

Appendix A

References

References

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Appendix B

Water Quality Benchmarks for Receiving Water

Water Quality Benchmarks for Receiving Water

Table 1. Narrative Objectives and Toxicity

Constituent	Watershed ^[1]	Narrative Objective ^[2]	Source
Flow	CC, SCR, VR	None	None
pH	CC, SCR, VR	The pH of inland surface waters shall not be depressed below 6.5 or raised above 8.5 as a result of waste discharges. Ambient pH levels shall not be changed by more than 0.5 pH units from natural conditions as a result of waste discharges.	Basin Plan (LARWQCB 1994)
Temperature	CC	For waters designated WARM, water temperature shall not be altered by more than 5°F above the natural temperature. At no time shall WARM-designated waters be raised above 80°F as a result of waste discharges.	Basin Plan (LARWQCB 1994)
	SCR, VR	For waters designated COLD, water temperature shall not be altered by more than 5°F above the natural temperature.	Basin Plan (LARWQCB 1994)
Dissolved Oxygen	CC	The dissolved oxygen content of all surface waters designated as WARM shall not be depressed below 5 mg/L as a result of waste discharges.	Basin Plan (LARWQCB 1994)
	SCR, VR	The dissolved oxygen content of all surface waters designated as COLD and SPWN shall not be depressed below 7 mg/L as a result of waste discharges.	Basin Plan (LARWQCB 1994)
Turbidity	CC, SCR, VR	Waters shall be free of changes in turbidity that cause nuisance or adversely affect beneficial uses. Increases in natural turbidity attributable to controllable water quality factors shall not exceed the following limits: <ul style="list-style-type: none"> Where natural turbidity is between 0 and 50 NTU, increases shall not exceed 20%; Where natural turbidity is greater than 50 NTU, increases shall not exceed 10%. 	Basin Plan (LARWQCB 1994)
Total Suspended Solids (TSS)	CC, SCR, VR	Wastes shall not contain suspended material in concentrations that cause nuisance or adversely affect beneficial uses.	Basin Plan (LARWQCB 1994)
Pesticides	CC, SCR, VR	No individual pesticide or combination of pesticides shall be present in concentrations that adversely affect beneficial uses.	Basin Plan (LARWQCB 1994)
Toxicity	SCR, VR	All waters shall be free of toxic substances in concentrations that are toxic to, or that produce detrimental physiological responses in human, plant, animal or aquatic life. There shall be no chronic toxicity in ambient waters outside mixing zones.	Basin Plan (LARWQCB 1994)
Toxicity	CC	1.0	TMDL ^[3]

[1] CC = Calleguas Creek Watershed SCR = Santa Clara River Watershed VR = Ventura River Watershed

[2] "Natural" or "ambient" conditions must be determined on a case-by-case basis.

[3] *Total Maximum Daily Load for Toxicity, Chlorpyrifos and Diazinon in Calleguas Creek, its Tributaries and Mugu Lagoon* (July 2005).

Table 2. Salts and Nutrients (all values listed in units of mg/L, unless otherwise noted)

Watershed Reach	Chloride ^[1]	Sulfate ^[1]	TDS ^[1]	Nitrogen ^[2]	Ammonia ^[3]	Phosphate
VR Between Camino Cielo and Casitas Vista Rd.	60	300	800	5	Dependent on pH, temperature	None
SCR Reach 1	None	None	None	None	None	None
SCR Reach 2	150	600	1200	10 ^{T2}	[see Nitrogen]	None
SCR Reach 3	100 ^{T1}	650	1300	10 ^{T2}	[see Nitrogen]	None
SCR Reach 4	100	600	1300	10 ^{T2}	[see Nitrogen]	None
CC Reach 3	1300 lbs/day ^{T4}	250	850	9 ^{T3}	Dependent on pH, temperature	None
CC Above Potrero Rd.	150	250	850	9 ^{T3}	Dependent on pH, temperature	None

Watersheds: VR = Ventura River SCR = Santa Clara River CC = Calleguas Creek

[1] All chloride, sulfate and TDS objectives listed are contained in the Basin Plan, Table 3-8 unless indicated by “T”, which indicates a TMDL Load Allocation (LA) – see indicated TMDL footnotes below.

[2] The Nitrogen objective listed for VR (Basin Plan) is as Nitrate-N + Nitrite-N.

[3] Ammonia objectives for VR (COLD, SPWN) and CCW (WARM) are based on the April 2002 Basin Plan Amendment equations and are calculated based on the pH and temperature of the receiving water measured at the time of sample collection for ammonia analysis (COLD = the cold freshwater habitat beneficial use as defined in the Basin Plan; SPWN = the spawning, reproduction and/or early development beneficial use as defined in the Basin Plan; WARM = warm freshwater habitat beneficial use as defined in the Basin Plan.) Ammonia objectives are 30-day averages.

T1: SCR Reach 3 Chloride TMDL: Amendment to the *Water Quality Control Plan for the Los Angeles Region* to Update the Chloride Objective for Reach 3 at Santa Paula in the Lower Santa Clara River (LARWQCB, November 6, 2003).

T2: SCR Reaches 2, 3, 4 Nitrogen TMDL: Amendment to the *Water Quality Control Plan for the Los Angeles Region* to Include a TMDL for Nitrogen Compounds in the Santa Clara River (LARWQCB, August 7, 2003). Load Allocation listed is as Ammonia-N + Nitrate-N + Nitrite-N.

T3: CCW Nitrogen TMDL: Amendment to the *Water Quality Control Plan for the Los Angeles Region* to Include a TMDL for Nitrogen Compounds and Related Effects in Calleguas Creek (LARWQCB, October 24, 2002). Load Allocation listed is as Nitrate-N + Nitrite-N.

T4: CCW Chloride TMDL: Total Maximum Daily Load for Chloride – Calleguas Creek Watershed (USEPA, March 22, 2002). No Load Allocations are in effect under storm conditions.

Table 3. Organochlorine Pesticides (all values listed in units of ug/L)

Constituent	CC, SCR Watersheds		VR Watershed	
	Benchmark	Source ^[1]	Benchmark	Source ^[1]
Aldrin	0.00014	CTR HHO	0.00013	CTR HHWO
Alpha-BHC	0.013	CTR HHO	0.0039	CTR HHWO
Beta-BHC	0.046	CTR HHO	0.014	CTR HHWO
Gamma-BHC (Lindane)	0.063	CTR HHO	0.019	CTR HHWO
Delta-BHC	None	None	None	None
Chlordane-alpha	None	None	None	None
Chlordane-gamma	None	None	None	None
Chlordane, sum	0.00059	CTR HHO	0.00057	CTR HHWO
2,4' -DDD	None	None	None	None
2,4' -DDE	None	None	None	None
2,4' -DDT	None	None	None	None
4,4' -DDD	0.00084	CTR HHO	0.00083	CTR HHWO
4,4' -DDE	0.00059	CTR HHO	0.00059	CTR HHWO
4,4' -DDT	0.00059	CTR HHO	0.00059	CTR HHWO
Dieldrin	0.00014	CTR HHO	0.00014	CTR HHWO
Endosulfan I	0.056	CTR AFWC	0.056	CTR AFWC
Endosulfan II	0.056	CTR AFWC	0.056	CTR AFWC
Endosulfan Sulfate	240	CTR HHO	110	CTR HHWO
Endrin	0.036	CTR AFWC	0.036	CTR AFWC
Endrin Aldehyde	0.81	CTR HHO	0.76	CTR HHWO
Endrin Ketone	None	None	None	None
Toxaphene	0.0002	CTR AFWC	0.0002	CTR AFWC

Watersheds: CC = Calleguas Creek SCR = Santa Clara River VR = Ventura River

[1] CTR = California Toxics Rule (USEPA, May 18, 2000).

HHO = Human Health for Consumption of Organisms Only (30-day average)

HHWO = Human Health for Consumption of Water and Organisms (MUN-designation) (30-day average)

AFWC = Aquatic Life, Freshwater Chronic (4-day average)

Table 4. Organophosphorus Pesticides (all values listed in units of ug/L)

Constituent	Calleguas Creek Watershed		SCR, VR Watersheds	
	Benchmark	Source ^[1]	Benchmark	Source
Bolstar	None	None	None	None
Chlorpyrifos	0.810	TMDL	None	None
Demeton	None	None	None	None
Diazinon	0.138	TMDL	None	None
Dichlorovos	None	None	None	None
Dimethoate	None	None	None	None
Disulfoton	None	None	None	None
Ethoprop	None	None	None	None
Fenchlorophos	None	None	None	None
Fensulfothion	None	None	None	None
Fenthion	None	None	None	None
Malathion	None	None	None	None
Merphos	None	None	None	None
Methyl Parathion	None	None	None	None
Mevinphos	None	None	None	None
Phorate	None	None	None	None
Tetrachlorvinphos	None	None	None	None
Tokuthion	None	None	None	None
Trichloronate	None	None	None	None

Watersheds: SCR = Santa Clara River VR = Ventura River

[1] Total Maximum Daily Load for Toxicity, Chlorpyrifos, and Diazinon in the Calleguas Creek, its Tributaries and Mugu Lagoon (LARWQCB, July 7, 2005).

Load Allocations listed for chlorpyrifos and diazinon are chronic, 4-day averages.

Table 5. Pyrethroid Pesticides (all values listed in units of ug/L)

Constituent	CC, SCR, VR Watersheds	
	Benchmark	Source
Bifenthrin	None	None
Cyfluthrin	None	None
Cyhalothrin	None	None
Cypermethrin	None	None
Deltamethrin/Tralomethrin	None	None
Esfenvalerate/Fenvalerate	None	None
Fenpropathrin	None	None
Fluvalinate	None	None
Permethrin	None	None
Resmethrin	None	None

Watersheds: SCR = Santa Clara River VR = Ventura River

Appendix C

Supporting Documents for Field Procedures

Appendix C

Attachment 1: Standard Operating Procedure for Flow Measurement

Current Measurement (Flow Measurement) Standard Operating Procedures

Version Date 07/20/06

If conditions safely permit, current-meter measurements are best made by wading. Measurements are made by recording velocity and depth at increments across the channel. The channel cross section should be defined such that:

1. It is perpendicular to the direction of flow.
2. Velocity and depth measurements should be spaced apart such that no more than 10% of the flow passes through any one cross section.

For water depths less than 2.5 ft, velocities are measured at a depth equal to 0.6 times the depth of the water at the measurement location, which is the theoretical depth at which the velocity is equal to the depth-averaged velocity. For water depths greater than 2.5 ft, velocities are measured at 0.2 and 0.8 times the depth, and the average is calculated and assumed to be equal to the depth-averaged velocity.

While taking velocity measurements, field personnel should stand in a position that least affects the velocity of the water passing the current meter. That position is usually obtained by facing the bank so that the water flows against the side of the leg. The current meter should be placed ahead of and upstream from the feet. In all cases, the wading rod, to which the current meter is affixed, should be held in a vertical position with the meter parallel to the direction of flow while the velocity is being observed. Personnel should avoid standing in the water if their feet and legs occupy a significantly large percentage of a narrow cross section. In very small streams, measurements should be taken while standing on the bank or an elevated plank or other support, rather than in the water.

When the flow is too low for a reliable measurement of discharge by current meter, typically one inch deep, the discharge is determined by use of (1) a volumetric method of measurement or (2) the float method, both of which are described below.

VOLUMETRIC MEASUREMENTS

Some monitoring locations may be free-flowing, which allows for collection of the entire flowing stream of water into a container of known volume. The time it takes to fill the known volume is measured using a stopwatch and recorded on the field log. The time it takes to fill the container should be measured three times and averaged to ensure that the calculated discharge is representative. For free-flowing outfalls, the estimated flow rate, Q , is calculated by:

$$Q = (\text{Filled-container volume}) / (\text{Average time to fill container})$$

FLOAT MEASUREMENTS

In cases where flows are too shallow to use a current meter and it is not possible to collect the entire flow into a container, a float and stopwatch may be used. Typically, floats consist of debris collected near the site (e.g. a leaf) or objects that are already floating in the stream of water. The width of the flowing water (not the entire part of the channel that is damp) is measured using a tape measure, along with the depth of the flowing stream in the middle of the channel. The average velocity of the stream is calculated by measuring the time it takes the float to travel a pre-measured distance, normally 10 feet, at least three times and recorded on the field log.

For sheet flows, the estimated flow rate, Q, is calculated by:

$$Q = f \times (\text{Flowing Width}) \times (\text{Water Depth}) \times (\text{Average Velocity})$$

The coefficient f is used to account for friction effects of the channel bottom. The value of f typically ranges from 0.60 – 0.90.

