Assurance Officer	Jim Sumner		07-01-21

Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
12-01-00	0	Internal	Jim Sumner (ETS)	Original document
07-10-10	1	External (NC DENR) Internal	Lance Ferrell (NC DENR) Jim Sumner (ETS)	• Table Q5.1: preservation in the Daphnid, *Ceriodaphnia dubia*, Survival and Reproduction by North Carolina Modification changed to Ice, < 6°C.
08-01-11	2	External (NC DENR) Internal	Lance Ferrell (NC DENR) Jim Sumner (ETS)	• Table Q5.1: holding time for Daphnid, *Ceriodaphnia dubia*, Survival and Reproduction by North Carolina Modification changed to 36-hours. • Updated references to Table Q5.1.
04-01-13	3	Internal	Jim Sumner (ETS)	• Updated procedure and references to the approved analytical method identified in USEPA Method Update Rule II (MUR II), May 18, 2012. • Included requirements for initial demonstration of capability and ongoing demonstration of capability.
07-01-13	4	External (NC DENR) Internal	Lance Ferrell (NC DENR) Jim Sumner (ETS)	• Changed requirement for outlier results in reference toxicant tests to: "If an outlier occurs, two additional reference toxicant tests are performed as soon as possible." instead of within the same month.
09-28-16	5	External (TVA) Internal	Rick Sherrard, Donald Snodgrass (TVA) Jim Sumner (ETS)	• Corrected typographical errors.
10-01-17	6	Internal	Jim Sumner (ETS)	• Updated procedure to include NELAP requirements. • Additional guidance included in SOP. • Method number revised based on 2017 MUR. • MDL procedure updated. • WET control charts are determined using log transformed data. • Rounding data occurs after all calculations have been completed. • PE or QCS samples must be analyzed as any other sample received in the laboratory.
07-01-21	7	Internal	Jim Sumner (ETS)	• Updated procedure and references to the approved analytical method identified in USEPA Method Update Rule, May 19, 2021.

Subject: Work Processes

Scope and Application

The purpose of this procedure is to ensure that work processes affecting quality are planned, authorized, and performed under controlled conditions, and in accordance with written and approved procedures and instructions.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Definitions

Acceptance Criteria: Specified limits placed on characteristics of an item, process, or service defined in standards, procedures, or other required documents.

Accuracy: The degree of agreement of a measurement with an accepted reference or true value. Accuracy is a measure of the bias of the system; accuracy may be a measure of the bias in a single data point or the bias in a population of data points.

Calibration: The comparison of a measurement system or device of unverified accuracy to a measurement system or device of known accuracy to detect or correct any variation from requirement performance specifications of the unverified measurement system or device.

Calibration Data: Recorded measurement data that are used for quality and safety control, for development, analysis, or measurement system support, or for validation of product conformance to requirements.

Calibration Standard: A reference standard traceable to the National Institute of Standards and Technology (NIST), National Bureau of Standards (NBS), USEPA, or other approved institution.

Measuring and Test Equipment (MT&E): Devices or systems used to calibrate, measure, gage, test, inspect, or control in order to acquire research, development, test operational data for process control, safety and to determine compliance with design specifications or other technical requirements.

Precision: The degree of mutual agreement between independent measurements of the same property, made under similar conditions. Standard deviation, relative standard deviation, relative percent difference, minimum significant difference (or percent minimum significant difference) is used to express precision.

Qualification (personnel): The characteristics or abilities gained through education, training, or experience, as measured against established requirements, such as standards or tests, which qualify an individual to perform a required function.

Reference Standard: The highest level of calibration standard for a specific technology within an organization. Reference standards are typically calibrated by an outside agency.

Technical Logbook: A permanently bound logbook used to record all original data, calculations, notes, sketches, ideas, experimental endeavors, and the results thereof.

Traceability: The ability to trace the history, application, or location of an item and like items or activities by means of recorded identification.

Requirements

Data Entry and Recording of Technical Information

The requirements for recording technical information in notebooks, logbooks, and on data collection forms include:

- All entries are to be written legibly using an indelible black ink pen.
- Data entries must be traceable to the date and the person making the entry (refer to Policy P4: Employee Handwriting Sample).
- Entries should be made in chronological order.
- Entries must not be obliterated or backdated. Care must be taken not to obscure any information.
- Correction fluid and erasures are not permitted.
- A single line will be used to mark through errors.
- The person making the alteration or amplification will initial and date the new entry.
- Data forms will be completely filled in. Spaces left blank, where applicable, will be filled in with “not applicable” (NA) or with a line, initialed and dated.
- Data forms will be uniquely identified (e.g., consecutively numbered).

Permanently bound logbooks are used for recording original data, calculations, notes, comments, calibration results, chemical analyses, culture maintenance, toxicity test data and results, as well as, other information pertinent to functions performed within Environmental Testing Solutions.

Logbooks will be stored in the laboratory, while in use. Completed logbooks will be placed in the Laboratory Safe (fireproof) for 2 years then placed in the attic for storage for a minimum of 3 years.

Significant Figures

To avoid reporting results that are inaccurate or deceiving, "significant figures" are used. Significant figures give an indication of the reliability of the analytical method used. Reported values for drinking water and wastewater tests contain **2** significant figures. A value is made up of significant figures when it contains all digits known to be true and one last digit in doubt. For example, if a value is reported as 18.4 mg/L, the "18" must be a firm value while the "0.4" is somewhat uncertain and may be "0.3" or "0.5".

The number of significant figures for a method is established by testing replicate samples and determining variability. For example, if an effluent sample is tested 4 times for ammonia and the results are: 0.209, 0.207, 0.211, and 0.206, the average should be reported as 0.21 and not 0.208 since there is variability in the hundredth's digit.

Rounding

The rounding of numbers is a necessary part of analytical reporting. All instruments, glassware, and methods have limits on significant figures which must be taken into consideration when reporting results. Numbers that are not significant must be dropped by rounding off, after all calculations are made. If the digit 6, 7, 8, or 9 is dropped, round up one unit. If the digit 0, 1, 2, 3, or 4 is dropped, do not change the preceding digit. If the digit 5 is dropped, round to the even value. For example, 12.4 is rounded off to 12, 12.7 is rounded off to 13 and 12.5 is rounded to 12.

Internal Quality Control

The purpose of quality control (QC) is to reduce variability in executing procedures, in taking measurements, and in obtaining field and laboratory data. In addition, QC assesses whether activities are performed and/or measuring and test equipment (M&TE) and test systems being used to collect data are meeting established acceptance criteria and the project data quality requirements. QC samples may be used to (1) monitor sample collection and handling techniques, (2) evaluate equipment and container sterilization processes, (3) assess the sensitivity of test organisms and the credibility of the test system, (4) measure the precision of analytical methods, and (5) document equipment calibration. Internal QC may include control samples, split samples, duplicate samples, replicates, certified standards, and other certified reference materials.

Project data quality requirements include, but are not limited to:

- comparability (adherence to approved methods, performance evaluation studies)
- representativeness (meaningful sampling plan and collection, proper sample preservation, adherence to holding times, maintaining chain-of-custody, decontamination and cleanliness control)

- accuracy (performance evaluation samples, blanks and laboratory control samples, matrix spike recoveries, reference toxicant tests)
- precision (sample duplicates and replication, percent minimum significant differences and coefficient of variation)
- sensitivity (minimum detection limits, reference toxicant tests, percent minimum significant differences)
- completeness (percent of data that meets data quality objectives)

Comparability

Adherence to approved methods: Testing is performed in accordance with US Environmental Protection Agency (USEPA), Standard Methods for the Examination of Water and Wastewater (SM), and/or applicable state regulatory agency established guidelines and methods. Appropriate methods are referenced in applicable standard operating procedures.

Certified methods used for performing chemical and physical measurements of water samples are summarized in Table Q5.1.

Table Q5.1. Certified methods used for performing chemical and physical measurements of samples.

Drinking Water Testing:

Analysis	Method	Reference	Certifying Authority
Coliform, Total (P/A)	Colilert, SM 9223 B-2016	2017[a], SOP-B6	North Carolina Department of Health and Human Services
E. coli (P/A)	Colilert, SM 9223 B-2016	2017[a], SOP-B6	
E. coli Enumeration	Colilert by Quanti-Tray, SM 9223 B-2016	2017[a], 2000[b], SOP-B10	
Nitrate	SM 4500 NO_3^- D-2016	2017[a], SOP-C18	

[a]Standard Methods for the Examination of Water and Wastewater, 23rd Edition, 2017. American Public Health Association, 800 I Street, NW, Washington DC 20001-3710.

[b]IDEXX Quanti-Tray / 2000, 1 IDEXX Drive, Westbrook, ME, 04092.

Subject: Work Processes

Wastewater Testing:

Parameter	Methodology	Method Number	Reference
Temperature, °C	Thermometric	SM 2550 B-2010	2017[a], SOP-C1
Oxygen, Dissolved, mg/L	Electrode	SM 4500-O G-2016	2017[a], SOP-C2
Hydrogen Ion (pH), pH units	Electrometric measurement	SM 4500-H+ B-2011	2017[a], SOP-C3
pH, Liquid/Solid Waste	Electrometric measurement	SW846 9040C-2004 SW846 9045D-2004	On-line[b], SOP-C3
Specific conductance, µmhos/cm at 25°C	Wheatstone bridge	SM 2510 B-2011	2017[a], SOP-C4
Salinity	Wheatstone bridge	SM 2510 B-2011	2017[a], SOP-C5
Alkalinity, as $CaCO_3$, mg/L	Electrometric titration to pH 4.5	SM 2320 B-2011	2017[a], SOP-C6
Hardness, Total as $CaCO_3$	Titrametric (EDTA)	SM 2340 C-2011	2017[a], SOP-C7
Chlorine, Total residual, mg/L	Electrode	ORION 97-70-1977	2008, SOP-C8
Residue – non-filterable (TSS), mg/L	Gravimetric, 103-105°C post washing of residue	SM 2540 D-2015	2017[a], SOP-C9
Residue – filterable (TDS), mg/L	Gravimetric, 180°C	SM 2540 C-2015	2017[a], SOP-C10
Residue – volatile (TVS), mg/L	Gravimetric, 550°C	SM 2540 E-2015	2017[a], SOP-C11
Residue – settleable, mg/L	Volumetric, (Imhoff cone)	SM 2540 F-2015	2017[a], SOP-C12
Solids, Percent and Percent Moisture	180°C	SM 2540 G-2015	2017[a], SOP-C13
Biochemical Oxygen Demand (BOD_5), mg/L	Dissolved oxygen depletion	SM 5210 B-2016	2017[a], SOP-C14
Carbonaceous Biochemical Oxygen Demand (BOD_5), mg/L	Dissolved oxygen depletion with nitrification inhibitor	SM 5210 B-2016	2017[a], SOP-C14
Ammonia (as N), mg/L	Electrode	SM 4500 NH_3 D-2011	2017[a], SOP-C15
Chloride, mg/L	Titrimetric (mercuric nitrate)	SM 4500 Cl^- C-2011	2017[a], SOP-C16
Turbidity, NTU	Nephelometric	SM 2130 B-2011	2017[a], SOP-C17
Chemical Oxygen Demand (COD), mg/L	Spectrophotometric	HACH 8000-2003	2003[d], SOP-C19
Sulfate (as SO4), mg/L	Turbidimetric	SM 4500 SO_4^{2-} E-2011	2017[d], SOP-C20
Coliform (fecal), number per 100 mL	Membrane filter	SM 9222 D-2015	2017[a], SOP-B7
Coliform (fecal)	Most probable number (MPN)	SM 9221 E-2014	2017[a], SOP-B8

[a]Standard Methods for the Examination of Water and Wastewater, 23rd Edition, 2017. American Public Health Association, 800 I Street, NW, Washington DC 20001-3710.

[b]USEPA. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods (SW-846). On-line. US Environmental Protection Agency, Cincinnati, OH.

[c]Thermo Electron Corporation. 2008. Orion Residual Chlorine Electrode Instruction Manual, Orion 97-70. Thermo Electron Corporation, 166 Cummings Center Beverly, MA 01915.

[d]HACH Water Analysis Handbook 4th edition, revision 2. 2003. HACH Company, Loveland, CO 80539

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Toxicity Testing:

Analysis	Method	Reference	Certifying Authority
Daphnid, *Ceriodaphnia dubia*, Acute	EPA 2002.0	2002[b], SOP-AT9	North Carolina Department of Environmental Quality
Daphnid, *Ceriodaphnia dubia*, Survival and Reproduction	EPA 1002.0	2002[a], SOP-AT11	South Carolina Department of Health and Environmental Control
Daphnid, *Ceriodaphnia dubia*, Survival and Reproduction by North Carolina Modification	EPA 1002.0	2002[a], 1998[c], 1998[d], SOP-AT12, SOP-AT13	
Fathead Minnow, *Pimephales promelas*, Acute	EPA 2000.0	2002[b], SOP-AT18	Commonwealth of Kentucky Energy and Environmental Cabinet
Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth	EPA 1000.0	2002[a], SOP-AT20	
Mysid Shrimp, *Americamysis bahia*, Acute	EPA 2007.0	2002[b], SOP-AT41	
Mysid Shrimp, *Americamysis bahia*, Survival and Growth	EPA 1007.0	2002[e], SOP-AT43	
Inland Silverside, *Menidia beryllina*, Acute	EPA 2006.0	2002[b], SOP-AT47	
Inland Silverside, *Menidia beryllina*, Larval Survival and Growth	EPA 1006.0	2002[e], SOP-AT49	
Sheepshead Minnow, *Cyprinodon variegatus*, Acute	EPA 2004.0	2002[b], SOP-AT53	
Sheepshead Minnow, *Cyprinodon variegatus*, Larval Survival and Growth	EPA 1004.0	2002[e], SOP-AT55	

[a]USEPA. October 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th ed. EPA-821-R-02-013. US Environmental Protection Agency, Cincinnati, OH.