Appendix C

Attachment 2: Standard Operating Procedure for Water Sample Collection

Ambient Water Sample Collection Standard Operating Procedures

Version Date 07/20/06

Monitoring Event Preparation

Monitoring event preparation includes preparation of field equipment, placing bottle orders, and contacting the necessary personnel regarding site access and schedule. The following steps shall be completed two weeks prior to each sampling event:

1. Contact laboratories to order containers and to coordinate sample transportation details.
2. Confirm scheduled monitoring date with field crew(s), and set-up sampling day itinerary including sample drop-off.
3. Prepare equipment (see Table 1).
4. Prepare container labels and apply to containers.
5. Prepare the monitoring event summary and field log sheets to indicate the type of field measurements, field observations and samples to be collected at each of the stations.
6. Verify that field measurement equipment is functioning properly (*i.e.*, check batteries, calibrate, etc.)

Table 1 provides a checklist of field equipment to prepare prior to each sampling event.

Table 1. Field Equipment Checklist

X	Monitoring Plan	X	Tape Measure	X	Clean Secondary Container(s)
X	Sample Containers plus Extras with Extra Lids	X	Paper Towels or Rags in a Box	X	Peristaltic Pump
X	Pre-Printed and Extra Labels	X	Safety Equipment	X	Extra Pump Batteries
X	Event Summary Sheets	X	First Aid Kit	X	1 length of Clean Tubing per Site
X	Field Log Sheets	X	Cellular Telephone	X	Field Measurement Equipment
X	Chain of Custody Forms	X	Gate Keys		
X	Bubble Wrap	X	Hip Waders		
X	Coolers with Ice	X	Plastic Trash Bags		
X	New Powder-Free Nitrile Gloves	X	Distilled/DI Wash Bottles		
X	Pens	X	Blank Water		
X	Stop Watch	X	Sealable Plastic Bags		
X	Camera	X	Grab Pole		

Monitoring Event Summary and Post Event Summary

A monitoring event summary sheet will be produced for the sampling crew prior to each sampling event. The event summary sheet shall outline sampling requirements at each monitoring site, including a list of samples to be collected and quality control (QC) sample collection requirements. This summary will act as a guide to help field crews prepare for and track sample collection during each event. Additionally, the event summary sheet will list required containers and processing and storage requirements.

A post monitoring event summary report will be produced by the field crew subsequent to each monitoring event. This summary will serve as a guide for quality assurance personnel to qualify data. The post event summary will contain chain-of-custody (COC) forms submitted with samples and field log sheets.

Bottle Order/Preparation

Sample container orders will be placed with the appropriate analytical laboratory at least two weeks prior to each sampling event. Containers will be ordered for all water samples, including quality control samples, as well as extra containers in case the need arises for intermediate containers or a replacement. The containers must be the proper type and size and contain preservative as appropriate for the specified laboratory analytical methods.

The field crew must inventory sample bottles upon receipt from the laboratory to ensure that adequate bottles have been provided to meet analytical requirements for each monitoring event. After each event, any bottles and tubing used to collect water samples will be cleaned by the laboratory and either picked up by or shipped to the field crew.

Sample Container Labeling

All samples will be pre-labeled before each sampling event to the extent practicable. Pre-labeling sample bottles and jars simplifies field activities, leaving only sample collection time and date and field crew initials to be filled out in the field. Custom labels will be produced using blank water-proof labels. This approach will allow the stations and analytical constituent information to be entered in advance and printed as needed prior to each monitoring event.

Labels will be applied to the appropriate sample containers in a dry environment, as labels usually do not adhere to wet bottles. The labels will not be applied to container caps. Field labels shall contain the following information:

<ul style="list-style-type: none"> • Program Name • Station ID • Sample ID 	<ul style="list-style-type: none"> • Date • Time • Sampling Personnel 	<ul style="list-style-type: none"> • Analytical Requirements • Preservation Requirements • Laboratory Conducting Analysis
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Sample Collection

Sampling Technique

Samples will be collected in a manner that minimizes the possibility of sample contamination. These sampling techniques are summarized below:

- Samples are collected only into rigorously pre-cleaned sample containers.
- At least two persons are required on a sampling crew.
- Clean, powder-free nitrile gloves must be worn while collecting samples and must be changed whenever something not known to be clean has been touched.

- To reduce the potential for contamination and to ensure crew safety, field crews must observe the following precautions while collecting samples:
 1. Smoking is prohibited.
 2. Collecting samples near a vehicle, running or otherwise, is prohibited.
 3. Eating or drinking during sample collection is prohibited.
 4. Sampling personnel should avoid breathing, sneezing or coughing in the direction of an open sample container.
 5. Do not allow rain water to drip from rain gear or any other surface into sample containers.
 6. Do not eat or drink during sample collection.
 7. Do not breathe, sneeze or cough in the direction of an open sample container.

Water Sample Collection

Grab samples will be collected at approximately mid-stream, mid-depth at the location of greatest flow (where feasible) by direct submersion of the sample container. This is the preferred method for grab sample collection. However, due to sampling station configurations and safety concerns, direct filling of sample containers may not always be feasible, especially during wet events. Monitoring station configuration will dictate grab sample collection technique. Grab samples will be collected directly into the appropriate containers as outlined in the Project QAPP.

The grab sample techniques that may be employed are described below.

Direct Submersion: Hand Technique

Where practical, all grab samples will be collected by direct submersion at mid-stream, mid-depth using the following procedures.

1. Wear clean powder-free nitrile gloves when handling containers and lids. Change gloves if soiled or if the potential for cross-contamination occurs from handling sampling materials or samples.
2. Use pre-labeled sample containers as described in the Sample Container Labeling section.
3. Remove the lid, submerge the container to mid-stream/mid-depth, let the container fill and secure the lid.
4. Place sample on ice.
5. Collect the remaining samples including quality control samples, if required, using the same protocols described above.
6. Fill out the COC form, note sample collection time on the field log sheet, and deliver samples to the appropriate laboratory.

Intermediate Container Technique

Samples for which the introduction of a secondary container is acceptable, and which will be collected from an open channel, may be collected with the use of a specially cleaned

intermediate container following the steps listed below. A secondary container could include a container of similar composition to the sample container or a pre-cleaned pitcher of the same material as the sample container.

1. Wear clean powder-free nitrile gloves when handling bottles and lids. Change gloves if soiled or if the potential for cross-contamination occurs from handling sampling materials or samples;
2. Use pre-labeled sample containers as described in the Sample Container Labeling section;
3. Submerge the intermediate container to mid-stream/mid-depth (if possible), let the container fill, and quickly transfer the sample into the individual sample container(s) and secure the lid(s);
4. Place the sample(s) on ice;
5. Collect remaining samples including quality control samples, if required, using the same protocols described above; and,
6. Fill out the COC form, note sample collection time on the field log sheet, and deliver the samples to the appropriate laboratory.

Pumping

Samples for which the use of a peristaltic pump is acceptable and/or necessary because of sampling station configuration, and which will be collected from an open channel, may be collected with the use of a peristaltic pump and specially cleaned tubing following the steps listed below. Pumping may not be used to collect samples analyzed for ammonia.

1. Wear clean powder-free nitrile gloves when handling bottles, lids, and pump tubing. Change gloves if soiled or if the potential for cross-contamination occurs from handling sampling materials or samples;
2. Use pre-labeled sample containers as described in the Sample Container Labeling section;
3. Attach pre-cleaned tubing into the pump, exercising caution to avoid allowing tubing ends to touch any surface known not to be clean. A separate length of clean tubing must be used at each sample location for which the pump is used;
4. Place one end of the tubing below the surface of the water. To the extent possible, avoid placing the tubing near the bottom of the channel so that settled solids are not pumped into the sample container.
5. Hold the other end of the tubing over the opening of the sample container, exercising care not to touch the tubing to the sample container.
6. Pump the necessary sample volume into the sample container and secure the lid;
7. Place the sample on ice;
8. Collect remaining samples including quality control samples, if required, using the same protocols described above; and
9. Fill out the COC form, note sample collection time on the field log sheet, and deliver the samples to appropriate laboratory.

Field Measurements and Observations

Field measurements (listed in **Error! Reference source not found.**) will be collected and observations will be made at each monitoring site after all samples associated with the site are collected. Field measurements will include flow, pH, temperature, dissolved oxygen, turbidity, and conductivity. Measurements (except for flow) will be collected at approximately mid-stream, mid-depth at the location of greatest flow (if feasible). All field measurement results and comments regarding site observations will be recorded on a field log sheet for each site.

Flow measurements will be collected using a velocity meter or will be estimated at each sampling station after all other samples are collected. When a velocity meter is unavailable or flow is not sufficiently deep to use a velocity meter, depth, width, and velocity will be estimated to provide an estimate of flow. Depth will be estimated using the average of several depth measurements taken across the width of the channel. Width will be measured by extending a tape measure from one bank to the other. Velocity will be estimated by measuring the time it takes a floating object (*e.g.*, stick, orange peel) to travel a known distance.

If at any time the collection of field measurements by wading appears to be unsafe, field crews will not attempt to collect mid-stream, mid-depth measurements. Rather, field measurements will be made either directly from a stable, unobstructed area at the channel edge, or by using a telescoping pole and intermediate container to obtain a sample for field measurements and for filling sample containers.

In addition to field measurements, observations will be made at each monitoring site. Observations will include color, odor, floating materials as well as observations of contact and non-contact recreation. All comments on field observations will be recorded on the field log sheet.

Field Protocols

Field crews (2 persons per crew, minimum) will be mobilized for sampling only when weather conditions and flow conditions are considered to be safe. For safety reasons, sampling will be scheduled to occur during daylight hours. Sampling events will proceed in the following manner:

1. Before leaving the base of operations, confirm number and type of sample bottles as well as the complete equipment list.
2. Proceed to the first monitoring site.
3. Record the general information on the field log sheet.
4. Collect the samples indicated on the event summary sheet in the manner described in this QAPP. Collect additional volume and blank samples for field-initiated Quality Control (QC) samples as necessary. Place filled sample containers in coolers and carefully pack and ice samples as described in this QAPP. Using the log sheet, confirm that all appropriate bottles were filled.
5. Collect field measurements and observations, and record these on the field log sheet.

6. Repeat the procedures in steps 3, 4, and 5 for each of the remaining monitoring sites.
7. Complete the chain of custody forms using the field log sheets.
8. After sample collection is completed at all monitoring sites, deliver and/or ship samples to the appropriate laboratory.

Appendix D

Supporting Documents for Toxicity Testing

Appendix D

Attachment 1: Standard Operating Procedure for Chronic *Ceriodaphnia dubia* Bioassay

Revision #2 (Date last modified: 1/14/05 8:06 PM by Pacific EcoRisk)

Effective Date: December 8, 2002

Accepted: _____

***Ceriodaphnia dubia* (Cerio)**

Chronic Survival and Reproduction Bioassay

Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA Guidelines described in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA-821-R-02-013 and EPA-600-4-91-002)

1.0 INTRODUCTION

This test is based on a seven-day static-renewal exposure of < 24 hr old (neonate, first instar) *Ceriodaphnia dubia* to different concentrations of effluents and/or receiving waters. The test endpoints are survival and reproduction.

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Food: Selenastrum, YCT and the vitamins Thiamin, Biotin and B₁₂
2. Control/dilution (80:20) Water: Calistoga spring water and Evian spring water, mixed at a ratio of 80:20, respectively.
3. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
4. Thermometer: ASTM certified, for documenting test water temperature.
5. Beakers: (2) 1-L beakers and (4) 250-mL beakers.
6. "Cerio" Test containers: 30-mL plastic cups. Cups must be appropriately-cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
7. Cerio board: Foam Board containing pre-cut holes to support replicate cups in waterbath.
8. Water quality test cups: 100-mL plastic cups for water quality analysis.
9. De-ionized water: for rinsing of probes, etc.
10. Wash bottles: for rinsing of probes, etc.
11. Volumetric and graduated flasks and pipettes: for making up dilution series and reference toxicant test solutions.
12. Transfer pipettes, wide-bore: for transfer of organisms to and from test containers.
13. Cubitainers may be necessary for the client's collection of effluent.
14. Temperature controlled water bath under cool white fluorescent lighting.
15. ACS reagent CuSO₄ (copper sulfate), for use as reference toxicant.

2.2 Ordering and Holding of Test Organisms

1. Test organisms should be ordered far enough in advance so as to ensure arrival of < 24 hrs old animals on test set-up day. Approximately 25-33% more animals should be ordered than are actually needed for the test, so as to allow for some attrition of organisms that are stressed from the shipping, etc.

Order cerios from: - Aquatic Research Organisms (603) 926-1650

2. For additional instruction on the receipt and handling of the test organisms, see the “**Test Organism Receipt and Handling S.O.P.**”
3. Alternatively, <24 hour old neonates can be obtained from in-house Stock Cultures (See ***Ceriodaphnia dubia* Culture Maintenance SOP** for methods).

2.3 Collection and Holding of Water Samples

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or “blue ice type product) to bring the temperature to 4°C. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in water quality measurements should be taken. For instruction on the log-in of incoming samples, see the “**Test Sample(s) Log-In Procedures**”. The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 1 Liter of sample will be needed each day.