[b]USEPA. October 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed. EPA-821-R-02-012. US Environmental Protection Agency, Cincinnati, OH.

[c]North Carolina Department of Environmental Quality, Water Sciences. Biological Laboratory Certification / Criteria Procedures, Version 3.0. December 2010.

[d]North Carolina Department of Environmental Quality, Water Sciences. North Carolina Phase II Chronic Whole Effluent Toxicity Test Procedure, Version 3.0. December 2010.

[e]USEPA. October 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, 3rd ed. EPA-821-R-02-014. US Environmental Protection Agency, Cincinnati, OH.

Performance Evaluation (PE) or Single-blind QC check samples (QCS): Performance evaluation or single-blind QC check samples are performed to determine the accuracy of each type of analysis performed by the laboratory. These samples are provided by an approved proficiency testing (PT) provider and are analyzed in the same manner as any other sample received in the laboratory. The frequency of these PE samples is outlined in QAP-Q3.

Representativeness

Sample collection, proper sample preservation and adherence to holding times: Samples are collected in accordance with SOP-G3 (samples are currently not collected by Environmental Testing Solutions personnel) and processed in accordance with SOP-G4 and/or the specific test SOP.

Sample requirements for chemical and physical measurements of water samples are summarized in Table Q5.1.

Table Q5.1. Sample requirements for chemical and physical measurements of water samples.

Drinking Water Testing:

Analysis	Matrix	Container Type	Minimum Sample Volume	Preservation	Maximum Holding Time
Coliform, Total (P/A)	Water	Sterile Plastic	125 mL	Na_2SO_4	30 hours
E. coli (P/A)	Water	Sterile Plastic	125 mL	Na_2SO_4	30 hours
E. coli Enumeration	Water	Sterile Plastic	250 mL	Na_2SO_4, Ice, < 10°C	30 hours
Nitrate	Water	Plastic	250 mL	Ice, < 6.0°C	48 hours

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Wastewater Testing:

Analysis	Matrix	Container Type	Minimum Sample Volume	Preservation	Maximum Holding Time
Temperature	Water	Polyethylene	250 mL	None required	Immediately
Oxygen, dissolved	Water	Polyethylene	250 mL	None required	Analyze within 15 minutes
Hydrogen ion (pH)	Water	Polyethylene	250 mL	None required	Analyze within 15 minutes
pH, Solid	Solid	Polyethylene	25 g	None required	Analyze within 15 minutes
Specific Conductance	Water	Polyethylene	250 mL	Cool, ≤ 6°C	28 days
Salinity	Water	Polyethylene	250 mL	Cool, ≤ 6°C	28 days
Alkalinity	Water	Polyethylene	250 mL	Cool, ≤ 6°C	14 days
Hardness	Water	Polyethylene	250 mL	HNO_3 to pH < 2	6 months
Chlorine, total residual	Water	Polyethylene	250 mL	None required	Analyze within 15 minutes
Residue, nonfilterable (TSS)	Water	Polyethylene	1000 mL	Cool, ≤ 6°C	7 days
Residue, filterable (TDS)	Water	Polyethylene	1000 mL	Cool, ≤ 6°C	7 days
Residue, volatile (TVS)	Water	Polyethylene	1000 mL	Cool, ≤ 6°C	7 days
Residue, settleable	Water	Polyethylene	1000 mL	Cool, ≤ 6°C	48 hours
Solids, Percent and Percent Moisture	Solid	Polyethylene	25 g	Cool, ≤ 6°C	7 days
Biochemical oxygen demand	Water	Polyethylene	1000 mL	Cool, ≤ 6°C	48 hours
Biochemical oxygen demand, carbonaceous	Water	Polyethylene	1000 mL	Cool, ≤ 6°C	48 hours
Ammonia	Water	Polyethylene	250 mL	Cool, ≤ 6°C H_2SO_4 to pH < 2	28 days
Chloride	Water	Polyethylene	250 mL	None required	28 days
Turbidity	Water	Polyethylene	250 mL	Cool, ≤ 6°C	48 hours
Chemical Oxygen Demand	Water	Polyethylene	250 mL	Cool, ≤ 6°C H_2SO_4 to pH < 2	28 days
Sulfate	Water	Polyethylene	250 mL	Cool, ≤ 6°C	28 days
Fecal Coliform by Membrane Filtration	Water	Sterile Plastic	250 mL	0.008% $Na_2S_2O_3$ Cool, < 10°C	8 hours
Fecal Coliform by Multiple Tube Fermentation	Water Solid	Sterile Plastic Sterile Plastic	250 mL 100 g	$Na_2S_2O_3$ Cool, < 10°C Cool, < 10°C	8 hours 24 hours
Total Coliform by Multiple Tube Fermentation	Water	Sterile Plastic	250 mL	0.008% $Na_2S_2O_3$ Cool, < 10°C	8 hours

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Toxicity Testing:

Analysis (Matrix = Water)	**Number of Samples Required**	**Container Type**	***Minimum Sample Volume for Each Collection**	**Preservation**	**Maximum Holding Time**
Daphnid, *Ceriodaphnia dubia*, Acute	1	Plastic	1 liter	Ice, < 6ºC	36 hours
Daphnid, *Ceriodaphnia dubia*, Survival and Reproduction	3	Plastic	1 gallon	Ice, < 6ºC	36 hours
Daphnid, *Ceriodaphnia dubia*, Survival and Reproduction by North Carolina Modification	2	Plastic	1 liter (Pass/Fail) 1 gallon (Phase II)	Ice, < 6ºC	36 hours
Fathead Minnow, *Pimephales promelas*, Acute	1	Plastic	1 gallon	Ice, < 6ºC	36 hours
Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth	3	Plastic	2 gallons (sample 1, 2) 3 gallons (sample 3)	Ice, < 6ºC	36 hours
Mysid Shrimp, *Americamysis bahia*, Acute	1	Plastic	1 gallon	Ice, < 6ºC	36 hours
Mysid Shrimp, *Americamysis bahia*, Survival and Growth	3	Plastic	2 gallons	Ice, < 6ºC	36 hours
Inland Silverside, *Menidia beryllina*, Acute	1	Plastic	1 gallon	Ice, < 6ºC	36 hours
Inland Silverside, *Menidia beryllina*, Larval Survival and Growth	3	Plastic	2 gallons (sample 1, 2) 3 gallons (sample 3)	Ice, < 6ºC	36 hours
Sheepshead Minnow, *Cyprinodon variegatus*, Acute	1	Plastic	1 gallon	Ice, < 6ºC	36 hours
Sheepshead Minnow, *Cyprinodon variegatus*, Larval Survival and Growth	3	Plastic	2 gallons (sample 1, 2) 3 gallons (sample 3)	Ice, < 6ºC	36 hours

*Sample volumes are approximate and will vary dependent on permit and study requirements.

Maintaining chain-of-custody: A sample is considered to be under a person's custody if any of the following conditions are met:

1. The sample is in the person's physical possession.
2. The sample is in line of sight of the person after they have taken possession.
3. The sample is secured by that person so that any tampering can be detected through a custody seal.
4. A sample is secured by the person in possession, in an area which only authorized personnel can enter.

The custody of samples must be traceable from the time they are collected until final disposition, including archival where required. Samples will be identified so that the location where they were obtained, the date and time they were obtained, and the person(s) who obtained them can be traced.

Sample custody will be documented throughout sample collection, shipping, and analysis. SOP-G4 provides instructions for documenting sample custody and for completing chain-of-custody forms.

Decontamination and cleanliness control: All sample containers and collection devises are to be triple rinsed with site water before collecting water samples (per SOP-G3). Bottles used to collect samples are not reused and are discarded after use. All laboratory glassware and plasticware that is reused is cleaned in accordance with SOP-G1. Consumables (i.e. medicine cups, Solo cups and cubitainers) used in toxicity tests are evaluated using the applicable test species.

Accuracy

Method Blanks (MB): Method blanks monitor the purity of reagent water and the cleanliness of glassware and equipment. Blanks may also be analyzed after samples of high concentration that could cause carryover to the following sample. Blanks are performed with each batch of samples analyzed. Blanks must be less than half the reporting limit of the analysis being performed.

Laboratory control standards (LCS) or laboratory fortified blanks (LFB): Laboratory control standards (LCSs) are the equivalent of laboratory fortified blanks (LFBs) at ETS. LCSs are reagent water samples to which a known concentration of the analyte of interest has been added. LCSs are used to demonstrate that calibration curves are accurate, that the analysis is being properly run, that calibration standards are accurate, and to verify the results of analyses on client samples. LCSs are analyzed at a minimum every 20 samples. The accuracy of the LCS is calculated and reported in terms of the reference standard recovery (%RS). The %RS must be between 90 and 110%.

%RS is defined as follows:

$$\%RS = \frac{\text{Value Obtained}}{\text{True Value}} \times 100\%$$

Matrix spike recovery: Analyzing matrix spike samples determines the ability of a method or technique to recover a known quantity of the analyte in a typical sample which may contain impurities. If interferences are present in the sample, results may be obtained which are significantly higher or lower than the actual concentration. Matrix spike samples are randomly selected based on available sample volume or according to client/project instructions. Matrix spike samples are analyzed at a minimum every 20 samples. The accuracy of matrix spike analyses is calculated and reported in terms of the percent recovery (%R).

%R on a spiked sample is defined as follows:

$$\%R = \frac{\text{Concentration of Spiked Sample} - \text{Concentration of Unspiked Sample}}{\text{Concentration of Spike Added to Sample}} \times 100\%$$

For methods, where it is impossible to spike a sample for that particular parameter, known samples are analyzed to check the analyst's ability to produce a similar result to what is expected.

Initial Demonstration of Capability (IDC): Each new analyst must conduct an IDC before analyzing any sample to demonstrate proficiency in performing the method and obtaining acceptable results for each analyte. The IDC also demonstrates that laboratory modifications to a method will produce results as precise and accurate as those produced by the reference method.

IDC is performed as follows:

Follow all SOP requirements for the specific analyte, including calibration curve, blanks, LCS and/or matrix spikes. Perform 4 laboratory control standards (LCSs) at a concentration between 10 times the MDL and the midpoint of the calibration curve. This LCS will be prepared by the laboratory supervisor and the true value will not be known to the analyst. The laboratory supervisor will determine if the IDC is within the acceptance criteria identified below.

The accuracy of each LCS is calculated and reported in terms of the reference standard recovery (%RS). The %RS must be between 90 and 110% for each value.

%RS is defined as follows:

$$\%RS = \frac{\text{Value Obtained}}{\text{True Value}} \times 100\%$$

The precision of the LCSs is calculated and reported in terms of the relative standard deviation (%RSD). The %RSD must be ≤ 15%.

%RSD is defined as follows:

$$\%RSD = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100\%$$

Ongoing Demonstration of Capability (ODC): Each analyst must conduct an ODC yearly to demonstrate continued proficiency in performing the method and obtaining acceptable results for each analyte. The ODC also demonstrates that laboratory modifications to a method will produce results as precise and accurate as those produced by the reference method. The ODC is performed as indicated above for the IDC.

Reference toxicants for toxicity tests: Reference toxicant tests serve to: (1) determine the sensitivity of the test organisms over time; and (2) assess the comparability of within- and between-laboratory test results. Reference toxicant test results are used to identify potential sources of variability, such as test organism health, differences among batches of organisms, changes in laboratory water or food quality, and performance by laboratory analysts.

Potassium chloride, sodium chloride and copper (as copper sulfate) are reference toxicants selected by Environmental Testing Solutions based on the following:

1. Toxicant provides precise and reliable measures of toxicological sensitivity.
2. Toxicant disposal is not legally or environmentally problematic.
3. Toxicant produces a concentration-response effect for the test organism.
4. Toxicant is quantifiable.
5. Toxicant does not pose an unacceptable health hazard to laboratory personnel.
6. Toxicant is readily available.
7. Toxicant has extensive literature information on toxicological sensitivity to test organisms.
8. Toxicant is available in a pure form and soluble.
9. Toxicant is stable in solution, during storage, and has a stable toxicity with normal changes in synthetic water quality.

Control charts are used to monitor the ongoing sensitivity, precision and accuracy of toxicity tests performed in the laboratory. Charts are prepared for each combination of reference toxicant, test species, test condition, and endpoint. For establishing control charts, toxicity endpoints from at least five tests must be used. A running plot is maintained for the toxicity values from successive tests with a given reference toxicant, and endpoints (LC_{50}'s or IC_{25}'s) are examined to determine if they are within prescribed limits. Control charts are used to evaluate the cumulative trend of results from a series of tests and therefore should not be used as the only criterion for rejection of individual effluent or receiving water tests. The mean and upper and lower control limits (± 2 standard deviations, 95% confidence intervals) are re-calculated with each successive test result using log-transformed data. For consistency, data plotted in control charts are converted to g/L toxicant using the antilog. After two years of data collection, or a minimum of 20 data points, the control chart is maintained using only the 20 most recent data points.

In addition to the standard deviation, the calculated CV (i.e., standard deviation / mean) of the LC_{50} or IC_{25} values for the 20 most recent data points is also compared to the distribution of laboratory CVs

reported nationally for reference toxicant testing. If the calculated CV for our laboratory exceeds the 75th percentile of CVs reported nationally, the 75th and 90th percentiles are used to calculate warning and control limits, respectively, and options for reducing variability are investigated. If the CV for the set of LC_{50} or IC_{25} values is less than the 90th percentile reported nationally, the 90th percentile CV is used to set control limits. If the CV for the set of LC_{50} or IC_{25} values is less than the 75th percentile, warning limits are not set using the 75th percentile. In this instance, 10th and 25th percentile CVs reported nationally are used to set warning and control limits.

Outliers, values falling outside the upper and lower control limits, and trends of increasing or decreasing sensitivity, are readily identified in control charts. At the $P_{0.05}$ probability level (± 2 standard deviations), one in 20 tests is expected to fall outside of the control limits by chance alone. If more than one out of 20 reference toxicant tests fall outside the control limits, sources of variability are investigated and corrective actions are taken to reduce identified sources of variability.

If an outlier occurs, two additional reference toxicant tests are performed as soon as possible. In instances when the cause for the outlier can be documented (e.g., analyst error, test system failure or invalid due to test acceptability requirements), the outlier test is invalidated and excluded from future calculations of control limits.

If two or more consecutive tests do not fall within the control limits, the results must be explained and additional reference toxicant tests must be performed. Actions taken to correct the problem must be reported. In this situation, compliance testing may be invalidated.

Control limits of ± 2 standard deviations, by definition, will be exceeded 5% of the time, regardless of how well a laboratory performs. Highly proficient laboratories which develop a very narrow control limit may be unfairly penalized if a test which falls just outside the control limits is rejected. For this reason, the width of the control limits are considered in determining whether or not a reference toxicant test result falls “well” outside the expected range. The width of the control limits is evaluated by comparing the calculated CV (i.e., standard deviation / mean) of the LC_{50} or IC_{25} values for the 20 most recent data points to the distribution of laboratory CVs reported nationally for reference toxicant testing. In determining whether or not a reference toxicant test result falls “well” outside the expected range, the result is also compared with upper and lower bounds for ± 3 standard deviations, as any result outside these control limits would be expected to occur by chance only 1 out of 100 tests. When a result from a reference toxicant test is outside the 99% confidence intervals, an immediate investigation to assess the possible causes for the outlier is conducted.

Reference toxicant test results should not be used as the only criterion for the rejection of individual effluent or receiving water tests. Reference toxicant testing is used for evaluating the health and sensitivity of organisms over time and for documenting initial and ongoing laboratory performance. While reference toxicant test results should not be used as the only criterion for test rejection, effluent and receiving water test results are reviewed and interpreted in the light of reference toxicant test

results. The degree to which the reference toxicant test result fell outside of control chart limits, the width of the limits, the direction of the deviation (toward increased test organism sensitivity or toward decreased test organism sensitivity), the test conditions of both the effluent test and the reference toxicant test, and the objective of the test are reviewed.

Precision:

Sample duplicates for drinking water and wastewater tests: Analyzing duplicate samples determines the reproducibility of a method or technique. Duplicate samples are randomly selected based on available sample volume or according to client/project requirements. Duplicate samples for drinking water and wastewater tests are analyzed at a minimum every 20 samples. The precision of duplicate analyses is calculated and reported in terms of the relative percent difference (RPD) of the two values.

The RPD is calculated as follows:

$$RPD = \frac{\text{Absolute Value of the Difference of the Two Results}}{\text{Average of Two Results}} \times 100\%$$

or:

$$RPD = \frac{|A - B|}{[(A + B)/2]} \times 100\%$$

where: A = sample result
B = duplicate result

Control charts are used to monitor the ongoing duplicate precision in the laboratory. Duplicate analyses are collected yearly for each technician performing a given analysis in order to determine acceptance criteria. Acceptance criteria is based on control charts using three standard deviations (99% confidence intervals) from the mean of a minimum of 10 data points with a maximum of 30 data points. Charts are constructed for low, mid, and high range values for each analysis and limits are expressed as a percentage or in terms of the units of the analysis. Literature or method guidance values are used for infrequently used or newly developed methods until sufficient data is available.

Replication in toxicity tests: The sensitivity of toxicity tests will depend in part on the number of replicates per concentration, the significance level selected, and the type of statistical analysis. If the variability remains constant, the sensitivity of the test will increase as the number of replicates is increased. The minimum recommended number of replicates varies with the objectives of the test and the statistical method used for analysis of the data.

The precision of each test method is monitored through the percent minimum significant difference (as discussed below). If test sensitivity bounds determined by the USEPA cannot be achieved, it may be necessary to increase the number of replicates tested.

Percent minimum significant difference and coefficient of variation in toxicity tests: Test precision is based on control coefficient of variation or CV and the "Percent Minimum Significant Difference" or PMSD. These measures of test precision quantify within-test variability, or the sensitivity of each test to detect toxic effects on the biological endpoint. In addition, CV indicates variability among replicates under non-toxic conditions and is an indicator of uniformity of the test organisms

CV is calculated as follows:

$$CV = \frac{\text{Control Standard Deviation}}{\text{Control Mean}}$$

PMSD is calculated as follows:

$$PMSD = \frac{(100 \times MSD)}{\text{Control Mean}}$$

where: MSD = "minimum significant difference"

$$MSD = \frac{(d / EMS)}{(2/r)}$$

where: d = critical value of Dunnett's statistic when comparing "k" treatments to a control
EMS = error mean square from the analysis of variance of the endpoint responses
r = the number of replicates at each concentration.

PMSD is interpreted as the minimum percent difference between control and treatment that can be declared statistically significant in a toxicity test. A significant effect occurs when (control mean - treatment mean) exceeds the MSD. Dividing by the control mean and multiplying by 100 states this relationship in terms of the percent difference between control and treatment. USEPA characterized the distribution of values of PMSD and control CV, where values from all laboratories and toxicants for a given method and endpoint were combined and sample percentiles were reported. The 90^{th} percentiles reported by UPEPA are used as the upper control limits (or upper PMSD bounds or limits on the insensitivity of a test). The 10^{th} percentile may be used as a lower PMSD bound for declaring a significant difference or a lower limit to test sensitivity. A lower bound objectively specifies a limit to

the test sensitivity achieved in practice. If no more than ten percent of tests are more precise than this lower bound, then in practice, the analytical method rarely detects toxic effects of this small magnitude.

USEPA selected the PMSD to characterize endpoint variability for toxicity test methods because it integrates variability from several concentrations (always including the control), and it represents the MSD used in the hypothesis test. The control CV, by itself, does not fully represent the variability affecting a hypothesis test or point estimate. The PMSD also represents the variability affecting point estimates because it is calculated using the EMS for the endpoint measurement.

Percent minimum significant difference is used to assess the overall precision in the toxicity tests performed in the laboratory. Reference toxicant tests are used to characterize method variability because, in contrast to effluent samples, fixed concentrations of known toxicants are used. Only with this standardization is it possible to conclude that variability of the effect concentration estimates is derived from the sources discussed above, rather than from changes in the toxicant. These reference toxicant tests must be conducted using the same test conditions (type of dilution water, temperature, test protocol, and species) that are used for toxicity tests conducted by the laboratory. Control charts are used to monitor the ongoing precision through PMSDs at Environmental Testing Solutions. Acceptance criteria are based on control charts using two standard deviations (95% confidence intervals) from the mean of 20 PMSD data points. Upper and lower PMSD bounds established by the USEPA are impractical for use by the laboratory due to the large range between the PMSD bounds.

Environmental Testing Solutions' internal precision and accuracy data requirements for each toxicity method are summarized in Table Q5.2.

Table Q5.2: Internal precision and accuracy data requirements for each toxicity test method.

Toxicity Testing:

Analysis	Control Average (USEPA Guidance Criteria)			Average PMSD %
	Survival %	Reproduction (offspring/female) or Growth (mg/initial organisms)	CV %	
Daphnid, *Ceriodaphnia dubia*, Acute	**100** (≥90)			
Daphnid, *Ceriodaphnia dubia*, Survival and Reproduction	**100** (≥80)	**30.2** (≥15)	**4.9** (<42)	**6.0** (<47)
Fathead Minnow, *Pimephales promelas*, Acute	**100** (≥90)			
Fathead Minnow, *Pimephales promelas*, Larval Survival and Growth	**100** (≥80)	**0.637** (≥0.25)	**6.0** (<20)	**11.0** (<30)
Mysid Shrimp, *Americamysis bahia*, Acute	**100** (≥90)			
Mysid Shrimp, *Americamysis bahia*, Survival and Growth	**100** (≥80)	**0.261** (≥0.20)	**11.6** (<28)	**13.1** (<37)
Inland Silverside, *Menidia beryllina*, Acute	**100** (≥90)			
Inland Silverside, *Menidia beryllina*, Larval Survival and Growth	**100** (≥80)	**1.813** (≥0.50)	**8.5** (<18)	**16.7** (<28)

Note: Data based on reference toxicant test control charts. CV = coefficient of variation of control reproduction or growth, PMSD = percent minimum significant difference.

Sensitivity:

Method detection limit and reporting limit for drinking water and wastewater tests: The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that is distinguishable from the method blank results.

The MDL procedure (described below) is not applicable to methods that do not produce results with a continuous distribution, such as methods for whole effluent toxicity, presence/absence methods and microbiological methods that involve counting colonies. The MDL procedure also is not applicable to measurements such as biochemical oxygen demand, pH, specific conductance, titration methods and any method where low-level spiked samples cannot be prepared. Spiked samples are prepared from a clean reference matrix, such as deionized water, spiked with a known and consistent quantity of the analyte. MDL determinations using spiked samples may not be appropriate for all gravimetric methods

(e.g., residue or total suspended solids), but an MDL based on method blanks can be determined in such instances.

The MDL is determined as follows:

- Select a spiking level, typically 2 – 10 times the estimated MDL.
- Process a minimum of seven spiked samples and seven method blank samples through all steps of the method. The samples used for the MDL must be prepared in at least three batches on three separate calendar dates and analyzed on three separate calendar dates. The most recent available data for method blanks and spiked samples must be used. Statistical outlier removal procedures should not be used to remove data for the initial MDL determination, since the total number of observations is small and the purpose of the MDL procedure is to capture routine method variability. However, documented instances of gross failures (e.g., instrument malfunctions, mislabeled samples, cracked vials) may be excluded from the calculations, provided that at least seven spiked samples and seven method blanks are available. The rationale for removal of specific outliers must be documented and maintained on file with the results of the MDL determination.
- Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.
- Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.
- The MDL is the higher of the MDL_s (method detection limit calculated from spike samples) and MDL_b (method detection limit calculated from method blanks). As detection sensitivity improves, background contamination in the laboratory, consumable supplies and equipment can be more important in determining the detection limit than the sensitivity of the instrument.

  Calculate the MDL_s as follows:

$$MDL_s = T_{(n-1,\ 1-\alpha = 0.99)} \times S_s$$

  Where:
  S_s = sample standard deviation of the replicate spiked sample analyses.
  $T_{(n-1,\ 1-\alpha = 0.99)}$ = student's t-value appropriate for a single-tailed 99% percentile t statistic and a standard deviation estimate with n-1 degrees of freedom .

Calculate the MDL_b as follows:

1. If none of the method blanks provide numerical results for an individual analyte, the MDL_b does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of ND (not detected).

2. If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set MDL_b to the level that is no less than the 99th percentile of the method blank results. For "n" method blanks where n ≥ 100, sort the method blanks in rank order. The (n * 0.99) ranked method blank result (round to the nearest whole number) is the MDL_b.

   For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are: 1.5, 1.7, 1.9, 5.0, and 10, then 164 x 0.99 = 162.36 which rounds to the 162nd method blank result. Therefore, MDL_b is 1.9 for n =164 (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result).

3. If all of the method blanks for an individual analyte give numerical results, then the MDL_b is calculated as follows:

   $$MDL_b = Mean + T_{(n-1,\ 1-\alpha = 0.99)} \times S_b$$

   Where:
   Mean = mean of the method blank results (use zero in place of the mean of the mean is negative.
   S_s = sample standard deviation of the replicate method blank sample analyses
   $T_{(n-1,\ 1-\alpha = 0.99)}$ = student's t-value appropriate for a single-tailed 99% percentile t statistic and a standard deviation estimate with n-1 degrees of freedom

- Select the greater of MDL_s or MDL_b as the MDL.
- Refer to: USEPA. December 2016. Definition and Procedure for the Determination of the Method Detection Limit, Revision 2. EPA-821-R-16-006. US Environmental Protection Agency, Cincinnati, OH. for additional guidance and documenting ongoing data collection and verification.

Reporting Limit (RL): The RL is used to describe the lowest concentration of a substance that can be reliably measured and reported in a sample.

The reporting limit is defined as a value approximately 3 to 10 times the MDL and is equal or greater than the lowest point used on the calibration curve. The RL is considered to be the lowest level than can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions.

Environmental Testing Solutions uses the RL to establish reporting limits that have a high degree of confidence, with less probability of reporting false negative or false positive results. In cases where data must be reported below the RL, as requested by the client/study requirements, the data will be qualified as to the relatively higher degree of uncertainty of those values.

Completeness:

Percentage of tests that meet data quality objectives: Environmental Testing Solutions strives to maintain the highest level of quality in all client/studies performed in the laboratory. Data that does not meet data quality objectives are identified as deficiencies where corrective action is required (QAP-Q3)

In general, the basic elements of quality control include:

- technical competence of project personnel
- use of appropriate equipment and instruments for data collection
- good sampling practices
- good measurement practices
- use of controlled, approved procedures
- test inspection
- documentation
- training

Where appropriate, acceptance criteria have been established and identified and are described in relevant SOPs, in instructions for calibration/standardization of laboratory equipment, and/or are included on laboratory bench sheets and calibration forms.

Preparation of Standard Operating Procedures (SOPs)

Standard operating procedures (technical procedures) will be prepared for routine tasks or activities that document the methods and requirements associated with that task or activity. An SOP is needed when an activity or task needs to be standardized to ensure repeatability, or when issues of safety, environmental protection or quality assurance are in question. Procedures describing work processes will be of a detail commensurate with its complexity or importance. Maintenance, calibration, and/or standardization of M&TE used in Environmental Testing Solutions, and the documentation resulting

from these activities are described in these procedures. Procedures are based on the equipment manufacturer's instructions for calibration, standardization, and/or maintenance.