3.0 TEST INITIATION

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read the attached “**Summary of Test Conditions for *Ceriodaphnia dubia***”.

1. Label (1) 1-L beaker for the Control treatment and (1) 1-L beaker for the 100% sample treatment.
2. Fill the Control beaker with 800 mL of Control water and fill the 100% sample beaker with 600 mL of the 100% sample; volume requirements may need to be adjusted for each client. Check the D.O. level of the Control water and the 100% sample and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels.
3. Add food into the 800 mL of Control and 600 mL of sample water:
 - a. *Selenastrum* (algae) (13 mL/L): 10.4 mL in Control; 7.8 mL in 100% sample
 - b. YCT (7 mL/L): 5.6 mL in Control; 4.2 mL in 100% sample
4. Label (4) 250-mL beakers with the appropriate intermediate test dilutions. See client NPDES permit for specific dilution requirements.
5. Prepare 10 replicate “cerio” test cups for each treatment dilution according to client requirements (e.g.: Control, 6.25%, 12.5%, 25%, 50%, 100%). Label the cups with their treatment and replicate I.D. (i.e. as A-J) using a Sharpie pen.
6. Label (1) water quality test cup (~100-mL size) for each treatment using a Sharpie pen.
7. Using the Control water and the 100% effluent that have been spiked with food, prepare

200-mL volumes of test solution at each of the intermediate test treatment concentrations.

8. For each treatment, dispense 25-50 mL into a water quality test cup and record the initial water quality (pH, D.O., conductivity, and temperature) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels.
9. Place the replicate “cerio” test cups onto a foam support board. Then, beginning with the Control treatment and working up through the concentration series, aliquot 15 mL of test media into each of the 10 replicate cups at each treatment **using a clean 60-cc syringe**.
10. Use the Sharpie pen and trace the water level line onto each container. Place the foam support board with the dilution containers into the water bath to allow test waters to acclimate to test temperature.
11. Identify 10 adult cerio females from the brood board cultures that have had 8 or more offspring within the past 24 hours. There must not be more than an 8-hour deviation in the ages of the neonates.
12. Using a wide-bore transfer pipette, *carefully* capture and randomly allocate 1 neonate cerio into each of the test containers. Each of the neonates from one adult will be used to load one replicate (e.g. all A replicates) from each treatment concentration (this is done so that any anomalies, such as a high proportion of males in a particular replicate, can be addressed by omitting that particular replicate from the statistical analysis).
 - a. The organisms are delicate. When transferring, release organisms under the surface of the water. Make sure that each individual is uninjured. Replace injured individuals.
 - b. Be consistent with the volume of stock water used to transfer the organisms. Take care to avoid excessive dilution of the test treatments.
13. Place the foam support board, now containing the test replicate cups, into a temperature controlled water bath at 25°C under cool-white fluorescent lighting on a 16L:8D photoperiod.
14. Place transparent plastic cover sheet over the top of the test replicate cups on the foam support board.
15. Record the water bath temperature onto the test data sheet.

4.0 MAINTAINING THE TEST

4.1 *Each day*

1. Check the D.O. level of the Control water and the highest concentration of treatment media and make sure that there is enough oxygen (at least 4.0 mg/mL); if not, you must aerate the sample until the D.O. reaches adequate levels.
2. Prepare the appropriate beakers, test cups, and test solutions with the appropriate amounts of food, as above in section 3.0 Test Initiation (steps 1-7).
3. Record the “new” water quality measurements (temperature, pH, D.O. and conductivity) before pouring into new cerio test cups (section 3.0, step 8).
4. Fill each new “cerio” test cup with 15 mL of new test solution using **a clean 60-cc syringe**

(section 3.0, step 9) and trace the water level onto each container (section 3.0, step 10).

5. Pull the foam support board containing the test replicate cups out of the water bath, and examine each replicate cup. Record observations of dead organisms onto the test data sheets.
6. Using a pipette, carefully transfer each original test organism into its designated new test replicate cup (see section 3.0, step 12).
7. Examine each old test replicate cup to determine the number of neonates (if any) and record the count onto the test data sheet. Discard neonates after counting. Save ~25-50 mL of old test solution for each treatment and measure the “old” pH and D.O., recording the data onto the test data sheets.
8. Place the test organisms within their new test replicate containers onto a foam support board, and return the test replicates to the water bath. Record the water bath temperature onto the test data sheets.

5.0 TEST TERMINATION

Test are terminated when 60% or more of the surviving females in the controls have produced their third brood or at the end of 8 days, whichever occurs first. All observations on organisms' survival and number of offspring should be completed within two hours of test termination.

1. Pull the foam support board containing the test replicate cups out of the water bath, and examine each replicate cup. Record observations of dead organisms onto the test data sheets.
2. Examine each old test replicate cup to determine the number of neonates (if any) and record the count onto the test data sheet. Save ~25-50 mL of old test solution for each treatment and measure the “old” pH and D.O., recording the data onto the test data sheets.
3. Count the number of total offspring produced by each individual test organism and record onto the test data sheets. Any animal not producing young should be examined to determine if it is male.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenance, and termination are identical to those above. Information regarding the *Ceriodaphnia* reference toxicity test is presented in the “**Chronic *Ceriodaphnia dubia* Reference Toxicity Test SOP**”.

7.0 DATA ANALYSIS

The two endpoint data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analysis are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Test acceptability criteria for the *Ceriodaphnia dubia* chronic test includes:

1. 80% or greater control survival
2. Production of three broods of offspring by >60% of surviving control females
3. An average of ≥ 15 offspring per surviving control female.
4. Identified males must be excluded for analysis of the reproduction endpoint, but may be used for the survival endpoint. If 50% or more of the surviving organisms in a replicate block are males, the entire replicate block is excluded from analysis of the reproduction endpoint.
5. The entire test is invalid if fewer than 8 control replicates remain after excluding males.

9.0 QUALITY CONTROL

1. Control water, consisting of a mixture of commercial spring waters for tests and cultures.
2. All equipment is calibrated and operated as described in each applicable equipment SOP.
3. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The *Ceriodaphnia dubia* chronic toxicity test poses little risk to those performing it. Care should be taken in the preparation of the reference toxicant spiking solution. Review the MSDS for the reference toxicant. After the ref-tox spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR CHRONIC <i>CERIODAPHNIA DUBIA</i> REPRODUCTION AND GROWTH TEST (TEST METHOD 1002.0)	
1. Test type	Static renewal
2. Temperature	25 ± 1°C
3. Light quality	Ambient laboratory illumination
4. Light intensity	50-100 ft-c (10-20 $\mu\text{E}/\text{m}^2/\text{s}$)
5. Photoperiod	16 hours light: 8 hours darkness
6. Test chamber size	30 mL
7. Test solution volume	15 mL
8. Renewal of test solutions	Daily
9. Age of test organisms	Less than 24 hour old neonates (8 hour release)
10. No. of organisms per test chamber	One
11. No. of replicate chambers per concentration	Ten
12. No. of organisms per concentration	Ten
13. Feeding regime	Algae(13mL/L) & YCT (7mL/L) 0.1 mL daily
14. Test chamber cleaning	New cups daily. Rinse with deionized water.
15. Test chamber aeration	None
16. Dilution water	According to NPDES permit.
17. Test Concentrations	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
18. Test duration	7 days or until 60% of the surviving females in the control have 3 broods
19. Test endpoint	% survival and reproduction
20. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours (no sample > 72 hours is used in test)
21. Test acceptability	80% control survival, 60% of surviving females in control have three broods, and an average of 15 neonates per surviving control female. Exclude males for repro.
22. Sample volume required	1 Liter per day

Appendix D

Attachment 2: Standard Operating Procedure for Chronic *Hyalella azteca* Bioassay

***Hyalella azteca* 10-Day Water Toxicity Test**

1.0 OBJECTIVE

In laboratory tests designed to determine the toxicity of low-salinity water samples, *Hyalella azteca* are exposed to test solutions for 10 days, after which the percentage mortality is determined. Observed effects may be related to the presence of contaminants or to naturally occurring factors. In order to correctly interpret toxicity results, concentrations of chemical contaminants should be analyzed, as well as other water quality parameters, such as dissolved oxygen, pH, conductivity, ammonia, hardness, alkalinity, and temperature.

In this procedure, water samples collected from field stations are divided into randomly numbered replicate test containers in the laboratory. Ten *H. azteca* are placed into each replicate container. Each beaker is monitored daily for mortality, and is renewed at 5 days. After a 10-day exposure, survival is counted and recorded to give an estimate of sample toxicity. Because the test measures effects on an early life-stage of an ecologically important species possessing relatively stringent water quality requirements, the results constitute a good basis for decisions concerning either hazard evaluation or the suitability of estuarine waters for aquatic life (EPA 1993).

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (MPSL SOP 1.3).

2.1 Culture

- Pipettes, tubing, and clean air system
- Short buckets or trays
- Granite Canyon well water ($23 \pm 1^\circ\text{C}$)
- YCT for feeding, purchased from Aquatic Biosystems (Fort Collins, CO)
- *Hyalella azteca* (9-14 days old, supplied by Chesapeake Cultures (Gloucester, VA))

2.2 Test Initiation/Termination

- Environmental chamber ($23 \pm 1^\circ\text{C}$, ambient laboratory illumination for 16 hours/day)
- 300-mL clean glass beakers (5 per sample)
- 250-mL clean plastic tripour beakers (3 per reference toxicant concentration)
- Clear plastic sheets to cover beakers
- 1000-mL volumetric flask for reference toxicant concentrations
- 10-mL and micropipettors and pipettes for reference toxicant concentrations

***Hyaella azteca* 10-Day Water Toxicity Test**

- Cadmium chloride stock solution (100,000 µg/L Cd)
- Randomization sheet to arrange and identify test containers
- Data sheets
- Gloves and appropriate safety gear (see MPSL lab safety manual)
- Sample vials for reference toxicant analysis (new polyethylene 30 mL, acid washed)
- Dissecting microscope for counting neonates
- Disposable plastic pipettes with cut-off tips (for handling animals)

2.3 Water Quality

- Meters, probes, spectrophotometer, digital titrator and standards for measuring pH, dissolved oxygen, hardness, alkalinity, ammonia, and conductivity
- Thermometers (glass spirit thermometer and continuously recording thermometer)
- Graduated pipettes (10 mL) and hand pipette pump for water quality sampling
- Water quality vials (30 mL glass)
- Gloves and appropriate safety gear (see MPSL lab safety manual)

2.4 Dilution Water

In every step of this procedure, use Granite Canyon well water.

3.0 EXPERIMENTAL DESIGN

Aquatic toxicity tests can be used as screening tools or as part of more comprehensive studies to assess water quality. Careful consideration must be given to site characteristics, reference site selection, field replication, choice of synoptic measures, seasonal factors, and comprehensive planning and peer review to determine that study designs are adequate to meet program objectives.

This laboratory toxicity test consists of five replicate test beakers for each sample concentration. Beakers are arranged randomly, and each receives ten *H. azteca*. The quality of test animals and testing conditions is determined through concurrent testing of reference toxicants (positive controls) and control water (negative controls). Testing of reference sites or receiving water is recommended to demonstrate the suitability of test sites in the absence of toxic contaminant concentrations. Test conditions of temperature and photoperiod are controlled as indicated below, and dissolved oxygen, pH, conductivity, and ammonia are measured at the beginning and end of the exposure. Temperature is measured continuously, and hardness and alkalinity are measured at the beginning of the test.

***Hyalella azteca* 10-Day Water Toxicity Test**

4.0 PREPARATION OF SAMPLES FOR TESTING

One day before test initiation, the volume necessary for test initiation should be placed in the constant temperature room (23°C) to allow oxygen concentrations to equilibrate below super-saturated levels; the minimum time should be 12 hours. Prepare five replicate 300-mL beakers for each sample to be tested. Consult the random number sheet to ensure proper randomization. Each container receives 100 mL of test solution.

5.0 CONTROLS

5.1 Dilution Control

The dilution control should consist of Granite Canyon well water.

5.2 Reference Toxicant Tests

For cultured organisms, conduct a concurrent reference toxicant at least monthly. The reference toxicant test indicates the sensitivity of the organisms and the suitability of the test methodology.

Reagent grade cupric chloride (CdCl_2) should be used as the reference toxicant for *H. azteca* tests, unless another toxicant is specified. Prepare a 100,000 $\mu\text{g/L}$ Cd stock solution by adding 0.1630 g of reagent grade CdCl_2 to a final volume of one liter of distilled water in a plastic volumetric flask. Cap tightly and mix thoroughly. Sample and log the reference toxicant stock solution at the beginning of the test for chemical verification of the copper concentration. Acidify samples for analysis in clean sample vials with 1% by volume 14N-reagent grade nitric acid..