At a minimum, standard operating procedures are reviewed yearly. Standard operating procedures are revised when it becomes necessary to:

- delete an existing requirement
- create a new requirement
- initiate a new responsibility
- redefine activities or tasks being performed

Standard operating procedures are controlled documents. Each procedure will be identified by a unique identification number (e.g., SOP-XN), revision date, date of issuance, and consecutive page numbers. SOPs will be reviewed and approved by the Laboratory Supervisor prior to issuance. Procedures that are revised will include a "Document Revision History" describing the revision or modification. Archived procedures will be maintained in electronic form.

Responsibilities

Laboratory Supervisor

- Ensures that project procedures and instructions are initiated, reviewed, revised, approved and controlled.
- Identifies the appropriate basic elements of quality control and includes them in the project QA plan or SOPs.
- Identifies project data requiring review and verification.
- Determines the frequency or degree of verification required (e.g., percentage of data requiring verification).
- Establishes acceptance criteria for appropriate operational procedures and activities.
- Ensures that data, calculations, and analyses collected or performed by project personnel are verified by a technically qualified person to ensure that technical and quality requirements are achieved.
- Ensures that verification activities are performed and the results are documented.
- Establishes guidelines for identifying, correcting, and/or rejecting data found to be out of compliance with acceptance criteria.
- Ensures that all necessary controls, processes, test equipment, tools and skills are provided to attain the required quality and the required verification of quality.
- Ensures that all activities related to the technical aspects and quality of the project is in compliance with Environmental Testing Solutions' requirements.

QA Officer

- Prepares and/or reviews project SOPs. At a minimum, reviews SOPs yearly.
- Assists the Laboratory Supervisor in selecting and specifying the appropriate frequency of data verification.

Laboratory Technician

- Assists the Laboratory Supervisor or QA Officer in the development of SOPs.
- Performs project tasks in accordance with controlled, approved procedures.
- Documents project-related activities in accordance with the requirements identified in this QA procedure and SOPs.
- Performs data review and verification activities in accordance with project requirements.
- Notifies the Laboratory Supervisor and/or QA Officer of data found to be out of compliance with established acceptance criteria.

Required Records

Chain-of-custody forms and standard operating procedures are quality records and will be maintained in accordance with QAP-Q4.

The results of internal quality control checks are documented in logbooks or on project internal surveillance reports and will be maintained in accordance with QAP-Q4.

Subject: Design

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		10-01-17
Quality Assurance Officer	Jim Sumner		10-01-17

Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
12-01-00	0	Internal	Jim Sumner (ETS)	Original document
06-01-11	1	Internal	Jim Sumner (ETS)	• Corrected typographical errors.
10-01-17	2	Internal	Jim Sumner (ETS)	• Updated procedure to NELAP format.

Scope and Application

The purpose of this procedure is to ensure that all items, processes, and procedures are designed using sound scientific and experimental principles. This procedure also addresses the requirements for identifying and following appropriate USEPA standard methodology (or other approved methodology) for analytical procedures.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Definitions

Design Input: Those criteria, parameters, bases, or other design requirements upon which detailed final design is based.

Design Output: Experiment design, method design, computer programming design, and other documents used to define technical requirements of experiments, analytical methods, systems, and computer programs.

Design Process: Technical and management processes that commence with identification or design input and that lead to and include the issuance of design output documents (new standard operating procedures, study and/or research study plans).

Subject: Design

Design Review: Documented evaluation of a design by a team, including personnel other than the original designers. It is a critical evaluation to determine whether the proposed design will fulfill the established design criteria and perform satisfactorily in service.

Deviation: A departure from specified requirements.

Analytical Methods

Refer to QAP-Q5

Deviations from Standard Analytical Methods

Example: The holding time specified for the analysis of total residual chlorine (TRC; ORION 97-70) is exceeded routinely. The requirement for measuring TRC of water samples collected and analyzed for toxicity is based on the USEPA test method, which assumes analysis of samples after receipt by the laboratory performing the toxicity test. In this way, the concentration of TRC in water samples more closely reflects the conditions under which the tests are conducted.

Deviations from Testing Protocols

Every effort will be made to avoid deviating from approved testing protocols. However, deviations may occur. Deviations are documented on applicable laboratory benchsheets, chain-of-custody forms and in appropriate test reports. Documentation required includes the date the deviation occurred, a description of the deviation, and the initials of the person noting the deviation.

Responsibilities

Laboratory Director

- Serves as a peer reviewer, when requested.

Laboratory Supervisor

- With assistance from the QA Officer, selects and specifies the appropriate quality standards needed for the design project.
- Specifies applicable design inputs, such as materials, cost, performance requirements, regulatory requirements, codes and standards.
- Coordinates design efforts among and within participating groups and organizations (including assigning responsibilities and establishing working relationships among internal and external organizations).

Subject: Design

- Ensures that experimental design inputs are documented in sufficient detail to support experimental design activities.
- Ensures that documentation developed during the design process is maintained.
- References analytical procedures or methodologies used in the project QA plan or standard operating procedures and provides justification for specific deviations from acceptable methods.

QA Officer

- Reviews and/or approves, as requested, design documents generated.
- Performs QA audits and surveillances of appropriate phases of the project design.
- Assists the Laboratory Supervisor in selecting and specifying the appropriate quality standards needed for the project.

Laboratory Technician

- Provides input for the development and/or evaluation of experimental designs.

Required Records

Deviations from testing protocols are documented on applicable test information sheets in technical notebooks and in test reports, as appropriate.

Subject: Procurement

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		10-01-17
Quality Assurance Officer	Jim Sumner		10-01-17

Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
12-01-00	0	Internal	Jim Sumner (ETS)	Original document
06-01-11	1	Internal	Jim Sumner (ETS)	• Corrected typographical errors.
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Scope and Application

The purpose of this procedure is to ensure that procured items and services meet established technical and quality requirements and expectations and perform as specified. Technical and quality requirements may include, but are not limited to, specifications, standards, tests, inspections and codes.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Definitions

Complex Items: Items that have quality characteristics, not wholly visible in the end item, for which contractual conformance must be established progressively through precise measurements, tests, and controls that are applied to the item individually or in conjunction with other items during purchasing, manufacturing, assembly and functional operation.

Critical Application: An application in which the failure of the item or service could cause personal injury, harms the environment, or jeopardizes security.

Technical Reviewer: A responsible technical person with delegated authority from the Laboratory Director to approve the organization's procurement packages for technical adequacy.

Noncomplex Items: Items with quality characteristics for which simple inspection and testing of the end product are sufficient to determine conformance to contractual requirements.

Subject: Procurement

Noncritical Application: Any application other than a critical application.

Procurement Document: Specifications, special provisions, or other instructions used to define requirements of the services, equipment, or supplies to be procured.

Specification: A document that specifies requirements for the procurement; including, as applicable scope, design documents, specific codes and standards, supplier quality assurance program requirements, personnel qualification and certification, materials, fabrication, inspection and tests, item identification, packaging, shipping, handling, documentation and records.

Supplier: Any individual or organization who furnishes items or services in accordance with a procurement document. An all-inclusive term used in place of any of the following: vendor, seller, contractor, subcontractor, fabricator and consultant.

Requirements

The purchase of items or services by Environmental Testing Solutions personnel will comply with established procurement procedures. The procedures address procurement planning, supplier selection, bid evaluation, control of supplier-generated documents, supplier performance evaluations and requirements for technical review.

Procurement documents for critical applications, items, or services and complex items will receive an independent quality review by the QA Officer and the Laboratory Director to determine that all quality requirements have been included in the specifications.

Responsibilities

Requester

- Determines the item(s) or service to be procured.

QA Officer

- Determines procurement quality controls and the level of quality effort appropriate for critical or complex items and services.
- Serves as an independent verifier of the critical application determinations.
- Ensures that technical requirements for critical applications and services are consistent with laboratory policies, objectives, or requirements.
- Review procurement documents for contractual accuracy.

Required Records

Documented quality reviews of procurement suppliers are quality records and will be maintained.

Subject: Inspection and Acceptance Testing

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		10-01-17
Quality Assurance Officer	Jim Sumner		10-01-17

Document Revision History

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Scope and Application

The purpose of this quality assurance procedure is to ensure that inspection and acceptance testing, applicable to the project, is conducted using established acceptance and performance criteria. This procedure also identifies the requirements for ensuring that measuring and test equipment (M&TE) used to collect and process data and/or perform tests is calibrated, verified, and maintained.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Definitions

Acceptance Criteria: Specified limits placed on characteristics of an item, process, or service defined in codes, standards, or other requirements documents.

Accuracy: The degree of agreement of a measurement with an accepted reference or true value. Accuracy is a measure of the bias of the system; accuracy may be a measure of the bias in a single data point or the bias in a population of data points.

Calibration: The comparison of a measurement system or device of unverified accuracy to a measurement system or device of known accuracy to detect or correct any variation from requirement performance specifications of the unverified measurement system or device.

Calibration Data: Recorded measurement data that are used for quality and safety control, for development, analysis, or measurement system support, or for validation of product conformance to requirements.

Calibration Standard: A reference standard traceable to the National Institute of Standards and Technology (NIST), the National Bureau of Standards (NBS), the US Environmental Protection Agency (USEPA), or other approved institution.

Inspection: Examination or measurement to verify when an item or activity conforms to specified requirements.

Measuring and Test Equipment (M&TE): Devices or systems used to calibrate, measure, gage, test, inspect, or control in order to acquire research, development, test, or operational data for process control/safety and to determine compliance with design specifications or other technical requirements.

Reference Standard: The highest level of calibration standard for a specific technology within an organization. Reference standards are typically calibrated by an outside agency.

Tolerance (M&TE): The allowable deviation from a standard; the range of variation permitted in maintaining a specified dimension.

Traceability (M&TE): The ability to relate measurements to National Standards or a nationally accepted measurement system through an unbroken chain of comparisons; also used to describe the tracking of M&TE to its applications.

Uncertainty (M&TE): The quadratic summation of random and systematic errors. Normally expressed at the 95% confidence level when multiple measurements are performed.

Verification: The act of reviewing, inspecting, testing, checking, auditing, or otherwise determining and documenting when items, processes, services, or documents conform to specified requirements.

Inspections

Inspections are periodically conducted to ensure compliance with Environmental Testing Solutions' procedures and requirements. The inspection of project equipment and instruments used to collect and process data is addressed in the Measuring and Test Equipment section of this procedure. Additional inspection activities, e.g., receiving inspections, audits and surveillances, are addressed in other procedures within this quality assurance plan.

Subject: Inspection and Acceptance Testing

Testing

Appropriate tests will be conducted to ensure that items and/or processes perform in accordance with their intended use. Tests may include, but are not limited to, bench tests, preoperational tests, post-modification tests and operational tests. Tests may be performed by organizations or groups involved in the design of equipment or systems. All testing should be conducted using established methods, requirements and acceptance criteria. Tests of equipment and instruments used to collect and process project data are addressed in the Measuring and Test Equipment section of this procedure.

Measuring and Test Equipment (M&TE)

Measuring and test equipment and test systems will be calibrated or verified, as appropriate, at either prescribed intervals or before use. The users of M&TE are required to:

- assume responsibility for the control of M&TE used within the project
- determine and document calibration intervals for M&TE used within the project
- determine and document acceptance criteria and/or uncertainty criteria for M&TE used within the project
- establish procedures for performing calibration and/or verification of M&TE used within the project
- perform calibration and/or verification of M&TE used within the project
- coordinate with calibration and verification organizations to maintain current calibration of M&TE not calibrated or verified by project personnel
- determine the impact of M&TE found to be out of tolerance
- implement procedures for segregating and identifying M&TE found to be out of tolerance
- develop and implement corrective actions for M&TE found to be out of tolerance

M&TE calibrated and/or verified daily or weekly do not require a label indicating the calibration status; however, the results of calibration/verification activities will be recorded in logbooks. The results of calibration/verification activities must be traceable to specific M&TE. M&TE calibrated less frequently, i.e., monthly, quarterly, semiannually, or annually, will be labeled. The label provides the identification of the M&TE, calibrating organization, calibration data (results or status) and identification of the person performing the calibration.

Identification and Segregation of Suspect Equipment

M&TE that is suspect in having a defect or out of calibration (i.e., fails to meet established acceptance or uncertainty criteria) will be identified as out of tolerance and immediately removed from use. M&TE identified as out of tolerance will be tagged (or other appropriate method) as “Do Not Use.”

Subject: Inspection and Acceptance Testing

Responsibilities

Laboratory Supervisor

- Ensures that M&TE used within the project is properly identified and documented.
- Ensures that instructions for the maintenance, calibration, and/or verification of project M&TE are included in standard operating procedures and are accessible to project personnel.
- Determines and documents the frequency of calibration/verification intervals of project M&TE.
- Determines and documents the acceptance/uncertainty criteria for project M&TE.
- Establishes procedures for identifying (tagging) and segregating M&TE found to be out of tolerance to ensure that the M&TE is not inadvertently used until repaired or replaced.
- Determines and documents the impact of M&TE found to be out of tolerance.
- Ensures that corrective actions are developed and implemented for M&TE found to be out of tolerance.
- Coordinates the calibration/verification of M&TE (not calibrated/verified by project personnel) with calibration organizations to ensure that calibration/verification is current.
- Ensures that calibration standards and/or reference standards are available for use in calibrating/verifying project M&TE.
- Ensures that calibration standards are traceable to nationally recognized standards.
- Ensures that data obtained from calibration/verification activities are maintained.
- Ensures that project personnel are trained and qualified in the performance of calibration/verification activities.
- Periodically assesses the competence of project personnel in the performance of calibration/verification activities.