Reference toxicant solutions should be three to five replicates of 0 (control), 5.6, 10, 18, 32, and 56 μg Cd/L. Other concentrations may be added between these if greater precision is desired for quality control chart purposes. Prepare 1000 mL of each concentration by adding stock solution (see dilution schedule) to a 1000-ml plastic volumetric flask and fill with culture water. Aliquot each concentration to randomly numbered test containers as indicated on the random number sheet, and into water quality vials. Start with the control solutions and progress to the highest concentration to minimize contamination. Place the reference toxicant test containers in the constant temperature room, cover, and equilibrate.

All tests (sample and reference toxicant) must use *H. azteca* from the same culture. They must be handled in the same way and delivered to the test containers at the same time.

***Hyalella azteca* 10-Day Water Toxicity Test**

6.0 TEST ORGANISMS

6.1 Laboratory Acclimation

Order amphipods to arrive between two and seven days before test initiation. Amphipods must be between 7 and 14 days old at test initiation. Place the amphipods in a culture tray containing well water at a temperature that varies by no more than 3°C from transport conditions. Acclimate the amphipods to test temperature and conductivity. Hold amphipods at test temperature for 48 hours prior to initiating sediment testing. Remove any dead or moribund animals. Make sure water in the tray is constantly aerated. Check the amphipods daily, and monitor the health of amphipods by observing appearance. If more than 5% of the amphipods appear unhealthy during the 48 hours prior to the test, reschedule the test and immediately arrange for another amphipod shipment. Renew the culture daily before the test with dilution water, and feed 10 mL YCT daily.

6.2 Amphipod Loading

Using a clean transfer pipette with the tip removed, transfer the amphipods from the culture tray into the test containers. Only transfer animals that are healthy and moving. Replace injured or stressed amphipods. Continue until each container has 10 animals. Maintain water temperature ($23 \pm 1^\circ\text{C}$) by sorting animals in the constant temperature room where the test is being held.

7.0 MONITORING THE TOXICITY TEST

7.1 Counting *Hyalella* Mortality

Test duration is 10 days. Check all test containers daily, and record the number of live animals. Also attempt to count the number dead to ensure that the total number of animals in the container at the start of the test was 10; if not, record this on the data sheet. Immobile *H. azteca* that do not respond to a stimulus are considered dead. The stimulus should be a gentle stream of water from a disposable pipette. *H. azteca* that exhibits a visible response is considered living. Remove dead animals.

7.2 Measuring Water Quality in Test Containers

Measure temperature, dissolved oxygen, pH, ammonia, and conductivity at the beginning and end of the test. Hardness and alkalinity should be measured at the beginning of the test. Sample the initial test solutions at the time of dilution preparation. Water quality should also be measured on old and new dilutions at the time of renewal. Renewal water quality parameters include dissolved oxygen, pH, conductivity, and temperature. Water quality should be measured only initially on reference toxicant tests.

***Hyalella azteca* 10-Day Water Toxicity Test**

7.3 Feeding

Test containers are fed 1.5 mL YCT every other day.

8.0 TEST SOLUTION RENEWAL

The test duration is 10 days. Because toxicity may change over short periods in test containers, the test solutions must be renewed after 5 days. Prepare new test solutions as in section 4. One day before solution renewal, the volume necessary for renewal should be placed in the constant temperature room (23°C) to allow oxygen concentrations to equilibrate below super-saturated levels. These samples must be aerated if dissolved oxygen concentrations exceed maximum values allowed.

9.0 TERMINATING THE TOXICITY TEST

After 10 days of exposure, final mortality counts are made.

Final water quality must be sampled at the termination of the test. Deliver a sample from each site into pre-labeled water quality containers. Measure and record temperature, dissolved oxygen, pH and conductivity of each sample. Only measure temperature for the reference toxicant test.

Take the completed data sheet to the office for data entry and analysis. Notify the data analyst that the data has arrived. Make sure the data sheets are placed in the proper location and that the person keeping track of the data knows where it is.

10.0 DATA HANDLING AND TEST ACCEPTABILITY

Immediately after test termination, check the data sheet to determine whether dilution water and conductivity controls have acceptable survival ($\geq 90\%$). If not, notify the project officer without delay. Tests with temperature, salinity, or dissolved oxygen measurements outside the specified ranges, may be considered conditionally acceptable based on the project officer's best professional judgment. Acceptable temperatures are $23 \pm 1^\circ\text{C}$; acceptable dissolved oxygen concentration is 60-100% saturation.

11.0 REFERENCES

US EPA. 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/4-90/027F. August 1993.

***Hyalella azteca* 10-Day Water Toxicity Test**

12.0 TEST SUMMARY

Species:	<i>Hyalella azteca</i>
Test Duration:	10 Days
Endpoint:	Survival
Renewals:	At day 5
Organism Source	Chesapeake Cultures (Gloucester, VA)
Age of Test Organisms:	7-14 Days
Test Salinity Range:	0-15 ‰
Test Temperature:	23 ± 1°C
Dilution Water:	Granite Canyon well water
Light intensity:	Ambient laboratory illumination (10-20 $\mu\text{E}/\text{m}^2/\text{s}$)
Photoperiod:	16 hour light: 8 hour dark
Replication:	5 (samples), 3 (reference toxicant)
Test Containers:	300-mL glass beakers
Test Solution Volume:	100 mL
Loading:	10 animals per beaker
Feeding:	In culture prior to test initiation and 1.5 mL YCT every other day
Water Quality:	pH, dissolved oxygen, temperature, conductivity, hardness, alkalinity, ammonia
Reference Toxicant:	cadmium chloride (CdCl_2)
Daily Monitoring:	count alive and remove dead
Acceptability Criteria:	mean survival in dilution water controls $\geq 90\%$

Appendix D

Attachment 3: Standard Operating Procedure for Chronic *Americamysis bahia* Bioassay

Revision #2 (Date last modified: 1/14/05 3:23 PM by Pacific EcoRisk)

Effective Date: December 8, 2002

Accepted: _____

Americamysis bahia
Chronic (7-Day) Survival Growth and Fecundity Bioassay
Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA Guidelines described in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition (EPA-821-R-02-014). See Addendum, for tests that require the guidelines set forth in previous editions (EPA/600/4-87/028 and EPA-600-4-91-003).

1.0 INTRODUCTION

This test is based on a seven-day static-renewal exposure of 7 day old *Americamysis bahia* to different concentrations of effluents and/or receiving waters during the life period when eggs are produced by the females. The test endpoints are survival, growth (measured as dry weight) and fecundity (measured as the number of mature females with eggs in the oviduct and brood sac).

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Food: Brine shrimp nauplii - The test organisms will need to be fed brine shrimp (*Artemia*) nauplii at least twice per day. These nauplii should be produced with in-house cultures that will require brine shrimp eggs, seawater (natural or artificial), and egg incubation containers. Incubation of the eggs should begin long enough prior to receiving the test organisms so as to assure a ready supply of newly-hatched nauplii as needed.
2. Aeration System: needed for aeration when D.O. drops below acceptable levels.
3. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
4. Thermometer: ASTM certified, for documenting test water temperature.
5. Test Containers: 400-mL glass beakers or 200-mL disposable plastic cups. Cups must be appropriately cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
6. De-Ionized water: for rinsing of probes, etc.
7. Wash Bottles: for rinsing of probes, etc.
8. Volumetric and Graduated Flasks and Pipettes: for making up dilution series and reference toxicant test solutions
9. Wide-bore transfer pipettes or small handheld dip nets: for transfer of organisms to and from test containers.
10. NITEX mesh sieves (150 μm & 500 μm); for concentrating organisms.
11. Dissecting microscope, for examination of organisms and enumeration of females with brood sacs.

12. Depression slide, for microscopic examination of live mysids.
13. Balance: capable of weighing to 0.01 mg.
14. Reference Weights: for calibration of balance.
15. Drying Oven: for drying fish at 105°C at test termination.
16. Desiccator: for holding dried fish.
17. Forceps: for transfer of organisms to weighing pans.
18. Aluminum Foil Weighing Pans: for drying and weighing of fish.
19. Cubitainers: may be necessary for the client's collection of effluent.
20. Artificial Sea Salt (Crystal Sea®): for salting up of effluent to acceptable test salinity.
21. ACS Reagent K₂CrO₄ (Potassium Dichromate), for use as reference toxicant.

2.2 *Ordering and Holding of Test Organisms*

1. Test organisms should be ordered far enough in advance so as to ensure arrival of 7 day old animals by the day of test set-up. Approximately 25-33% more animals should be ordered than are actually needed for generation of larvae, so as to allow for some attrition of organisms that are stressed from the shipping, etc.
Order mysids from: (1) - Aquatic Indicators: (904) 829-2780
(2) - Aquatic Biosystems Inc.: (303) 223-2938
(3) - Aquatox (501)767-9120
2. Order the juvenile mysids to be pre-adapted to the test salinity. This is important as the supplier may be culturing the mysids at a different salinity than the desired test salinity. If the mysids come in at a “non-test” salinity, they must begin acclimation ASAP. Place them in control water at the receiving salinity and immediately begin the adjust the holding salinity towards the test salinity.
3. For additional instruction on the receipt and handling of the test organisms, see the “**Test Organism Receipt and Handling SOP**”.

2.3 *Collection and Holding of Water Samples*

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or “blue ice type product) to bring the temperature to 4°C. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in water quality measurements should be taken. For instruction on the log-in of incoming samples, see the “**Test Sample(s) Log-In Procedures**”. The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 3 Liters of sample will be needed each day.

3.0 **TEST INITIATION**

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read

the attached “**Summary of Test Conditions for *Americamysis bahia***”.

1. Mysids should be fed artemia at least two hours prior to the start of the test.
2. Label an appropriate number of 400-mL beakers with the appropriate test treatments for your dilution series (e.g.: control, 5%, 10%, 25%, 50%, and 100%). You will need **8 replicate containers** per treatment. Label the beakers with their treatment and replicate I.D. using a Sharpie pen. See client NPDES permit for specific dilution requirements.
3. Label test cups for water quality measurements with treatment and replicate I.D. using a Sharpie pen.
4. Prepare dilutions (as needed) with filtered seawater or appropriately salted receiving water according to client NPDES permit. Always work from low to high concentration and rinse out any glassware 3X with dH₂O and one final with seawater prior to use.
5. For each treatment, record the initial water quality (pH, D.O., salinity, conductivity and temperature) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels.
6. Then, beginning with the Control treatment and working up through the concentration series, pour 150 mL of sample water into each of the 8 containers.
7. Use the Sharpie pen and trace the water level line onto each container (if using cups).
8. Clean the tank holding the test organisms as much as possible (i.e., remove uneaten food and any dead mysids) minimizing any disturbance of the live animals.
9. Using a net or sieve, concentrate some of the animals into a corner of the holding tank or into a smaller container of control water. Using a wide-bore transfer pipette, capture and randomly allocate **5 mysids** into each of the test containers following the randomization template.
 - a. The organisms are delicate. When transferring, release organisms under the surface of the water. Make sure that each individual is uninjured. Replace injured individuals.
 - b. Be consistent with the volume of water used to transfer the organisms. Take care to avoid excessive dilution of the test treatments. Note any excess dilution of the test solution.
10. Randomly place the test containers within the temperature-controlled water bath (26 ± 1°C) under a 16-hour light, 8-hour dark photoperiod at a light intensity of 50-100 foot-candles. Make sure that all of the necessary data are recorded upon the data sheets.

4.0 MAINTAINING THE TEST

1. The test organisms should be fed newly-hatched brine shrimp nauplii first thing in the morning.
2. At the time of the media renewal, pull the test containers from the water bath and arrange, in replicate # order, and by treatment. From one randomly selected container at each treatment, measure the “old” temperature, pH, D.O. and salinity/conductivity. Check these measurements to make sure that the water quality is within acceptable limits.
3. Remove the uneaten food and any dead animals. This can be accomplished either of two

methods: (1) siphon out the debris and approximately 80% of the “old” test media from each replicate container, being careful not to accidentally siphon any of the live animals; (2) use a transfer pipette to squirt water across the bottom of the replicate container, stirring up debris from the bottom in the process, and then quickly pour approximately 80% of the “old” media out, being careful not to pour out any live animals.

4. Count the number of live animals in each replicate and record the number on the data sheet.
5. Check the D.O. level of the Control water and the highest concentration of treatment media and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels. Carefully (and slowly) pour fresh media back into the replicate containers until the water level is at the 150-mL line. In order not to stress the animals while pouring in this media, tilt the containers and slowly pour in the new media down the side of the tilted container.
6. From one randomly selected replicate container at each treatment (beginning with the Control, and working upward through the concentration series), measure the “new” pH, D.O., and salinity/conductivity. Record these data on the data sheet and randomly place the replicate beakers back in the water bath. Sometime between 3 p.m. and 5 p.m., feed each replicate container with newly-hatched brine shrimp nauplii.