Laboratory Technician

- Perform calibration/verification activities in accordance with documented, approved procedures.
- Perform calibration/verification activities in accordance with established intervals.
- Document the results of calibration/verification activities.
- Ensure that the results of calibration/verification activities are within the established acceptance criteria and/or uncertainty criteria for the M&TE.
- Segregate and identify (by tagging or other appropriate method) M&TE found to be out of tolerance.
- Notify the Laboratory Supervisor, QA Officer, or other appropriate project personnel of M&TE found to be out of tolerance.

Required Records

Calibration and maintenance records are quality records and are maintained in accordance with QAP-Q4.

Subject: Management Assessment

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		10-01-17
Quality Assurance Officer	Jim Sumner		10-01-17

Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
12-01-00	0	Internal	Jim Sumner (ETS)	Original document
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Scope and Application

The purpose of this procedure is to provide management guidance in assessing the performance and effectiveness of the project for which they are responsible and for identifying problems that may impede the project's ability to achieve its QA objectives. This procedure establishes measures to promote improvement within the project and to develop and analyze performance information that focuses on performance improvement.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Subject: Management Assessment

Assessment Process

The assessment process should be ongoing and should evaluate client and employee perceptions relative to the following key issues.

- mission and key objectives of the project
- employees' role in the project
- client's expectations and the degree to which expectations are being met
- opportunities for improving quality and cost effectiveness
- recognizing and enhancing human resource expertise

Assessments should evaluate the impact on overall mission accomplishment, with emphasis on environment, safety and health issues, quality issues, budget impacts, and procedures compliance. It is an integral part of the project's self-assessment process and should focus on the following.

- leadership and communication
- quality planning
- quality improvement and communication
- project personnel capabilities and training
- client focus and feedback

Assurance of client satisfaction with project quality and performance is an important goal.
The Laboratory Supervisor will review the overall mission and project objectives with technical staff. This review should include an opportunity for project staff to make suggestions and offer concerns.

Self-Assessment Program

A description of the Environmental Testing Solutions' internal ("self') assessment program is contained in QAP-Q3 of this quality assurance plan.

Subject: Management Assessment

Performance and Trends

The Laboratory Supervisor will evaluate the performance of the project annually. The evaluation may address performance indicators in Table Q9.1, or project-specific issues identified by the Laboratory Supervisor. The purpose for conducting this evaluation is to:

- review and document project accomplishments, achievements and strengths
- review adherence to QA, technical and client requirements
- identify project weaknesses or deficiencies
- identify areas for improvement
- identify trends that may affect the project's ability to achieve its QA objectives

Table Q9.1. Performance indicators.

Training
Adherence to procedures
Nonconformance
Corrective actions resulting from surveillances or audits
Accuracy and precision
QC samples: type and quantity
Acceptance or uncertainty criteria
Reports generated
Publications
Surveillances and audits performed
Presentations
Procedures approved
Records (quality assurance and project-specific)

Subject: Management Assessment

Responsibilities

Laboratory Supervisor

- Identifies and/or develops performance indicators appropriate for the project.
- Assesses the performance of the project's accomplishment of its quality objectives through observations, interviews and document reviews.
- Identifies problems and evaluates trends for repetitive issues that may impede the project from achieving its objectives in accordance with quality, safety and environmental requirements.
- Assesses the achievement of client expectations through client feedback and communication.
- Takes action in response to trends or impacts identified from the assessment process that obstruct the project's ability to achieve its quality objectives.
- Evaluates the effectiveness of actions that result from management assessments and performance evaluations.
- Reviews with technical staff the results of management assessments and performance evaluations.

Laboratory Technician

- Participate in the assessment and performance evaluation process in a proactive manner.
- Provide the Laboratory Supervisor with suggestions for meeting the project's mission and quality objectives.
- Notify the Laboratory Supervisor of obstacles that impede the ability to meet the project's mission and quality objectives.

Required Records

The results of management assessments are quality records and will be maintained in accordance with QAP-Q4.

Subject: Independent Assessment

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		10-01-17
Quality Assurance Officer	Jim Sumner		10-01-17

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10-01-17	2	Internal	Jim Sumner (ETS)	• Updated procedure to NELAP format.

Scope and Application

The purpose of this procedure is to identify the documents involved in the performance of independent assessments (audits) and the responsibilities and requirements of Environmental Testing Solution personnel for providing assistance to personnel performing an independent assessment.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Definitions

Audit: A planned and documented activity performed to determine by investigation, examination, or evaluation of objective evidence the adequacy of and compliance with established procedures, instructions and other applicable documents, and the effectiveness of implementation.

Auditor: Any individual who performs any portion of an audit, including lead auditors, technical specialists, and others such as management representatives and auditors-in-training.

Independent Assessment: Performed by personnel not directly involved in the activity being assessed.

Lead Auditor: An individual qualified to organize and direct an audit, report audit findings and evaluate corrective action.

Surveillance: The act of monitoring or observing to verify whether an item or activity conforms to specified requirements.

Subject: Independent Assessment

Performance-Based Independent Assessments (Audits)

Planned and periodic independent assessments conducted by persons or organizations independent of Environmental Testing Solutions may be implemented to determine quality and process effectiveness and to promote improvement. The independent assessment should evaluate the implementation of the approved quality assurance program using a "performance based" approach (i.e., observe then evaluate).
Independent assessments of Environmental Testing Solutions' quality assurance program may be conducted by state regulatory agencies, USEPA , QA Officer, or clients.

A performance-based independent assessment should be based on the following concepts:

- Assessments are performed on activities that have a genuine impact on the project or project's performance.
- Assessments are performed in a manner that emphasizes safety and reliability.
- Assessments are performed by qualified personnel (QA Officer, clients, USEPA, or state regulatory agencies) who have the necessary technical capabilities to observe and evaluate an activity accurately.

Independent assessments may take the form of audits or surveillances. Personnel involved in performing the assessment (auditors) should evaluate the implementation of the approved quality assurance program and/or client requirements.

Deficiency Resolution and Results Tracking

The results of independent assessments are resolved by project management ("Corrective Actions") and tracked to completion.

Responses to deficiencies identified in the independent assessment should address the following.

- actions taken to resolve the problem
- identification of the cause of the problem ("root cause")
- actions that will be taken to prevent recurrence
- lessons learned
- actions to be taken for improvement

A follow-up review of deficient areas may be scheduled and conducted by the person(s) or organization performing the independent assessment.

Subject: Independent Assessment

Responsibilities

Laboratory Supervisor

- Provides documentation to the audit team as requested.
- Develops and implements corrective actions in response to deficiencies identified from the independent assessment.
- Confirms implementation and evaluates effectiveness of corrective actions developed.
- Provides documentation of the completion of corrective actions.

QA Officer

- Validates the proposed corrective action to ensure that the action(s) is appropriate to correct and prevent recurrence of the problem (i.e., root cause analysis, lessons learned, actions are achievable/closeable and can be documented).
- Verifies completion of corrective actions.
- Signs the corrective action plan indicating completion of verification activities.

Required Records

Records required documenting the identification, validation, implementation, completion, verification, and closure of corrective actions resulting from external audits or surveillances will be maintained.

Subject: Classification, Control and Use of Products and Materials

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		10-01-17
Quality Assurance Officer	Jim Sumner		10-01-17

Document Revision History

Effective Date	Revision number	Review Type	Evaluators	Revisions
05-23-11	0	Internal	Jim Sumner (ETS)	Original document
09-28-16	1	External (TVA) Internal	Rick Sherrard, Donald Snodgrass (TVA) Jim Sumner (ETS)	• Added *Pimephales* to the acute/chronic toxicity documentation of products and materials, which contain volatile or toxic substances.
10-01-17	2	Internal	Jim Sumner (ETS)	• Updated procedure to NELAP format.

Scope and Application

The purpose of this procedure is to classify and control the use of potentially volatile or toxic products and materials on the ETS grounds. This procedure is to ensure that volatile or toxic products and materials will not compromise the integrity of testing performed in the laboratory or health of laboratory cultures.

The scope of this procedure goes beyond just products and materials and includes applications or tasks which may generate volatile or toxic substances. It is the responsibility of all employees to be conscious and aware (and inform contractors) of how their actions may violate this procedure.

This procedure applies to all personnel or contractors on the ETS grounds (within the immediate vicinity of the building or within the building itself). Due to the importance of this procedure, failure to adhere to these requirements may be grounds for immediate termination of employment or contract with ETS.

Subject: Classification, Control and Use of Products and Materials

Definitions

Volatile: Product or material that evaporates rapidly, passing off readily in a vapor form.

Toxic: Product or material that is poisonous or has an inherent property that compromises the health or survival of living organisms.

Requirements

The purchase and use of products and materials by ETS personnel or contractors on the ETS grounds will comply with requirements contained within this document or more stringent requirements that are developed.

Classification and Characterization of Products or Materials

Material Safety Data Sheets (MSDSs) of all products and materials used or maintained on the ETS grounds must be maintained. MSDSs will be reviewed by the laboratory supervisor and evaluated for potential volatile or toxic substances. Products or materials, which contain volatile or toxic substances, must not be used on the ETS grounds until they are characterized. Characterization may include determining the acute or chronic toxicity of the product or material on *Ceriodaphnia dubia* survival and/or reproduction or *Pimephales promelas* survival and/or growth (based on the potential acute or chronic exposure).

Products or materials, which have been characterized as toxic, must be restricted and controlled. An alternative non-toxic product or material must be found and the toxic product or material must be removed from the ETS grounds. If an alternative cannot be found, it's use must be restricted and managed according to laboratory supervisor requirements. Restrictions may include, but are not limited to the following:

- scheduling during business hours that minimizes the amount and time exposure (in no instances may a toxic product or material be used after hours without prior approval by the laboratory supervisor)
- adequate ventilation
- monitoring by the laboratory supervisor

Restricted or toxic products or materials will be posted on the laboratory bulletin board (Exhibit 11.2).

Subject: Classification, Control and Use of Products and Materials

Required Records

MSDSs and Classification/Characterization Documentation (Exhibit Q11.1) are quality records and will be maintained.

Exhibits

Exhibit Q11.1: Classification/Characterization Documentation

Acute/chronic toxicity documentation of products and materials, which contain volatile or toxic substances.

Product or Material	Toxic or Volatile Substances	Potential Exposure (√)		Toxicity to *Ceriodaphnia* (√)		Toxicity to *Pimephales* (√)	
		Acute	Chronic	Pass	Fail	Pass	Fail

Note: Pass = non-toxic to *Ceriodaphnia dubia* or *Pimephales promelas* during an acute or chronic exposure.
Fail = toxic to *Ceriodaphnia dubia* or *Pimephales promelas* during an acute or chronic exposure.

Exhibit Q11.2: Restricted Products and Materials

Restricted Products and Materials

Spray Paints
Pesticides
Herbicides
*Biocides
Cigarettes or nicotine products
Perfumes and colognes

***Approved biocides include: bleach, reagent alcohol, and hand sanitizers.**

Subject: Statistical Analyses, Data Review and Verification

Approval

Title	Name	Signature	Date
Laboratory Supervisor	Kelley E. Keenan		10-16-18
Quality Assurance Officer	Jim Sumner		10-16-18

Document Revision History

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06-01-11	0	Internal	Jim Sumner (ETS)	Original document
11-24-14	1	External (NC DENR) Internal	Carol Hollenkamp (NC DENR) Jim Sumner (ETS)	• Included special considerations in the analysis of Pass/Fail acute tests. • Included references.
09-28-16	2	External (TVA) Internal	Rick Sherrard, Donald Snodgrass (TVA) Jim Sumner (ETS)	• Corrected typographical errors. • Removed Exhibit Q12.3.
10-01-17	3	Internal	Jim Sumner (ETS)	• Updated procedure to NELAP format.
10-16-18	4	External (NC DWR) Internal	Carol Hollenkamp (NC DWR) Jim Sumner (ETS)	• Updated Exhibit Q12.2 to include EPA flow charts for survival analyses. • Updated Section B.2 to state: $\alpha = 0.01$ is used for North Carolina *Ceriodaphnia* Pass/Fail Chronic Toxicity Tests and *Americamysis bahia* Chronic growth analyses.

Scope and Application

To address the requirements and policies for performing statistical analyses as well as, the review, validation and verification of data generated by the laboratory.

This procedure applies to all personnel who are conducting drinking water, wastewater, and toxicity testing in Environmental Testing Solutions' facility.

Definitions

Statistics: Mathematical science dealing with the collection, analysis, and interpretation of numerical data using the theory of probability (methods for drawing inferences about characteristics of a population from the examination of a random sample).

Subject: Statistical Analyses, Data Review and Verification

Data Review: The evaluation process that determines the quality of reported results. It involves examination of raw data (i.e., instrument output) and quality control and method parameters by a professional with knowledge of tests performed.

Data Validation: A systematic process, performed external from the data generator, which applies a defined set of performance-based criteria to a body of data that may result in physical qualification of the data. Data validation occurs prior to drawing a conclusion from the body of data.

Data Verification: A systematic process of evaluating the completeness, correctness, consistency, and compliance of a set of facts against a standard or contract which is performed by either the data generator or by an entity external to the data generator.

References

USEPA. October 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, 5th ed. **EPA-821-R-02-012, Method 2002.0 for *Ceriodaphnia dubia*, Method 2021.0 for *Daphnia magna***. US Environmental Protection Agency, Cincinnati, OH.

USEPA. October 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 4th ed. **EPA-821-R-02-013, Method 1002.0**. US Environmental Protection Agency, Cincinnati, OH.

USEPA. October 2002. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, 3rd ed. **EPA-821-R-02-014, Method 1007.0**. US Environmental Protection Agency, Cincinnati, OH.

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination Program. EPA-833-R-00-003. US Environmental Protection Agency, Cincinnati, OH.

USEPA. 2001. Final Report: Inter-laboratory Variability Study of EPA Short-term Chronic and Acute Whole Effluent Toxicity Test Methods, Volumes 1 and 2 - Appendix. EPA-821-B-01-004 and EPA-821-B-01-005. US Environmental Protection Agency, Cincinnati, OH.