5.0. TEST TERMINATION

1. After 7 days, pull the test containers from the water bath. Measure and record the “old” pH, D.O., and salinity/conductivity. Count and record the number of live animals in each replicate container and remove the dead ones. Siphon or pour out approximately 90% of the old media and replace with control water.
2. Examine the **live** animals within 12 hours of termination. Using a microscope (240X), determine the number of immature animals, sex of the mature animals, and the presence or absence of eggs in the oviduct or brood sacs for surviving females. Record data on the data sheet.
3. Pour the remaining test solution containing the surviving mysids into a <300 μ m mesh screen submerged in deionized water so as to wash away debris that may alter final weights.
4. Euthanize the organisms from each replicate in an ice-bath with filtered seawater, rinse in de-ionized water and transfer the organisms onto a pre-dried and pre-weighed aluminum drying pan (the pans should be weighed as per the **Weighing of Test Organisms S.O.P.**). When all of the replicates have been transferred into their respective drying pans, place the pans into the drying oven, and dry at 105°C for at least 6 hrs.
5. After drying, place the aluminum pans into the dessicator and seal. Allow to cool at least 4 hrs, after which each pan must be weighed and the weight data recorded. The weight recorded for the empty pans minus the weight of the pans + dried animals = the pooled dry weight of the organisms for that replicate. Divide this number by the number of organisms in the replicate to obtain the mean dry weight for individual mysids in that replicate. For the control treatment, calculate the mean weight per surviving fish for each replicate to determine if the weights met test acceptability criteria.

6. At this point, there should be two endpoint data for each replicate: percentage survival and mean dry weight per individual.
7. Notify client immediately if toxicity is observed.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenance, and termination are identical to those above. Information regarding the *Americamysis bahia* reference toxicity test is presented in the “**Chronic (7-day) *Americamysis bahia* Reference Toxicity Test SOP**”.

7.0 DATA ANALYSIS

The three endpoint data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Test acceptability criteria for the *Americamysis bahia* chronic test are as follows: (1) 80% or greater survival in the control; (2) average dry weight of 0.20 mg/individual in controls; and (3) fecundity can be used if >50% of females in controls produce eggs.

9.0 QUALITY CONTROL

1. Control water, consisting of deionized water salted up to test salinity with Crystal Sea® sea salt, is used.
2. The test mysids shall be maintained in the laboratory 4-7 days prior to use in tests in order to monitor and examine them for health and quality.
3. All equipment is calibrated and operated as described in each applicable equipment SOP.
4. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The *Americamysis bahia* 7-day chronic toxicity test poses little risk to those performing it. Care should be taken in the preparation of the reference toxicant spiking solution. Review the MSDS for the reference toxicant. After the ref tox spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR MYSID, <i>AMERICAMYSIS BAHIA</i> , LARVAL SURVIVAL, GROWTH, AND FECUNDITY TEST (TEST METHOD 1007.0)	
1. Test type	Static renewal
2. Test duration	7 days
3. Salinity	20 to 30 ppt \pm 2 ppt
4. Temperature	26 \pm 1°C
5. Light quality	Ambient laboratory illumination
6. Light intensity	50-100 ft-c (10-20 μ E/m ² /s)
7. Photoperiod	16 hours light: 8 hours darkness
8. Test chamber size	400-mL
9. Test solution volume	150 mL
10. Renewal of test solutions	Daily
11. Age of test organisms	7 days old
12. No. of organisms per test chamber	Five (5)
13. No. of rep. chambers per concentration	Eight (8)
14. No. of organisms per concentration	Forty (40)
15. Feeding regime	Artemia nauplii twice daily
16. Test chamber cleaning	Siphon daily, immediately before test solution renewal
17. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
18. Dilution water	According to NPDES permit. Filtered (1 μ m) seawater, dH ₂ O salted up with Crystal Sea [®] sea salt.
19. Test concentration	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
20. Dilution factor	According to NPDES permit. None, or \geq 0.5 dilution series
21. Test endpoint	% survival and growth
22. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours (no sample > 72 hours is used in test)
23. Sample volume required	3 Liters per day
24. Test acceptability	80% or greater average survival in controls; average weight of control animals \geq 0.2 mg; fecundity may be used if > 50% of control females produce eggs.

Appendix D

Attachment 4: Standard Operating Procedure for Chronic *Pimephales promelas* Bioassay

Revision #2 (Date last modified: 1/14/05 8:05 PM by Pacific EcoRisk)

Effective Date: December 8, 2002

Accepted: _____

***Pimephales promelas* (Fathead Minnow)**
Chronic (7-Day) Survival and Growth Bioassay
Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA Guidelines described in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA-821-R-02-013 and EPA-600-4-91-002).

1.0 INTRODUCTION

This test is based on a seven-day static-renewal exposure of < 24 hr old *Pimephales promelas* to different concentrations of effluents and/or receiving waters during the larval life period. The test endpoints are survival and growth (measured as dry weight).

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Food: Brine shrimp nauplii - The test organisms will need to be fed brine shrimp (*Artemia*) nauplii at least twice per day. These nauplii should be produced with in-house cultures that will require brine shrimp eggs, seawater (natural or artificial), and egg incubation containers. Incubation of the eggs should begin long enough prior to receiving the test organisms so as to assure a ready supply of newly-hatched nauplii as needed.
2. Balance: capable of weighing to 0.01 mg.
3. Reference Weights: for calibration of balance.
4. Drying Oven: for drying fish at 105°C at test termination.
5. Desiccator: for holding dried fish.
6. Aeration System: needed for aeration when D.O. drops below acceptable levels.
7. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
8. Thermometer: ASTM certified, for documenting test water temperature.
9. Test Containers: 600 mL glass beaker. Beakers must be appropriately cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
10. De-Ionized water: for rinsing of probes, etc.
11. Wash Bottles: for rinsing of probes, etc.
12. Volumetric and Graduated Flasks and Pipettes: for making up dilution series and reference toxicant test solutions
13. Wide-bore transfer pipettes or small handheld dip nets: for transfer of organisms to and from test containers.

14. NITEX mesh sieves (150 μ m); for concentrating organisms.
15. Forceps: for transfer of organisms to weighing pans.
16. Aluminum Foil Weighing Pans: for drying and weighing of fish.
17. Cubitainers: may be necessary for the client's collection of effluent.
18. ACS Reagent CuSO₄ (Copper Sulfate), for use as reference toxicant.

2.2 *Ordering and Holding of Test Organisms*

1. Test organisms should be ordered far enough in advance so as to ensure arrival of < 24 hrs old animals by the day of test setup. Approximately 25-33% more animals should be ordered than are actually needed for the test, so as to allow for some attrition of organisms that are stressed from the shipping, etc.

Order fish from:

- (1) - Aquatox (501)767-9120
- (2) - Aquatic Biosystems Inc. (303)223-2938
- (3) - Aquatic Research Organisms (603)926-1650

2. For additional instruction on the receipt and handling of the test organisms, see the “**Test Organism Receipt and Handling S.O.P.**”

2.3 *Collection and Holding of Water Samples*

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or “blue ice type product) to bring the temperature to 4°C. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in water quality measurements should be taken. For instruction on the log-in of incoming samples, see the “**Test Sample(s) Log-In Procedures**”. The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 4 Liters of sample will be needed each day.

3.0 **TEST INITIATION**

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read the attached “**Summary of Test Conditions for *Pimephales promelas***”.

1. Fish should be fed *Artemia nauplii* at least two hours prior to the start of the test.
2. Label an appropriate number of 600-mL beakers with the appropriate test treatments for your dilution series (e.g.: control, 5%, 10%, 25%, 50%, and 100%). You will need 2-4 containers per treatment. Label the beakers with their treatment and replicate I.D. using colored tape and a Sharpie pen. See client NPDES permit for specific dilution requirements.
3. Label test cups for water quality measurements with treatment and replicate I.D. using a Sharpie pen.
4. Prepare dilutions (as needed) with lab water or receiving water according to client NPDES permit. Always work from low to high concentration and rinse out any glassware 3X with dH₂O prior to use.

5. For each treatment, record the initial water quality (**pH, D.O., conductivity, and temperature**) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels. **Total residual chlorine** analysis is required for the highest concentration of municipal effluents. **Alkalinity** and **hardness** are measured for the control when water is made and for effluent when sample(s) arrive at the laboratory.
6. Beginning with the Control treatment and working up through the concentration series, pour 400 mL of test media into each of the containers. Use the Sharpie pen and trace the water level line onto each container. Place dilution containers into the water bath to allow test waters to acclimate to test temperature.
7. Prior to loading any fish, clean the tank holding the test organisms as much as possible (i.e. remove uneaten food) minimizing any disturbance of the live animals.
8. Using wide-bore pipette (or dipnet for older fish), *carefully* capture and randomly allocate 10 fish into each of the test containers. This is best accomplished by gradually placing allocations of 2-4 animals into each container until 10 animals are in each.
 - a. The organisms are delicate. When transferring, release organisms under the surface of the water. Make sure that each individual is uninjured. Replace injured individuals.
 - b. Be consistent with the volume of water used to transfer the organisms. Take care to avoid excessive dilution of the test treatments. Note any excess dilution of the test solution.
9. Randomly place the test containers within the temperature-controlled water bath ($25 \pm 1^\circ\text{C}$), under a 16 hour light, 8 hour dark photoperiod at a light intensity of 50-100 foot-candles. Make sure that all of the necessary data are recorded upon the data sheets.
10. Feed each replicate with newly-hatched brine shrimp nauplii. Let the replicate containers (with the animals within) sit for one hour and then re-examine each replicate, removing any organism(s) that appear to have been unduly stressed or injured from the loading procedure, and replace with a freshly caught animal.

4.0 MAINTAINING THE TEST

1. The test organisms should be fed newly-hatched brine shrimp nauplii first thing in the morning.
2. At the time of the media renewal, pull the test containers from the water bath and arrange, in replicate # order, and by treatment. From one randomly selected container at each treatment, measure the “old” temperature, pH, D.O. and salinity/conductivity. Check these measurements to make sure that the water quality is within acceptable limits.
3. Remove the uneaten food and any dead animals. This can be accomplished either of two methods: (1) siphon out the debris and approximately 80% of the “old” test media from each replicate container, being careful not to accidentally siphon any of the live animals; (2) use a transfer pipette to squirt water across the bottom of the replicate container, stirring up debris from the bottom in the process, and then quickly pour approximately 80% of the “old” media out, being careful not to pour out any live animals.
4. Count the number of live animals in each replicate and record the number on the data sheet.

5. Check the D.O. level of the Control water and the highest concentration of treatment media and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels. Carefully (and slowly) pour fresh media back into the replicate containers until the water level is at the 400 mL line. In order not to stress the animals while pouring in this media, tilt the containers and slowly pour in the new media down the side of the tilted container.
6. From one randomly selected replicate container at each treatment (beginning with the Control, and working upward through the concentration series), measure the “new” pH, D.O., and conductivity. Record these data on the data sheet and randomly place the replicate beakers back in the water bath. Sometime between 3 p.m. and 5 p.m., feed each replicate container with newly-hatched brine shrimp nauplii.

5.0 TEST TERMINATION

1. After 7 days, pull the test containers from the water bath. Measure and record the “old” temperature, pH and D.O.. Siphon or pour out approximately 80% of the old media. Count and record the number of live animals in each replicate container. Pour the remaining test solution containing the surviving fish into a 500 μ m mesh screen submerged in deionized water so as to wash away debris that may alter final weights.
2. Carefully transfer the animals from each replicate onto a pre-dried and pre-weighed aluminum drying pan (the pans should be weighed as per the **Weighing of Test Organisms S.O.P.**). When all of the replicates have been transferred into their respective drying pans, place the pans into the drying oven, and dry at 105°C for at least 6 hours.
3. After drying, place the aluminum pans into the desiccator and seal. Allow to cool at least 4 hrs., after which each pan must be weighed and the weight data recorded. The weight recorded for the weight of the pans + dried animals minus the empty pans = the pooled dry weight of the organisms for that replicate. Divide this number by the number of organisms originally loaded in the replicate to obtain the mean dry weight for individual fish in that replicate. For the control treatment, calculate the mean weight per surviving fish for each replicate to determine if the weights met test acceptability criteria.
4. At this point, there should be two endpoint data for each replicate: percentage survival and mean dry weight per individual.
5. Notify client immediately if toxicity is observed.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenance, and termination are identical to those above. Information regarding the fathead minnow reference toxicity test is presented in the “**Chronic *Pimephales promelas* Reference Toxicity Test SOP**”.

7.0 DATA ANALYSIS

The two endpoint data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analysis are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Tests are acceptable if the mean survival in control treatments is at least 80%. The average dry weight of surviving control fish must be at least 0.25 mg.