North Carolina Department of Environment and Natural Resources, Division of Water Quality. Biological Laboratory Certification / Criteria Procedures, Version 3.0. December 2010.

North Carolina Department of Environment and Natural Resources, Division of Water Quality. Pass/Fail Methodology for Determining Acute Toxicity in a Single Effluent, Version 3.0. December 2010.

North Carolina Department of Environment and Natural Resources, Division of Water Quality. North Carolina *Ceriodaphnia* Chronic Whole Effluent Toxicity Procedure, Version 3.0. December 2010.

North Carolina Department of Environment and Natural Resources, Division of Water Quality. North Carolina Phase II Chronic Whole Effluent Toxicity Test Procedure, Version 3.0. December 2010.

Statistical Analyses

A. Acute Tests

1. Survival is statistically analyzed using ToxCalc® according to prescribed EPA methodologies (α = 0.05, see Exhibit Q12.1).

2. For North Carolina Pass/Fail acute tests, statistical comparisons are made with a *t*-Test, Modified *t*-Test for unequal variances, or Wilcoxon Rank Sum Test for non-parametric data (α = 0.01).

SPECIAL CONSIDERATIONS
(as indicated in: North Carolina Department of Environment and Natural Resources, Division of Water Quality. Pass/Fail Methodology for Determining Acute Toxicity in a Single Effluent, Version 3.0. December 2010.

F-test: The F test for equality of variances is not possible when the response proportions of all four replicates within the control or treatment set are equal. This results in a variance of 0 in the denominator of the F equation, which is not valid. When data pass the test for normality, but cannot be tested for equality of variances, the modified t test should be used, since the assumption of equality of variances cannot be made.

Wilcoxon Rank Sum Test: A critical value of 10 for a one-sided Wilcoxon Rank Sum test for tests with 2 treatments (control and the effluent treatment) and four replicates in each treatment is used. Some statistical programs (including ToxCalc) do not have a critical value or may use a different value for n=4, m=4 at the 0.01 level of significance. For instance, some statistical programs give a critical value of 9 for n=4, m=4 at the 0.01 level of significance. A critical value of 9 will cause all results to pass, even in cases with high mortality. This is because the lowest 4 ranks will always add up to at least 10. If the statistical program uses a critical value of 9 or does not have a critical value at n=4, m=4, a critical value of 10 may be substituted. Alternatively, a 0.05 level of significance may be chosen rather than the 0.01 level of significance. The 0.05 level of significance may only be chosen for non-normal acute pass fail tests with 4 replicates and two concentrations (control and effluent treatment).

t-value non-calculable: If all replicates of the control (Treatment 1) have identical responses equal to or less than 10 percent mortality and all replicates of the effluent concentration (Treatment 2) have equal responses though greater than that of the control response, the t value is not calculable. The Wilcoxon Rank Sum Test should be used in this case. In the Wilcoxon Rank Sum test, tied values are given an average rank.

All responses identical: If all replicates in both treatments have identical responses which are equal to or less than ten percent mortality, no statistical test is appropriate. When all responses are equal and mortality is equal or less than 10%, the test result should be reported as "PASS."

B. Chronic Tests

1. Survival is statistically analyzed using ToxCalc® according to prescribed EPA methodologies (α = 0.05, see Exhibit Q12.2).

2. Reproduction, Growth, or fecundity is statistically analyzed using ToxCalc® according to prescribed EPA methodologies (α = 0.05 unless otherwise specified by the facility permit, α = 0.01 is used for North Carolina *Ceriodaphnia* Pass/Fail Chronic Toxicity Tests and *Americamysis bahia* Chronic growth analyses). Reproduction, growth, and fecundity endpoints are calculated based on the initial number of organisms present in each replicate.

3. For North Carolina *Ceriodaphnia* chronic tests, reproduction is < 20.0% reduced from control reproduction, the test will be considered a non-significant. Percent reduction is calculated by subtracting the mean number of neonates produced by the treatment organisms from the mean number of neonates produced by the control organisms, dividing that number by the mean number of neonates produced by the control organisms, and multiplying by 100%.

Data Entry

Chemical Analyses in Association with Toxicity Tests:
Data for the chemical analyses performed in association with toxicity tests are entered directly from the laboratory benchsheets into Daily Chemical Analyses excel spreadsheets. These spreadsheets are used to calculate averages and minimum and maximum numbers for each chemical analysis performed. Encoding in the Daily Chemical Analysis spreadsheet automatically provides these calculations in summary tables which are imported into the test reports.

Subject: Statistical Analyses, Data Review and Verification

Data Review, Verification, and Validation

Data review and verification at Environmental Testing Solutions is an on-going process. The frequency and/or degree of verification and review are commensurate with the project data quality requirements. At a minimum, data generated from testing activities will be reviewed and verified by qualified project personnel to ensure that:

- Data have been accurately recorded, transcribed, and quantified.
- Procedures have been complied with.
- Data appear to be reasonable and consistent.
- Analyses were performed within specified parameters.

Ideally, final reports will be reviewed and approved by a qualified person other than the report preparer prior to issuance.

Additional data review and verification activities and requirements related to specific testing are described in applicable standard operating procedures.

At a minimum, whole toxicity test results are reviewed for the following:

- Examine the test results to verify that the test method and dilution series as required in the NPDES permit were used. To reduce test method and dilution series errors that do not conform to NPDES permit specifications, Environmental Testing Solutions includes pertinent test criteria on chain-of-custody forms. Environmental Testing Solutions requires the permittee to change any information on this form that is inaccurate. These chain-of-custody forms are reviewed upon sample receipt at the laboratory prior to initiating the toxicity test.
- Evaluate the test results against the permit requirements for toxicity to assess whether the limit or numeric monitoring trigger is being achieved.
- Examine the results to verify the sample was maintained at the proper temperature from time of collection to arrival at the laboratory. Also, determine if the sample meet the test initiation and renewal holding time requirements.
- Evaluate the test results for the effluent to verify that the laboratory met the test acceptability criteria (TAC) as specified for each test method or regulatory authority. All invalid tests must be repeated with a newly collected sample, as specified in the permit.
- Examine the “Summary of Test Conditions and TAC” section for the specific method to determine whether the required and recommended test conditions were met.
- Examine the statistical results to verify the recommended flowcharts for statistical analysis were followed. Any deviation from the recommended flowcharts for selection of statistical methods should be noted in the data report.

- Examine the concentration-response relationships as these must be reviewed to ensure that calculated test results are interpreted appropriately. All toxicity test results (from multi-concentration tests) reported under the NPDES program should be reviewed and reported according to USEPA guidance on the evaluation of concentration-response relationship.
- Test review of a given effluent or receiving water test must include review of the associated reference toxicant test and current control chart. Out-of-control reference toxicant test results are evaluated to determine appropriate corrective action.
- The within-test variability of individual tests should be reviewed. When NPDES permits require sublethal hypothesis testing endpoints from Methods 1000.0, 1002.0, 1003.0, 1004.0, 1006.0, and 1007.0 (e.g., growth or reproduction NOECs and LOECs), within-test variability must be reviewed and variability criteria must be applied as described in the Method Manuals Section on Test Review.

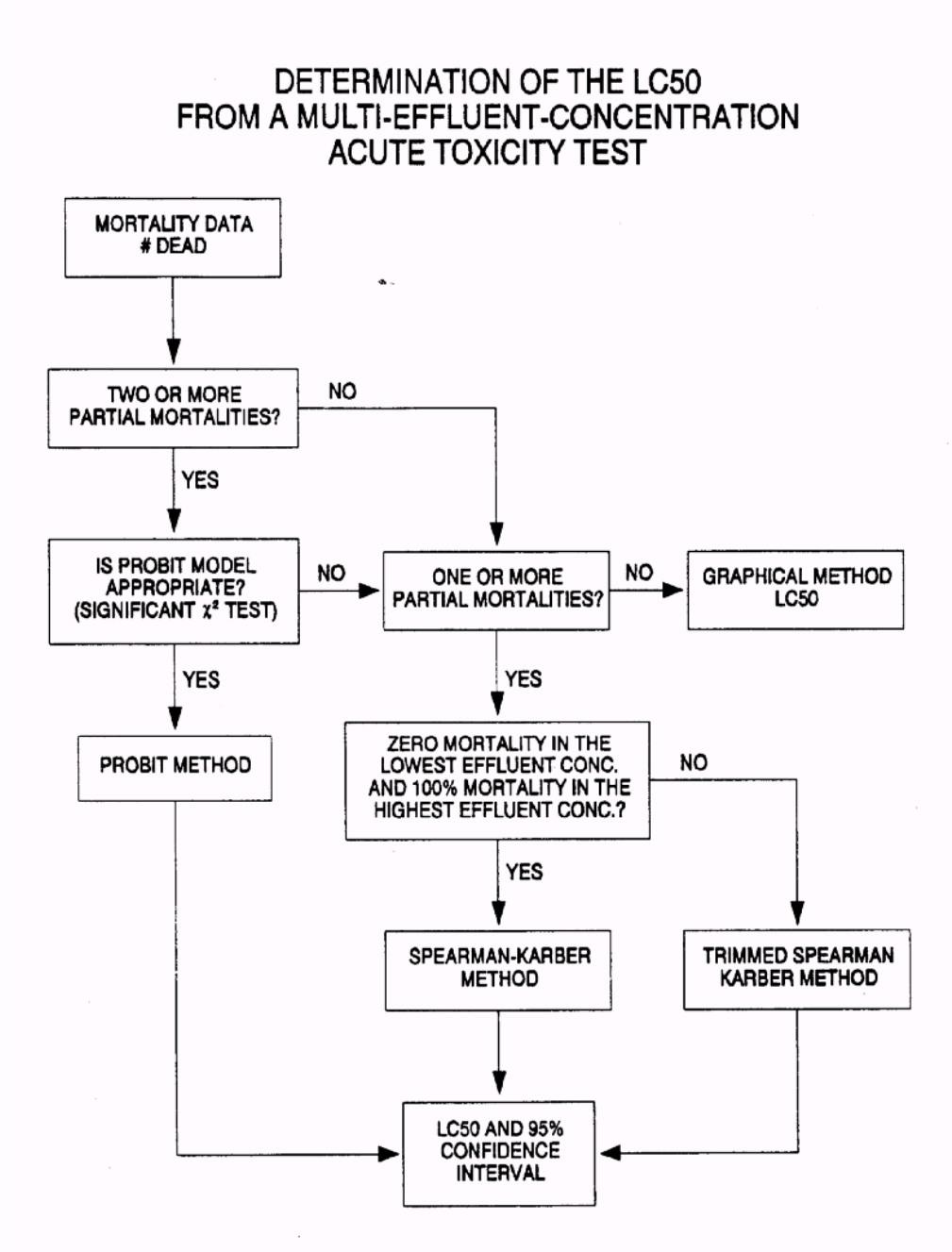
Environmental Testing Solutions does not validate data generated by laboratory activities; however, data validation may be conducted by other validation organizations or by the client. Environmental Testing Solutions will provide all pertinent information needed to complete the validation process.

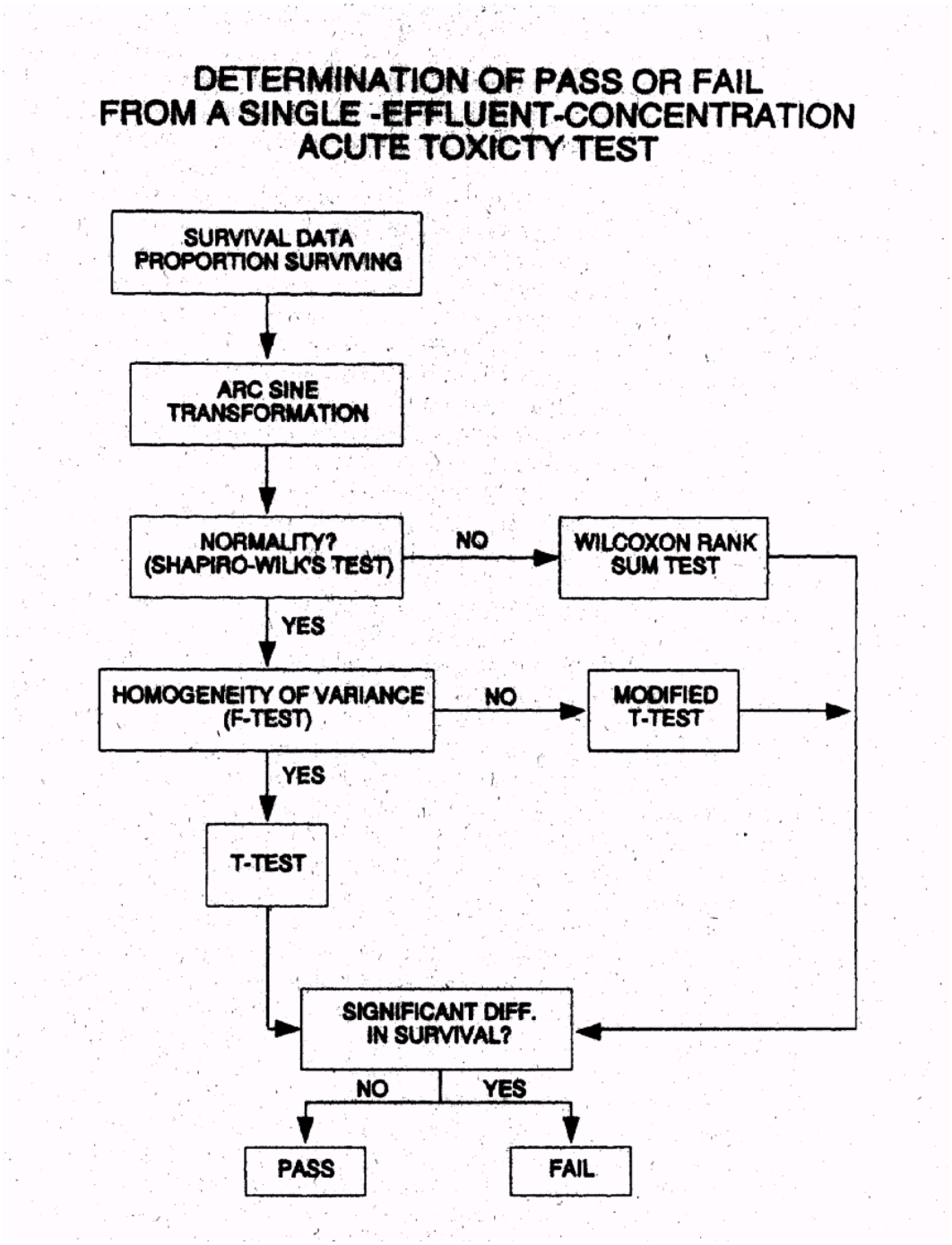
Exhibits

Exhibit Q12.1: EPA Acute Analysis Flow Charts.
Exhibit Q12.2: EPA Chronic Analysis Flow Charts.

Subject: Statistical Analyses, Data Review and Verification

Exhibit Q12.1: EPA Acute Analysis Flow Charts.




DETERMINATION OF PASS OR FAIL
FROM A SINGLE -EFFLUENT-CONCENTRATION
ACUTE TOXICTY TEST
SURVIVAL DATA
PROPORTION SURVIVING
ARC SINE
TRANSFORMATION
NORMALITY?
(SHAPIRO-WILK'S TEST)
NO
WILCOXON RANK
SUM TEST
YES
HOMOGENEITY OF VARIANCE
(F-TEST)
NO
MODIFIED
T-TEST
YES
T-TEST
SIGNIFICANT DIFF.
IN SURVIVAL?
NO
YES
PASS
FAIL

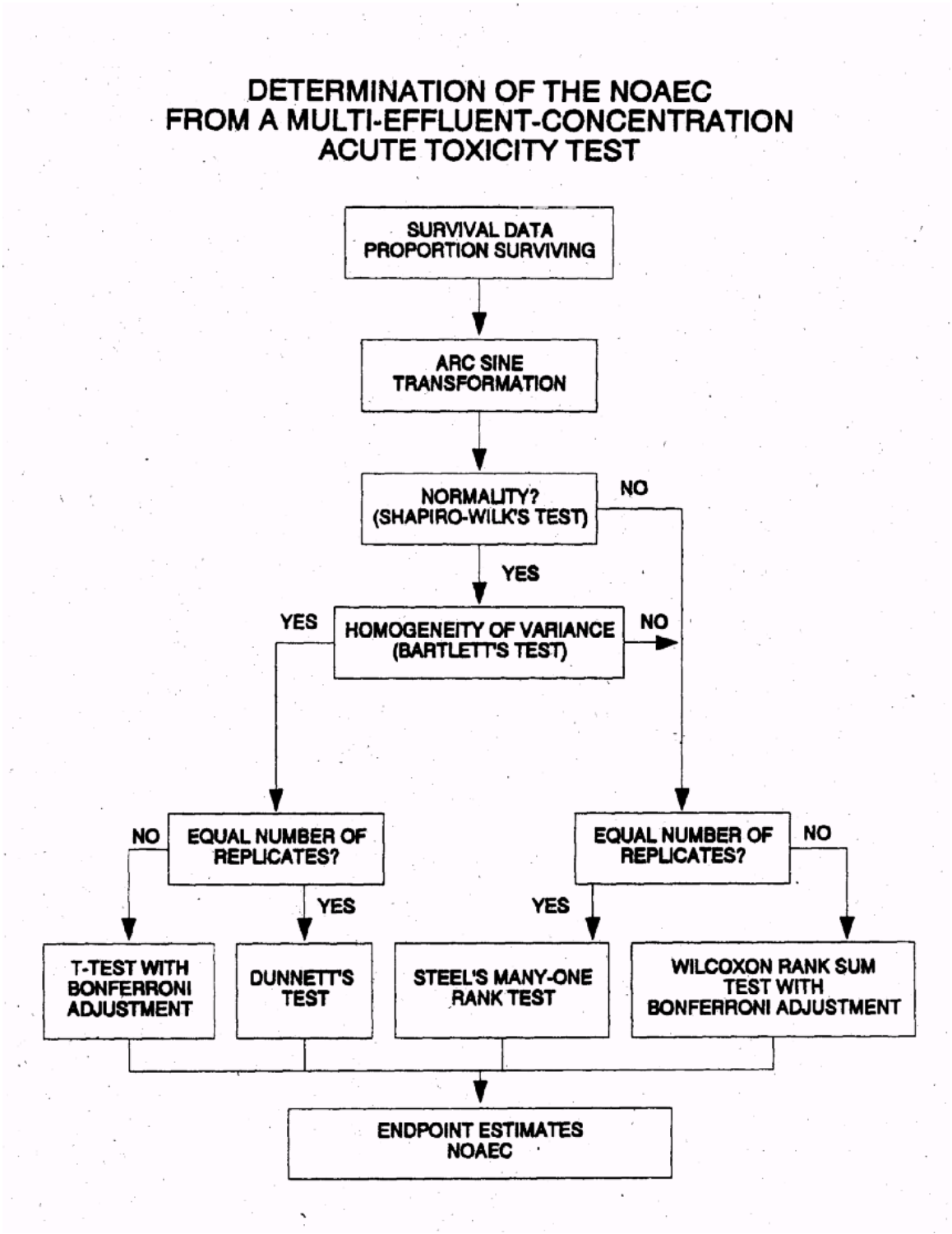
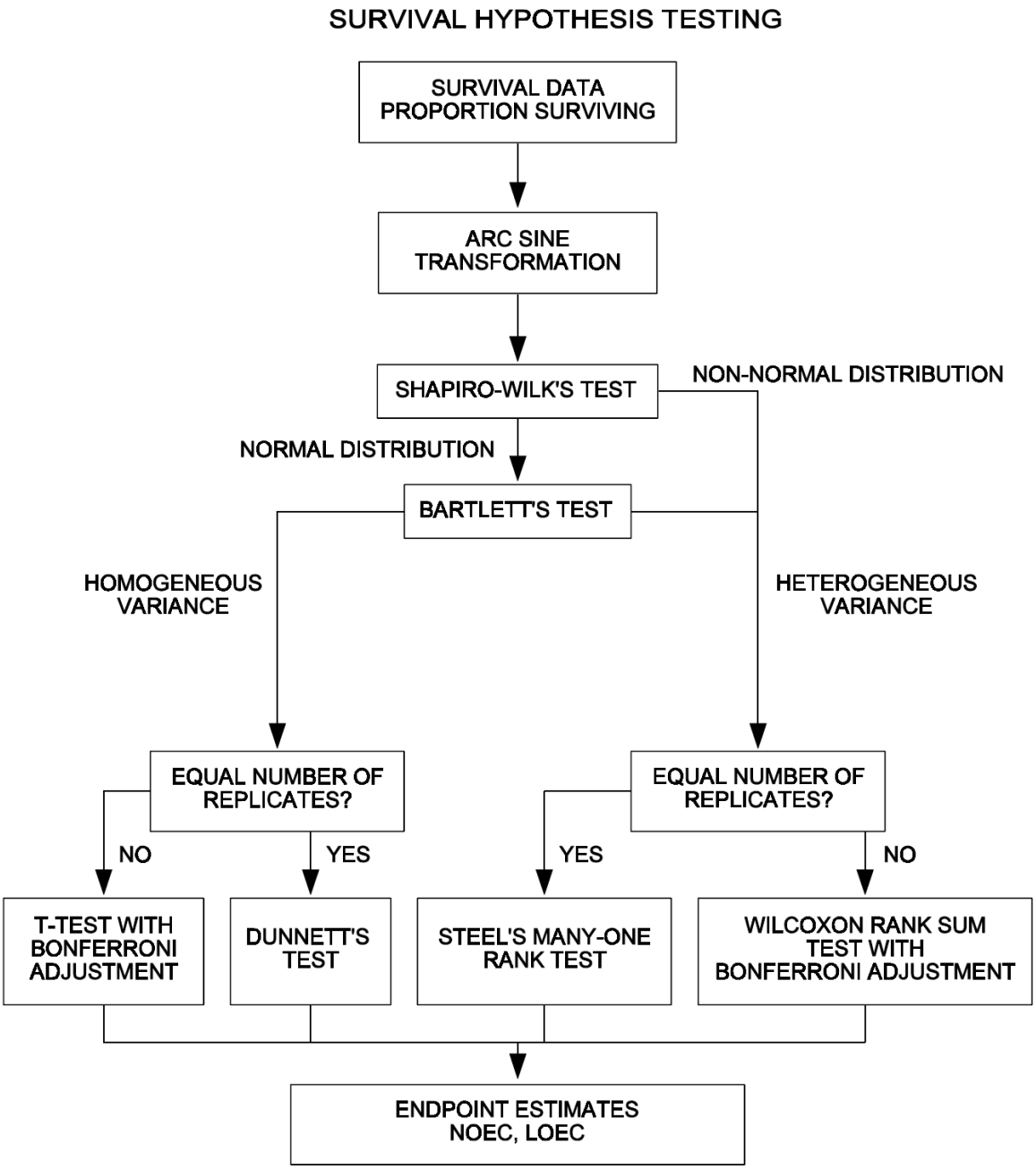
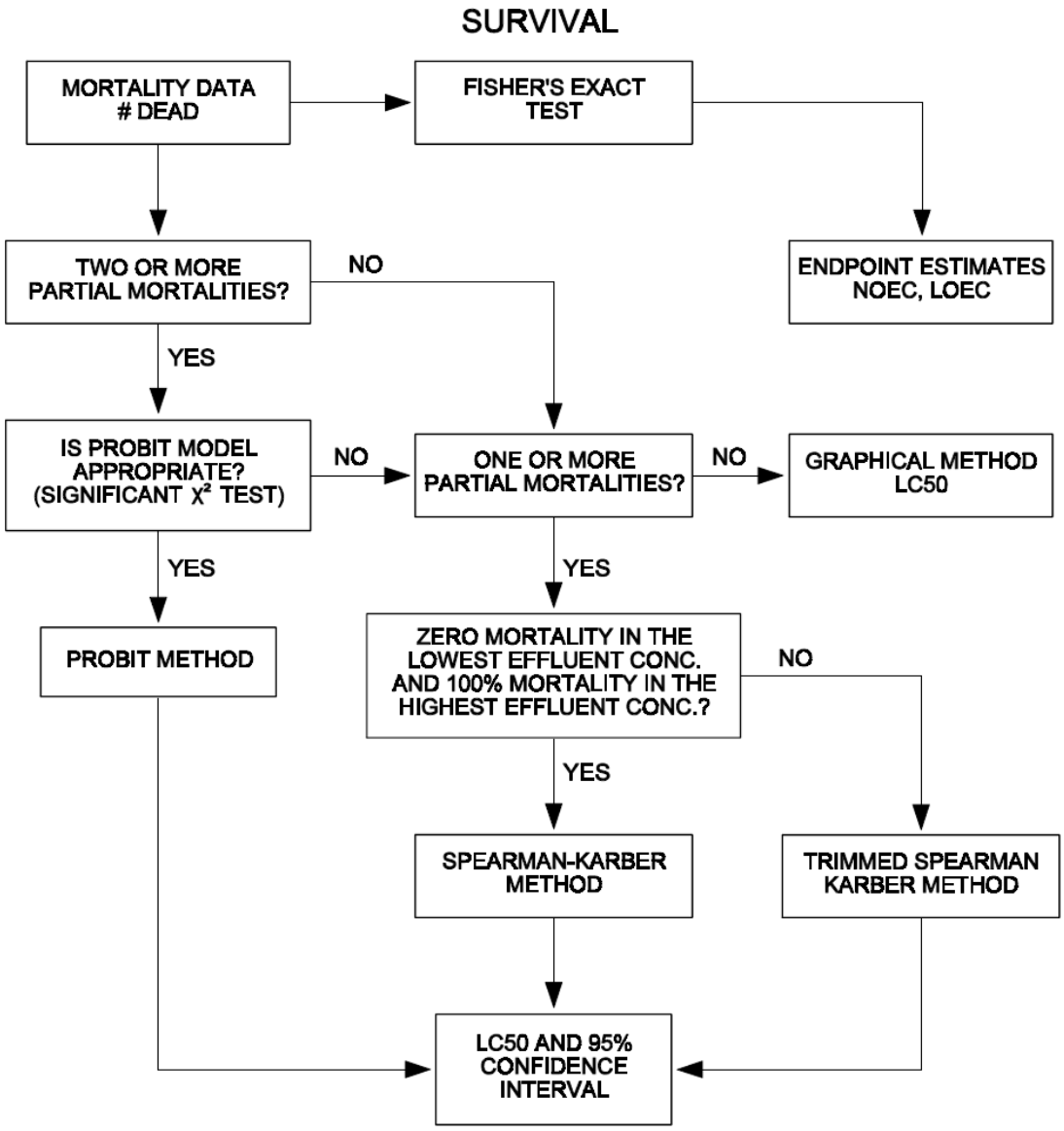

DETERMINATION OF THE NOAEC
FROM A MULTI-EFFLUENT-CONCENTRATION
ACUTE TOXICITY TEST
SURVIVAL DATA
PROPORTION SURVIVING
ARC SINE
TRANSFORMATION
NORMALITY?
(SHAPIRO-WILK'S TEST)
NO
YES
YES
HOMOGENEITY OF VARIANCE
(BARTLETT'S TEST)
NO
NO
EQUAL NUMBER OF
REPLICATES?
YES
EQUAL NUMBER OF
REPLICATES?
NO
YES
T-TEST WITH
BONFERRONI
ADJUSTMENT
DUNNETT'S
TEST
STEEL'S MANY-ONE
RANK TEST
WILCOXON RANK SUM
TEST WITH
BONFERRONI ADJUSTMENT
ENDPOINT ESTIMATES
NOAEC