9.0 QUALITY CONTROL

1. Control water, consisting of deionized water adjusted to moderate hardness with EPA salts, is used.
2. All equipment is calibrated and operated as described in each applicable equipment SOP.
3. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The fathead minnow 7-day chronic toxicity test poses little risk to those performing it. Care should be taken in the preparation of the reference toxicant spiking solution. Review the MSDS for the reference toxicant. After the ref tox spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR FATHEAD MINNOW, <i>PIMEPHALES PROMELAS</i> , LARVAL SURVIVAL AND GROWTH TEST (TEST METHOD 1000.0)	
1. Test type	Static renewal
2. Test duration	7 days
3. Temperature	25 ± 1°C
4. Light quality	Ambient laboratory illumination
5. Light intensity	50-100 ft-c (10-20 $\mu\text{E}/\text{m}^2/\text{s}$)
6. Photoperiod	16 hours light: 8 hours darkness
7. Test chamber size	600 mL
8. Test solution volume	400 mL
9. Renewal of test solutions	Daily
10. Age of test organisms	Newly hatched larvae should be less than 24 h old, but no more than 48 hours old (all hatched within 24 h)
11. # of organisms per test chamber	Ten
12. # of replicate chambers per concentration	Four
13. # of organisms per concentration	Forty
14. Feeding regime	Artemia nauplii twice daily
15. Test chamber cleaning	Siphon daily, immediately before test solution renewal
16. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
17. Dilution water	Moderately hard water (deionized water with reagent grade chemicals added to make EPA moderately hard synthetic water)
18. Dilution factor	≥ 0.5
19. Test endpoint	% survival and growth
20. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours (no sample > 72 hours is used in test)
21. Sample volume required	4 Liters per day
22. Test acceptability	80% or greater average survival in controls; average weight of control fish ≥ 0.25 mg

Appendix D

Attachment 5: Standard Operating Procedure for Chronic *Atherinops affinis* Bioassay

Revision #2 (Date last modified: 1/14/05 3:24 PM by Pacific EcoRisk)

Effective Date: December 8, 2002

Accepted: _____

***ATHERINOPS AFFINIS* (TOPSMELT)**
Chronic (7-Day) Survival and Growth Bioassay
Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA Guidelines described in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (EPA/600/R-95/136).

1.0 INTRODUCTION

This test is based on a seven-day static-renewal exposure of 9 -15 day old *Atherinops affinis* to different concentrations of effluents and/or receiving waters during the juvenile life period. The test endpoints are % survival and growth (measured as dry weight).

2.0 TEST PREPARATION

2.1 *Equipment and Supplies Needed*

1. Food: Brine shrimp nauplii - The test organisms will need to be fed brine shrimp (*Artemia*) nauplii at least twice per day. These nauplii should be produced with in-house cultures that will require brine shrimp eggs, seawater (natural or artificial), and egg incubation containers. Incubation of the eggs should begin long enough prior to receiving the test organisms so as to assure a ready supply of newly-hatched nauplii as needed.
2. Aeration System: needed for aeration when D.O. drops below acceptable levels.
3. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
4. Thermometer: ASTM certified, for documenting test water temperature.
5. Test Containers: 600 mL glass beaker. Beakers must be appropriately cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
6. De-Ionized water: for rinsing of probes, etc.
7. Wash Bottles: for rinsing of probes, etc.
8. Volumetric and Graduated Flasks and Pipettes: for making up dilution series and reference toxicant test solutions
9. Wide-bore transfer pipettes or small handheld dip nets: for transfer of organisms to and from test containers.
10. NITEX mesh sieves (150 μ m); for concentrating organisms.
11. Balance: capable of weighing to 0.01 mg.
12. Reference Weights: for calibration of balance.
13. Drying Oven: for drying fish at 105°C at test termination.

14. Desiccator: for holding dried fish.
15. Forceps: for transfer of organisms to weighing pans.
16. Aluminum Foil Weighing Pans: for drying and weighing of fish.
17. Cubitainers: may be necessary for the client's collection of effluent.
18. Artificial Sea Salt (Crystal Sea[®]): for salting up of effluent to acceptable test salinity.
19. ACS Reagent CuSO₄ (Copper Sulfate), for use as reference toxicant.

2.2 *Ordering and Holding of Test Organisms*

1. Test organisms should be ordered far enough in advance so as to ensure arrival of 9 to 15-day old animals by the day of test set-up. Approximately 25-33% more animals should be ordered than are actually needed for the test, so as to allow for some attrition of organisms that are stressed from the shipping, etc.

Order fish from: (1) - Aquatic Biosystems Inc. (303)223-2938

2. Order the juvenile fish to be pre-adapted to the test salinity. This is important as the supplier may be culturing the fish at a different salinity than the desired test salinity. If the fish come in at a "non-test" salinity, they must begin acclimation ASAP. Place them in control water at the receiving salinity and immediately begin the adjust the holding salinity towards the test salinity.
3. For additional instruction on the receipt and handling of the test organisms, see the "**Test Organism Receipt and Handling S.O.P.**"

2.3 *Collection and Holding of Water Samples*

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or "blue ice" type product) to bring the temperature to 4°C. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in water quality measurements should be taken. For instruction on the log-in of incoming samples, see the "**Test Sample(s) Log-In Procedures**". The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 4 Liters of sample will be needed each day.

3.0 TEST INITIATION

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read the attached "**Summary of Test Conditions for *Atherinops affinis***".

1. Feed fish *Artemia* nauplii at least two hours prior to the start of the test.
2. Label an appropriate number of 600-mL beakers with the appropriate test treatments for your dilution series (e.g.: control, 10%, 25%, 50%, and 100%). You will need 2-4 containers per treatment (test dependent). Label the beakers with their treatment and replicate I.D. using colored tape and a Sharpie pen. See client NPDES permit for specific

dilution requirements.

3. Label test cups for water quality measurements with treatment and replicate I.D. using a Sharpie pen.
4. Prepare dilutions (as needed) with filtered seawater or appropriately salted receiving water according to client NPDES permit. Adjust salinity of control and sample waters, if necessary, by adding Crystal Sea[®] Sea Salt into solution while stirring on a stir plate. Always work from low to high concentration and rinse out any glassware 3X with dH₂O and one final with control salinity water prior to use.
5. For each treatment, record the initial water quality (**pH, D.O., salinity, conductivity, and temperature**) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels. **Total residual chlorine** analysis is required for the highest concentration of municipal effluents. **Alkalinity** and **hardness** are measured for the control when water is made and for effluent when sample(s) arrive at the laboratory.
6. Beginning with the Control treatment and working up through the concentration series, pour 400 mL (200 mL minimum) of test media into each of the containers. Use the Sharpie pen and trace the water level line onto each container. Place dilution containers into the water bath to allow test waters to acclimate to test temperature.
7. Try to arrange the work schedule so that loading of organisms into the test containers will take place around 2-3 p.m. Prior to loading fish, clean the tank holding the test organisms as much as possible (i.e. remove uneaten food and any dead organisms) minimizing any disturbance of the live animals.
8. Using a net and pipette, *carefully* capture and randomly allocate 5 fish into each of the test containers.
 - a. The organisms are delicate. When transferring, release organisms under the surface of the water. Make sure that each individual is uninjured. Replace injured individuals.
 - b. Be consistent with the volume of water used to transfer the organisms. Take care to avoid excessive dilution of the test treatments. Note any excess dilution of the test solution.
9. Randomly place the test containers within the temperature-controlled (**20°C ±1°C**) water bath under a 16-hour light, 8-hour dark photoperiod at a light intensity of 50-100 foot-candles. Make sure that all of the necessary data are recorded upon the data sheets.
10. Feed each replicate with newly-hatched brine shrimp nauplii. Let the replicate containers (with the animals within) sit for one hour and then re-examine each replicate, removing any organism(s) that appear to have been unduly stressed or injured from the loading procedure, and replace with a freshly caught animal.

4.0 MAINTAINING THE TEST

1. The test organisms should be fed newly-hatched brine shrimp nauplii first thing in the morning.
2. At the time of the media renewal, pull the test containers from the water bath and arrange, in replicate # order, and by treatment. From one randomly selected container at each

treatment, measure the “old” temperature, pH, D.O. and salinity/conductivity. Check these measurements to make sure that the water quality is within acceptable limits.

3. Remove the uneaten food and any dead animals. This can be accomplished either of two methods: (1) siphon out the debris and approximately 80% of the “old” test media from each replicate container, being careful not to accidentally siphon any of the live animals; (2) use a transfer pipette to squirt water across the bottom of the replicate container, stirring up debris from the bottom in the process, and then quickly pour approximately 80% of the “old” media out, being careful not to pour out any live animals.
4. Count the number of live animals in each replicate and record the number on the data sheet.
5. Check the D.O. level of the Control water and the highest concentration of treatment media and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels. Carefully (and slowly) pour fresh media back into the replicate containers until the water level is at the 400 mL line. In order not to stress the animals while pouring in this media, tilt the containers and slowly pour in the new media down the side of the tilted container.
6. From one randomly selected replicate container at each treatment (beginning with the Control, and working upward through the concentration series), measure the “new” pH, D.O., and salinity/conductivity. Record these data on the data sheet and randomly place the replicate beakers back in the water bath. Sometime between 3 p.m. and 5 p.m., feed each replicate container with newly-hatched brine shrimp nauplii.

5.0 TEST TERMINATION

1. After 7 days, pull the test containers from the water bath. Measure and record the “old” pH, D.O., and salinity/conductivity. Siphon or pour out approximately 80% of the old media. Count and record the number of live animals in each replicate container.
2. Pour the remaining test solution containing the surviving fish into a 500 μ m mesh screen submerged in deionized water so as to wash away debris that may alter final weights.
3. Euthanize the organisms from each replicate in an ice-bath with filtered seawater, rinse in de-ionized water and transfer the organisms onto a pre-dried and pre-weighed aluminum drying pan (the pans should be weighed as per the **Weighing of Test Organisms S.O.P.**). When all of the replicates have been transferred into their respective drying pans, place the pans into the drying oven, and dry at 105°C for at least 6 hrs.
4. After drying, place the aluminum pans into the desiccator and seal. Allow to cool at least 4 hrs., after which each pan must be weighed and the weight data recorded. The weight recorded for the weight of the pans + dried animals minus the empty pans = the pooled dry weight of the organisms for that replicate. Divide this number by the number of organisms in the replicate to obtain the mean dry weight for individual topsmelt in that replicate. For the control treatment, calculate the mean weight per surviving fish for each replicate to determine if the weights met test acceptability criteria.
5. At this point, there should be two endpoint data for each replicate: percentage survival and mean dry weight per individual.

6. Notify client immediately if toxicity is observed.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenance, and termination are identical to those above. Information regarding the *Atherinops affinis* reference toxicity test is presented in the “**Chronic *Atherinops affinis* Reference Toxicity Test SOP**.”

7.0 DATA ANALYSIS

The survival and growth data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analysis are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Test acceptability criteria for the *Atherinops affinis* chronic test are as follows: 80% or greater survival in the controls and an average dry weight of the control larvae, when dried immediately after test termination, is equal to or greater than 0.85 mg per individual. The survival LC₅₀ for copper in the reference toxicant test must be <205 µg/L. There must be <25% and <50% MSD for survival and growth, respectively, relative to controls in the reference toxicant test.

9.0 QUALITY CONTROL

1. Control water, consisting of deionized water salted up to test salinity with Crystal Sea® sea salt, is used.
2. The test fish shall be maintained in the laboratory 4-7 days prior to use in tests in order to monitor and examine them for health and quality.
3. All equipment is calibrated and operated as described in each applicable equipment SOP.
4. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The *Atherinops affinis* 7 Day chronic toxicity test poses little risk to those performing it. Care should be taken in the preparation of the reference toxicant spiking solution. Review the MSDS for the reference toxicant. After the ref tox spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR TOPSMELT, <i>ATHERINOPS AFFINIS</i> , LARVAL SURVIVAL AND GROWTH TEST	
1. Test type	Static renewal
2. Test duration	7 days
3. Salinity	5 to 34 ppt \pm 2 ppt
4. Temperature	20 \pm 1°C
5. Light quality	Ambient laboratory illumination
6. Light intensity	50-100 ft-c (10-20 μ E/m ² /s)
7. Photoperiod	16 hours light: 8 hours darkness
8. Test chamber size	600-mL
9. Test solution volume	400 mL (200 mL minimum)
10. Renewal of test solutions	Daily
11. Age of test organisms	9-15 days old
12. No. of organisms per test chamber	Five
13. No. of rep. chambers per conc'n	Five
14. No. of organisms per conc'n	Twenty-five
15. Feeding regime	40 <i>Artemia</i> nauplii per larvae twice daily
16. Test chamber cleaning	Siphon daily, immediately before test solution renewal
17. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
18. Dilution water	According to NPDES permit. Filtered (1 μ m) seawater, dH ₂ O salted up with Crystal Sea [®] sea salt.
19. Test concentration	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
20. Dilution factor	According to NPDES permit. None, or \geq 0.5 dilution series
21. Test endpoint	% survival and growth
22. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours (no sample > 72 hours is used in test)
23. Sample volume required	4 Liters per day
24. Test acceptability	80% or greater average survival in controls; average weight of control fish \geq 0.85 mg; Cu LC ₅₀ \leq 205 μ g/L; <25% & <50% MSD for survival and growth.