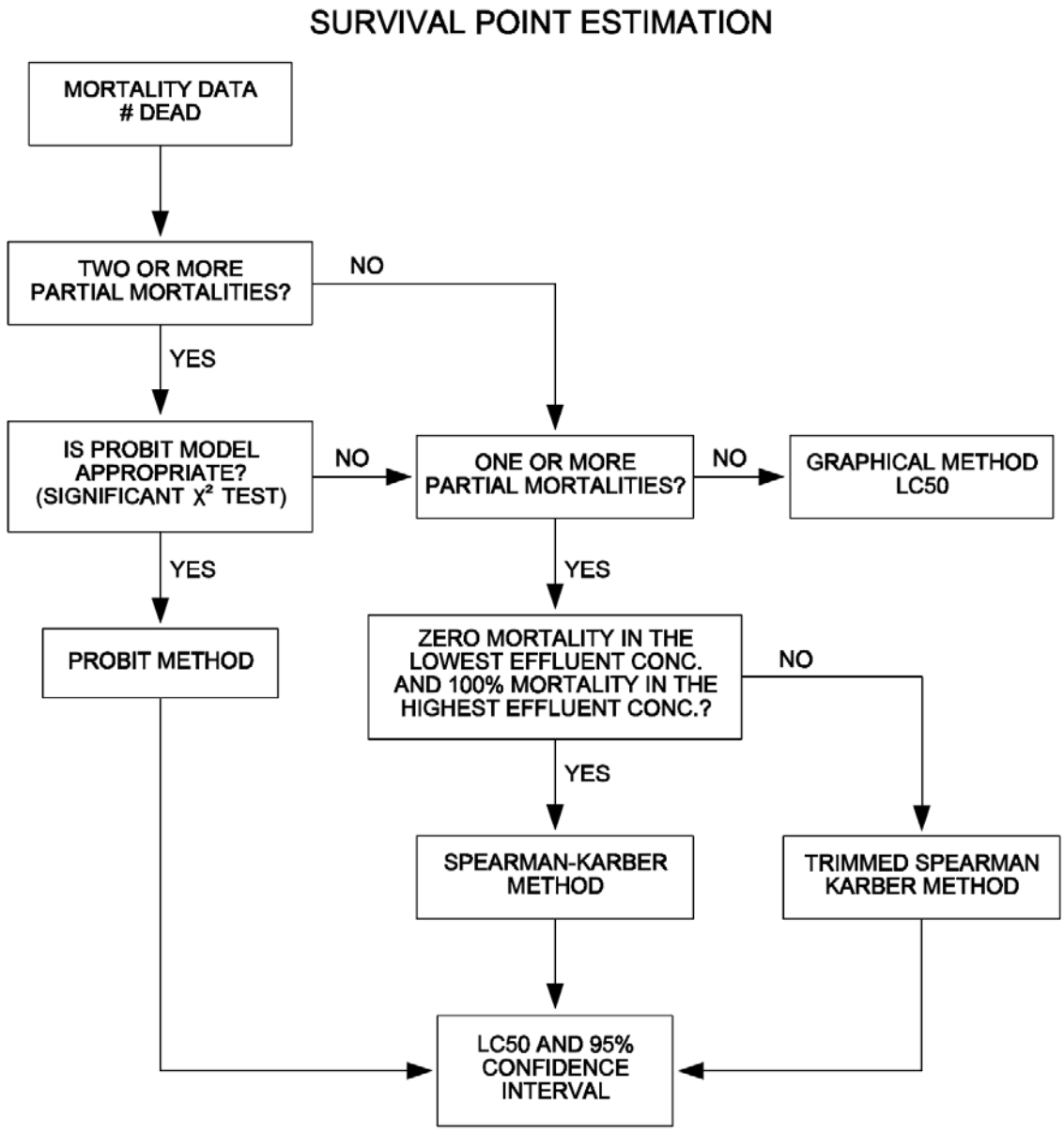
Exhibit Q12.2: EPA Chronic Analysis Flow Charts.

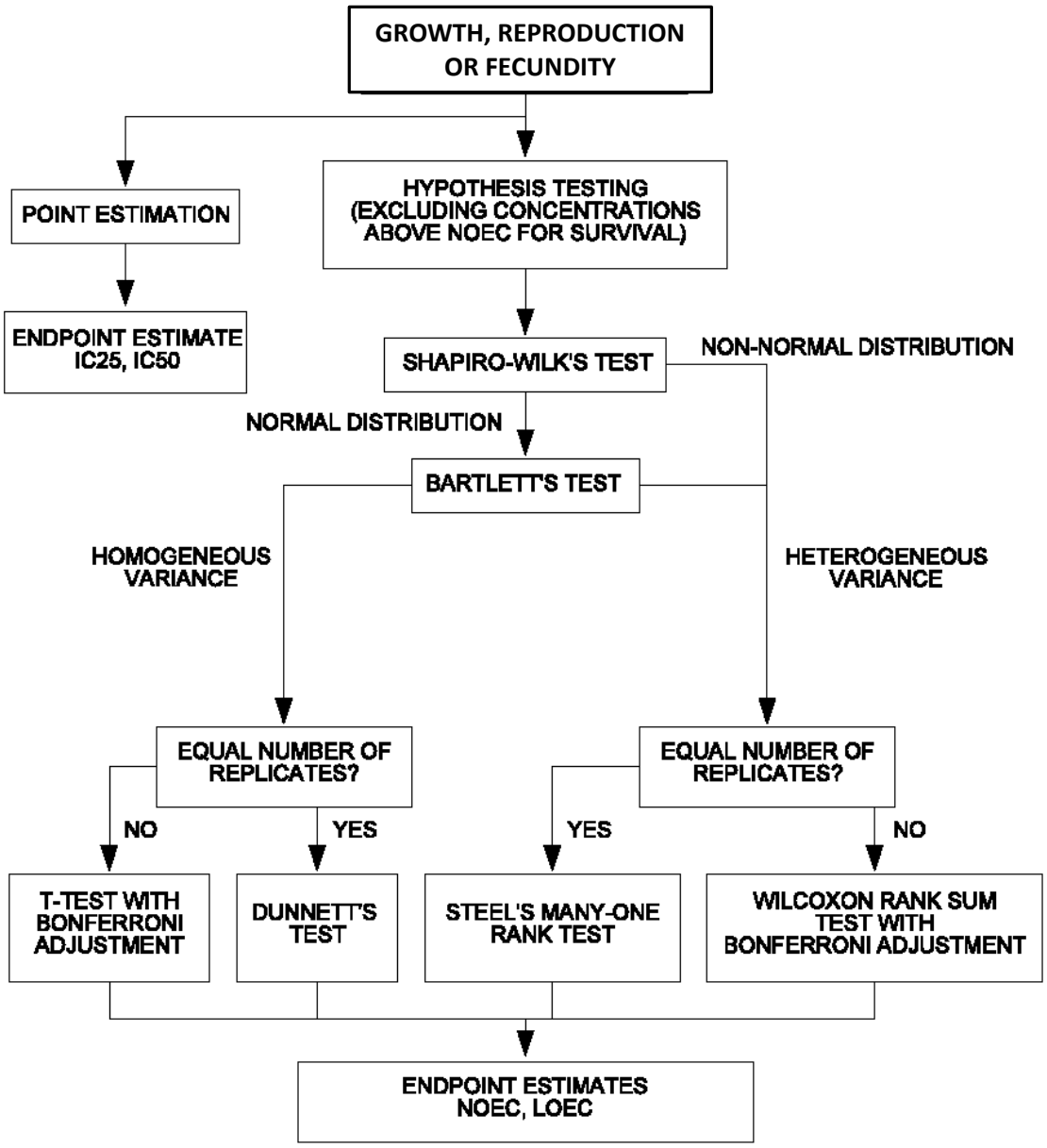


Subject: Statistical Analyses, Data Review and Verification

***Ceriodaphnia dubia* Survival Analyses**




SURVIVAL POINT ESTIMATION
MORTALITY DATA # DEAD
TWO OR MORE PARTIAL MORTALITIES?
NO
YES
IS PROBIT MODEL APPROPRIATE? (SIGNIFICANT X^2 TEST)
NO
ONE OR MORE PARTIAL MORTALITIES?
NO
GRAPHICAL METHOD LC50
YES
YES
PROBIT METHOD
ZERO MORTALITY IN THE LOWEST EFFLUENT CONC. AND 100% MORTALITY IN THE HIGHEST EFFLUENT CONC.?
NO
YES
SPEARMAN-KARBER METHOD
TRIMMED SPEARMAN KARBER METHOD
LC50 AND 95% CONFIDENCE INTERVAL


GROWTH, REPRODUCTION OR FECUNDITY
POINT ESTIMATION
HYPOTHESIS TESTING (EXCLUDING CONCENTRATIONS ABOVE NOEC FOR SURVIVAL)
ENDPOINT ESTIMATE IC25, IC50
SHAPIRO-WILK'S TEST
NON-NORMAL DISTRIBUTION
NORMAL DISTRIBUTION
BARTLETT'S TEST
HOMOGENEOUS VARIANCE
HETEROGENEOUS VARIANCE
EQUAL NUMBER OF REPLICATES?
EQUAL NUMBER OF REPLICATES?
NO
YES
YES
NO
T-TEST WITH BONFERRONI ADJUSTMENT
DUNNETT'S TEST
STEEL'S MANY-ONE RANK TEST
WILCOXON RANK SUM TEST WITH BONFERRONI ADJUSTMENT
ENDPOINT ESTIMATES NOEC, LOEC

APPENDIX H

SINGLE CONCENTRATION TOXICITY TEST - COMPARISON OF CONTROL WITH 100% EFFLUENT OR RECEIVING WATER

1. To statistically compare a control with one concentration, such as 100% effluent or the instream waste concentration, a t-test is the recommended analysis. The t-test is based on the assumptions that the observations are independent and normally distributed and that the variances of the observations are equal between the two groups.

2. Shapiro Wilk's test may be used to test the normality assumption (see Appendix B for details). If the data do not meet the normality assumption, the nonparametric test, Wilcoxon's Rank Sum Test, may be used to analyze the data. An example of this test is given in Appendix F. Since a control and one concentration are being compared, the K = 1 section of Table F.5 contains the needed critical values.

3. The F test for equality of variances is used to test the homogeneity of variance assumption. When conducting the F test, the alternative hypothesis of interest is that the variances are not equal.

4. To make the two-tailed F test at the 0.01 level of significance, put the larger of the two variances in the numerator of F.

$$F = \frac{S_1^2}{S_2^2} \quad \text{where } S_1^2 > S_2^2$$

5. Compare F with the 0.005 level of a tabled F value with n_1 - 1 and n_2 - 1 degrees of freedom, where n_1 and n_2 are the number of replicates for each of the two groups.

6. A set of *Ceriodaphnia dubia* reproduction data from an effluent screening test will be used to illustrate the F test. The raw data, mean and variance for the control and 100% effluent are given in Table H.1.

TABLE H.1. *CERIODAPHNIA DUBIA* REPRODUCTION DATA FROM AN EFFLUENT SCREENING TEST —

	Replicate											
	1	2	3	4	5	6	7	8	9	10	X	S^2
Control	36	38	35	35	28	41	37	33	.	.	35.4	14.5
100% Effluent	23	14	21	7	12	17	23	8	18	.	15.9	36.6

7. Since the variability of the 100% effluent is greater than the variability of the control, S^2 for the 100% effluent concentration is placed in the numerator of the F statistic and S^2 for the control is placed in the denominator.

$$F = \frac{36.61}{14.55}$$

8. There are 9 replicates for the effluent concentration and 8 replicates for the control. Thus, the numerator degrees of freedom is 8 and the denominator degrees of freedom is 7. For a two-tailed test at the 0.01 level of significance, the critical F value is obtained from a table of the F distribution (Snedecor and Cochran, 1980). The critical F value

for this test is 8.68. Since 2.52 is not greater than 8.68, the conclusion is that the variances of the control and 100% effluent are homogeneous.

9. **EQUAL VARIANCE T-TEST**

9.1 To perform the t-test, calculate the following test statistic:

$$t = \frac{\bar{Y}_1 - \bar{Y}_2}{S_p \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Where: $\bar{Y}_1$ = Mean for the control

$\bar{Y}_2$ = Mean for the effluent concentration

$$S_p = \sqrt{\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2}}$$

S_1^2 = Estimate of the variance for the control

S_2^2 = Estimate of the variance for the effluent concentration

n_1 = Number of replicates for the control

n_2 = Number of replicates for the effluent concentration

9.2 Since we are usually concerned with a decreased response from the control, such as a decrease in survival or a decrease in reproduction, a one-tailed test is appropriate. Thus, compare the calculated t with a critical t, where the critical t is at the 5% level of significance with $n_1 + n_2 - 2$ degrees of freedom. If the calculated t exceeds the critical t, the mean responses are declared different.

9.3 Using the data from Table H.1 to illustrate the t-test, the calculation of t is as follows:

$$t = \frac{35.4 - 15.9}{5.13 \sqrt{\frac{1}{8} + \frac{1}{9}}} = 7.82$$

Where:

$$S_p = \sqrt{\frac{(8-1)14.5 + (9-1)36.6}{(8+9-2)}} = 5.13$$

9.4 For an 0.05 level of significance test with 15 degrees of freedom the critical t is 1.754 (Note: Table D.5 for K = 1 includes the critical t values for comparing two groups). Since 7.82 is greater than 1.754, the conclusion is that the reproduction in the 100% effluent concentration is significantly lower than the control reproduction.