Appendix D

Attachment 6: Standard Operating Procedure for Chronic *Cyprinodon variegatus* Bioassay

Revision #2 (Date last modified: 1/14/05 3:23 PM by Pacific EcoRisk)

Effective Date: December 8, 2002

Accepted: _____

***Cyprinodon variegatus* (Sheepshead Minnow)**

Chronic (7-Day) Survival and Growth Bioassay

Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA Guidelines described in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms, Third Edition (EPA-821-R-02-014). See Addendum, for tests that require the guidelines set forth in previous editions (EPA/600/4-87/028 and EPA-600-4-91-003).

1.0 INTRODUCTION

This test is based on a seven-day static-renewal exposure of < 24 hr old *Cyprinodon variegatus* to different concentrations of effluents and/or receiving waters during the larval life period. The test endpoints are survival and growth (measured as dry weight).

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Food: Brine shrimp nauplii - The test organisms will need to be fed brine shrimp (*Artemia*) nauplii at least twice per day. These nauplii should be produced with in-house cultures that will require brine shrimp eggs, seawater (natural or artificial), and egg incubation containers. Incubation of the eggs should begin long enough prior to receiving the test organisms so as to assure a ready supply of newly-hatched nauplii as needed.
2. Aeration System: needed for aeration when D.O. drops below acceptable levels.
3. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
4. Thermometer: ASTM certified, for documenting test water temperature.
5. Test Containers: 600 mL glass beaker. Beakers must be appropriately cleaned and rinsed, and then pre-soaked for 24 hrs (overnight) in control water, before use in test.
6. De-Ionized water: for rinsing of probes, etc.
7. Wash Bottles: for rinsing of probes, etc.
8. Volumetric and Graduated Flasks and Pipettes: for making up dilution series and reference toxicant test solutions
9. Wide-bore transfer pipettes or small handheld dip nets: for transfer of organisms to and from test containers.
10. NITEX mesh sieves (150 μ m & 500 μ m); for concentrating organisms.
11. Balance: capable of weighing to 0.01 mg.
12. Reference Weights: for calibration of balance.

13. Drying Oven: for drying fish at 105°C at test termination.
14. Desiccator: for holding dried fish.
15. Forceps: for transfer of organisms to weighing pans.
16. Aluminum Foil Weighing Pans: for drying and weighing of fish.
17. Cubitainers: may be necessary for the client's collection of effluent.
18. Artificial Sea Salt (Crystal Sea®): for salting up of effluent to acceptable test salinity.
19. ACS Reagent CuSO₄ (Copper Sulfate), for use as reference toxicant.

2.2 Ordering and Holding of Test Organisms

1. Test organisms should be ordered far enough in advance so as to ensure arrival of < 24 hrs old animals on test set-up day. Approximately 25-33% more animals should be ordered than are actually needed for the test, so as to allow for some attrition of organisms that are stressed from the shipping, etc.
Order fish from: (1) - Aquatic Indicators (904)829-2780
(2) - Aquatic Biosystems Inc. (303)223-2938
(3) - Aquatic Research Organisms (603)926-1650
2. Order the juvenile fish to be pre-adapted to the test salinity. This is important as the supplier may be culturing the fish at a different salinity than the desired test salinity. If the fish come in at a “non-test” salinity, they must begin acclimation ASAP. Place them in control water at the receiving salinity and immediately begin to adjust the holding salinity towards the test salinity.
3. For additional instruction on the receipt and handling of the test organisms, see the “**Test Organism Receipt and Handling S.O.P.**”

2.3 Collection and Holding of Water Samples

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or “blue ice type product) to bring the temperature to 4°C. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in water quality measurements should be taken. For instruction on the log-in of incoming samples, see the “**Test Sample(s) Log-In Procedures**”. The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 6 Liters of sample will be needed each day.

3.0 TEST INITIATION

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read the attached “**Summary of Test Conditions for *Cyprinodon variegatus***”

1. Feed fish *Artemia* nauplii at least two hours prior to the start of the test.
2. Label an appropriate number of 600-mL or 1-L beakers with the appropriate test treatments for your dilution series (e.g.: control, 10%, 25%, 50%, and 100%). You will need 2-4

containers per treatment (test dependent). Label the beakers with their treatment and replicate I.D. using colored tape and a Sharpie pen. See client NPDES permit for specific dilution requirements.

3. Label test cups for water quality measurements with treatment and replicate I.D. using a Sharpie pen.
4. Prepare dilutions (as needed) with filtered seawater or appropriately salted receiving water according to client NPDES permit. Adjust salinity of control and sample waters, if necessary, by adding Crystal Sea® Sea Salt into solution while stirring on a stir plate. Always work from low to high concentration and rinse out any glassware 3X with dH₂O and one final with control salinity water prior to use.
5. For each treatment, record the initial water quality (**pH, D.O., salinity, conductivity, and temperature**) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels. **Total residual chlorine** analysis is required for the highest concentration of municipal effluents. **Alkalinity** and **hardness** are measured for the control when water is made and for effluent when sample(s) arrive at the laboratory.
6. Beginning with the Control treatment and working up through the concentration series, pour 500-750 mL of test media into each of the containers. Use the Sharpie pen and trace the water level line onto each container. Place dilution containers into the water bath to allow test waters to acclimate to test temperature.
7. Try to arrange the work schedule so that loading of organisms into the test containers will take place around 2-3 p.m. Prior to loading fish, clean the tank holding the test organisms as much as possible (i.e. remove uneaten food and any dead organisms) minimizing any disturbance of the live animals.
8. Using a net and pipette, *carefully* capture and randomly allocate 10 larval fish into each of the test containers. This is best accomplished by gradually placing allocations of 2-4 animals into each container until 10 animals are in each.
 - a. The organisms are delicate. When transferring, release organisms under the surface of the water. Make sure that each individual is uninjured. Replace injured individuals.
 - b. Be consistent with the volume of water used to transfer the organisms. Take care to avoid excessive dilution of the test treatments. Note any excess dilution of the test solution.
9. Let the replicate containers (with the animals within) sit for one hour and then re-examine each replicate, removing any organism(s) that appear to have been unduly stressed or injured from the loading procedure, and replace with a freshly caught animal.
10. Randomly place the test containers within the temperature-controlled (25°C ±1°C) water bath under a 16-hour light, 8-hour dark photoperiod at a light intensity of 50-100 foot-candles. Make sure that all of the necessary data are recorded upon the data sheets.

4.0 MAINTAINING A STATIC RENEWAL TEST

1. The test organisms should be fed newly-hatched brine shrimp nauplii first thing in the

morning.

2. At the time of the media renewal, pull the test containers from the water bath and arrange, in replicate # order, and by treatment. From one randomly selected container at each treatment, measure the “old” temperature, pH, D.O. and salinity/conductivity. Check these measurements to make sure that the water quality is within acceptable limits.
3. Remove the uneaten food and any dead animals. This can be accomplished either of two methods: (1) siphon out the debris and approximately 80% of the “old” test media from each replicate container, being careful not to accidentally siphon any of the live animals; (2) use a transfer pipette to squirt water across the bottom of the replicate container, stirring up debris from the bottom in the process, and then quickly pour approximately 80% of the “old” media out, being careful not to pour out any live animals.
4. Count the number of live animals in each replicate and record the number on the data sheet.
5. Check the D.O. level of the Control water and the highest concentration of treatment media and make sure that there is enough oxygen; if not, you must aerate the sample until the D.O. reaches adequate levels. Carefully (and slowly) pour fresh media back into the replicate containers until the water level is at the fill line (500-750 mL). In order not to stress the animals while pouring in this media, tilt the containers and slowly pour in the new media down the side of the tilted container.
6. From one randomly selected replicate container at each treatment (beginning with the Control, and working upward through the concentration series), measure the “new” pH, D.O., and salinity/conductivity. Record these data on the data sheet and randomly place the replicate beakers back in the water bath. Sometime between 3 p.m. and 5 p.m., feed each replicate container with newly-hatched brine shrimp nauplii.

5.0 TEST TERMINATION

1. After 7 days, pull the test containers from the water bath. Measure and record the “old” pH, D.O., and salinity/conductivity. Siphon or pour out approximately 80% of the old media. Count and record the number of live animals in each replicate container.
2. Pour the remaining test solution containing the surviving fish into a 500 μ m mesh screen submerged in deionized water so as to wash away debris that may alter final weights.
3. Euthanize the organisms from each replicate in an ice-bath with filtered seawater, rinse in de-ionized water and transfer the organisms onto a pre-dried and pre-weighed aluminum drying pan (the pans should be weighed as per the **Weighing of Test Organisms S.O.P.**). When all of the replicates have been transferred into their respective drying pans, place the pans into the drying oven, and dry at 105°C for at least 6 hrs.
4. After drying, place the aluminum pans into the desiccator and seal. Allow to cool at least 4 hrs, after which each pan must be weighed and the weight data recorded. The weight recorded for the weight of the pans + dried animals minus the empty pans = the pooled dry weight of the organisms for that replicate. Divide this number by the number of organisms in the replicate to obtain the mean dry weight for individual fish in that replicate. For the control treatment, calculate the mean weight per surviving fish for each replicate to

determine if the weights met test acceptability criteria.

5. At this point, there should be two endpoint data for each replicate: percentage survival and mean dry weight per individual.
6. Notify client immediately if toxicity is observed.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test may be run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenance, and termination are identical to those above. Information regarding the sheephead reference toxicity test is presented in the “**Chronic *Cyprinodon variegatus* Reference Toxicity Test SOP**”.

7.0 DATA ANALYSIS

The two endpoint data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Test acceptability criteria for the sheephead minnow chronic test are as follows:

80% or greater survival in the controls and an average dry weight of the control larvae, when dried immediately after test termination, equal to or greater than 0.60 mg per individual. If the larvae have been preserved in fixative, for no more than 7 days, the average dry weight per control individual should be equal to or greater than 0.50 mg.

9.0 QUALITY CONTROL

1. Control water, consisting of deionized water salted up to test salinity with Crystal Sea® sea salt, is used.
2. Test fish arriving as embryos shall be maintained in the laboratory 4-7 days prior to use in tests in order to monitor and examine them for health and quality.
3. All equipment is calibrated and operated as described in each applicable equipment SOP.
4. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The sheephead minnow 7-day chronic toxicity test poses little risk to those performing it. Effluent and/or receiving waters may contain pathogens so appropriate safety precautions should be used when handling these solutions. Care should be taken in the preparation of the reference toxicant spiking solution. Review the MSDS for the reference toxicant. After the ref tox spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR SHEEPSHEAD MINNOW, <i>CYPRINIDON VARIEGATUS</i> , LARVAL SURVIVAL AND GROWTH TEST (TEST METHOD 1004.0)	
1. Test type	Static renewal
2. Test duration	7 days
3. Salinity	20 to 32 ppt \pm 2 ppt
4. Temperature	25 \pm 1°C
5. Light quality	Ambient laboratory illumination
6. Light intensity	50-100 ft-c (10-20 μ E/m ² /s)
7. Photoperiod	16 hours light: 8 hours darkness
8. Test chamber size	600-mL to 1-L
9. Test solution volume	500 mL to 750 mL
10. Renewal of test solutions	Daily
11. Age of test organisms	<24 hours old
12. No. of organisms per test chamber	Ten (10)
13. No. of rep. chambers per concentration	Four (4)
14. No. of organisms per concentration	Forty (40)
15. Feeding regime	<i>Artemia</i> nauplii twice daily
16. Test chamber cleaning	Siphon daily, immediately before test solution renewal
17. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min
18. Dilution water	According to NPDES permit. Filtered (1 μ m) seawater, dH ₂ O salted up with Crystal Sea [®] sea salt.
19. Test concentration	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
20. Dilution factor	According to NPDES permit. None, or \geq 0.5 dilution series
21. Test endpoint	% survival and growth
22. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours (no sample > 72 hours is used in test)
23. Sample volume required	6 Liters per day
24. Test acceptability	80% or greater average survival in controls; average weight of control fish \geq 0.6mg

Appendix D

Attachment 7: Standard Operating Procedure for Chronic *Selenastrum capricornutum* Bioassay

Revision #2 (Date last modified: 1/14/05 8:05 PM by Pacific EcoRisk)

Effective Date: December 8, 2002

Accepted: _____

Selenastrum capricornutum
Algal Growth Bioassay
Standard Operating Procedures

This S.O.P. is based upon the U.S. EPA Guidelines described in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA-821-R-02-013 and EPA-600-4-91-002).

1.0 INTRODUCTION

This test is based on a 96-hour static exposure of the freshwater alga, *Selenastrum capricornutum*, to different concentrations of effluents and/or receiving waters. The test endpoint is growth (measured as cell density).

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Autoclave
2. Balance: capable of weighing to 0.01 mg.
3. Meters: D.O., pH and conductivity/salinity, needed to document test water quality.
4. Thermometer: ASTM certified, for documenting test water temperature.
5. Test Containers: 250-mL Erlenmeyer flasks with aluminum foil for covering. Flasks must be appropriately cleaned and rinsed
6. De-Ionized water: for rinsing of probes, etc.
7. Wash Bottles: for rinsing of probes, etc.
8. Volumetric and Graduated Flasks and Pipettes: for making up dilution series and reference toxicant test solutions
9. Pipettor: Eppendorf micro-pipettor with tips capable of pipetting 10-1000 μL .
10. Cubitainers: may be necessary for the client's collection of effluent.
11. ACS Reagent ZnCl_2 (Zinc Chloride), for use as reference toxicant.
12. Filter apparatus and 0.45 μm filters.
13. Chemicals necessary for making algal nutrient solutions.
14. Light meter

2.2 Ordering and Holding of Test Organisms

1. Test cultures should be ordered far enough in advance so as to ensure algal cultures are in log growth prior to the test set-up.

Order Algal cultures from:

Aquatic Research Organisms (603)926-1650 or
Botany Department, Univ. Of Texas (512)471-4019

3. For additional instruction on the receipt and handling of algal test cultures, see the “**Algal Test Culture Receipt and Handling S.O.P.**”

2.3 Collection and Holding of Water Samples

Grab or composite samples should be collected into appropriately-cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or “blue ice” type product) to bring the temperature to 4°C. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample log-in water quality measurements should be taken. For instruction on the log-in of incoming samples, see the “**Test Sample(s) Log-In Procedures**”. The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 4 Liters of sample will be needed each day.

3.0 TEST INITIATION

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read the attached “**Summary of Test Conditions for *Selenastrum capricornutum***”.

3.1 Water Preparation

3.1.1 Diluent

1. Figure out how much water you need. Remember to have extra for rinsing the flasks.
2. Deionized water raised to moderate hardness with EPA chemicals (EPAMH) is used as control and diluent for freshwater algal tests unless specified otherwise.
3. Prepare sterile filtered water (0.45 μm). If performing an effluent test or testing a volatile material positive pressure filtration must be used.
4. Add nutrients. These are stored in the refrigerator. 1 mL/L of each of the five nutrient solutions (A-E) should be added to the filtered water. See **Table 1** for more information on the nutrient solutions.
5. Adjust the diluent pH to 7.5 ± 0.2 with HCl or NaOH.

3.1.2 Test Sample Water

Follow same procedure as in 3.1.1, except test samples should **not** be pH adjusted.

1. Prepare sample
2. Positive pressure sterile filter sample
3. Add nutrients

3.2 Test Setup

1. Measure and record light intensity using a light meter throughout the water bath. Intensity should be $86 \pm 8.6 \mu\text{E}/\text{m}^2/\text{s}$ (400+40 foot candles). Replace bulbs if necessary to maintain light intensity within the acceptable range.
2. Prepare the test flasks for each concentration. You will need 5 containers per treatment. Label the flasks with their treatment and replicate I.D. Rinse each flask with control water.
3. Prepare dilutions of the test material.
4. For each treatment, record the initial water quality (pH, D.O., conductivity, and temperature) onto the data sheets; check to make sure that all parameters are within acceptable levels.
5. Set aside at least 25 mL of solution for each control and test concentration in clearly labeled vials. These will be used as blanks at test termination. Store these subsamples in the dark, cold storage to prevent any algal growth.
6. From the control and low, middle and high test concentrations, collect subsamples of approximately 100 mL for alkalinity and hardness measurements to be performed later.

3.3 Test Inoculation

3.3.1 Determination of Inoculation Volumes:

1. Inoculation density of *Selenastrum* should be 1×10^4 cells/mL
2. A 4 to 7 day old culture should be used so that the culture is in log growth phase.
3. Use a hemacytometer to count the culture. Add 10 μL of the culture to each side of the hemacytometer and allow algae to settle for five to ten minutes before counting. Count 3-5 subsamples on the hemacytometer. To determine algal density in the culture, simply multiply the average hemacytometer count by 10^4 .
4. The formula for determining inoculation is as follows:

$$\text{volume of inoculation} = (\text{volume to be inoculated}) \times \frac{(1 \times 10^4)}{(\text{algal density of culture})}$$

3.3.2 Test Inoculation

1. Inoculate each control, test and reference toxicant dilution container with the appropriate volume of algae as calculated in section 3.3.1.
2. Beginning with the Control treatment and working up through the concentration series, pour 100 mL of the inoculated solution into each of the 5 containers associated with each concentration. Each concentration will have four test replicates and one water chemistry replicate for daily pH measurements.
3. Randomly place the test containers within the temperature-controlled water bath. Make sure that all of the necessary data are recorded upon the data sheets.

4.0 MAINTAINING THE TEST

1. Swirl flasks twice daily and randomize the flasks positions in the water bath.
2. Once daily, measure and record pH of each concentration. The pH readings should be taken from the flasks designated for water chemistry measurements so that the test flasks do not get contaminated.
3. Measure water bath temperature once daily.

5.0 TEST TERMINATION

1. Take down the test after 96 hours. Keep flasks cold and in the dark to restrict any further growth. Remove the blanks that were collected for each concentration at test initiation from the cold storage.
2. Tests are taken down using the turbidity method on the Hach spectrophotometer DR 2010. After turning on spectrophotometer, enter program number 951 when prompted by the meter. Set wavelength at 750 nm.
3. Starting with the Control, pour 25 mL of the appropriate blank solution into a cuvette. Place the filled cuvette into the machine and press zero. After the blank has been zeroed, all readings for the corresponding replicates can be taken without having to re-zero.
4. Swirl the first Control flask so that the contents are thoroughly mixed. Pour 25 mL into a cuvette (that is paired with the cuvette used for zeroing), place the cuvette into the spectrophotometer, and press read. Record reading. Repeat the procedure twice more for the replicate. Read three measurements on each of the remaining Control replicates.
5. Use the blank for the next concentration up to zero the meter. Take three turbidity readings on each of the four replicates of that concentration. Repeat the procedure up through the concentration series.
6. Spectrophotometer method #951 automatically converts turbidity readings into cells *Selenastrum*/mL. If the normal turbidity method is used (#750), a calibration curve must be constructed to convert turbidity values into cells/mL.
7. Measure and record final water quality (pH, D.O., conductivity, and temperature) for each control, sample or reference toxicant concentration.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenance, and termination are identical to those above. Information regarding the algal growth reference toxicity test is presented in the “*Selenastrum capricornutum* Growth Reference Toxicity Test SOP”.

The toxicant generally used is Zinc chloride at concentrations of 1, 2.5, 5, 10 and 20 µg Zn/L.

7.0 DATA ANALYSIS

The cell density data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

1. The average algal cell density in the control replicates must be greater than or equal to 1×10^6 at the end of the test.
2. The algal cell density in the control replicates must not vary more than 20% among replicates (CV%).

9.0 QUALITY CONTROL

1. Control water (Deionized water raised to moderate hardness with EPA chemicals) with nutrients added, is used.
2. All equipment is calibrated and operated as described in each applicable equipment SOP.
3. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The *Selenastrum* chronic toxicity test poses little risk to those performing it. Care should be taken in the preparation of the reference toxicant spiking solution. Review the MSDS for the reference toxicant. After the ref-tox spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

Table 1. Nutrient Stock Solutions for Maintaining Algal Stock Cultures and Test Control Cultures.

Nutrient Stock Solution	Compound	Amount dissolved in 500 mL
		Distilled Water
MACRONUTRIENTS:		
SOLUTION A	MgCl ₂ •6H ₂ O	6.08 g
	CaCl ₂ •2H ₂ O	2.20 g
	NaNO ₃	12.750 g
SOLUTION B	MgSO ₄ •7H ₂ O	7.350 g
SOLUTION C	K ₂ HPO ₄	0.522 g
SOLUTION D	NaHCO ₃	7.50 g
MICRONUTRIENTS:		
SOLUTION E	H ₃ BO ₃	92.8 mg
	MnCl ₂ •4H ₂ O	208.0 mg
	ZnCl ₂	1.64 mg ^a
	FeCl ₃ •6H ₂ O	79.9 mg
	CoCl ₂ •6H ₂ O	0.714 mg ^b
	Na ₂ MoO ₄ •2H ₂ O	3.63 mg ^c
	CuCl ₂ •2H ₂ O	0.006 mg ^d
	Na ₂ EDTA•2H ₂ O	150.0 mg ^e
	Na ₂ SeO ₄	1.196 mg ^f

- a ZnCl₂ - Weigh out 164 mg and dilute to 100 mL. Add 1 mL of this solution to Stock E.
- b CoCl₂•6H₂O - Weigh out 71.4 mg and dilute to 100 mL. Add 1 mL of this solution to Stock #1.
- c Na₂MoO₄•2H₂O - Weigh out 36.6 mg and dilute to 10 mL. Add 1 mL of this solution to Stock E.
- d CuCl₂•2H₂O - Weigh out 60.0 mg and dilute to 1000 mL. Take 1 mL of this solution and dilute to 10 mL. Take 1 mL of the second dilution and add to Stock E.
- e Na₂EDTA•2H₂O - Use this chemical only for culturing and when specifically required by the project. EDTA will chelate metals and can alter the toxicity of the sample.
- f Na₂SeO₄ - Weigh out 119.6 mg and dilute to 100 mL. Add 1 mL of this solution to Stock E.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR ALGAL (<i>SELENASTRUM CAPRICORNUTUM</i>) GROWTH TEST (TEST METHOD 1003.0)	
1. Test type	Static
2. Test duration	96 hours
3. Temperature	25 ± 1°C
4. Light quality	“Cool white” fluorescent lighting
5. Light intensity	400 ± 40 ft-c (86 ± 8.6 μ E/m ² /s)
6. Photoperiod	Continuous illumination
7. Test chamber size	250 mL
8. Test solution volume	100 mL
9. Renewal of test solutions	None
10. Age of test organisms	4-7 days old
11. Initial cell density	10,000 cells/mL
12. No. of rep. chambers per concentration	Four (4)
13. Shaking rate	Twice daily by hand
14. Test chamber cleaning	Rinsed in algal culture media. None during test.
15. Dilution water	Algal culture media
16. Test Concentrations	According to NPDES permit. Effluents: 5 and a control Receiving Waters: 100% and a control.
17. Dilution factor	According to NPDES permit. Effluents: ≥ 0.5 dilution series Receiving Waters: None, or ≥ 0.5 dilution series
18. Test endpoint	Growth
19. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours (no sample > 72 hours is used in test)
19. Sample volume required	2 Liters
20. Test acceptability	1 x 10 ⁶ cells/mL in controls; control variability ≤ 20% (CV%)

Appendix D

Attachment 8: Standard Operating Procedure for Chronic *Thalassiosira pseudonana* Bioassay

Revision #2 (Date last modified: 1/14/05 3:24 PM by Pacific EcoRisk)

Effective Date: December 8, 2002

Accepted: _____

Skeletonema and Thalassiosira

Algal Growth Bioassay

Standard Operating Procedures

This S.O.P. is based upon the guidelines described in ASTM Standards on Aquatic Toxicology and Hazard Evaluation (E 1218-97a; PCN #03-547093-16).

1.0 INTRODUCTION

This test is based on a 96 hour static exposure of the marine diatoms, *Skeletonema costatum* or *Thalassiosira pseudonana* to different concentrations of effluents and/or receiving waters. The test endpoint is growth (measured as cell density).

2.0 TEST PREPARATION

2.1 Equipment and Supplies Needed

1. Autoclave
2. Balance, capable of weighing to 0.01 mg.
3. Reference Weights, for calibration of balance.
4. pH, D.O., and conductivity/salinity meter, needed to document test water quality.
5. ASTM certified Thermometer, for documenting test water temperature.
6. Test Containers, 250 mL Erlenmeyer flasks with cotton or foil stoppers. Flasks must be appropriately cleaned, rinsed and autoclaved.
7. De-Ionized water, for rinsing of probes, etc.
8. Wash Bottles, for rinsing of probes, etc.
9. Volumetric and Graduated Flasks and Pipettes, for making up dilution series and reference toxicant test solutions.
10. Transfer Pipettes, for transfer of mysids to and from test containers.
11. NITEX mesh sieves (150 μ m), for removing debris or predatory zooplankton.
12. Cubitainers may be necessary for the client's collection of effluent.
13. ACS Reagent CuSO₄ Copper Sulfate), for use as reference toxicant.
14. Filter system for water.

2.2 Ordering and Holding of Test Organisms

1. Test cultures should be ordered far enough in advance so as to ensure algal cultures are in log growth prior to the test set-up. Ideally, algae should be cultured at the desired test salinity for two weeks prior to the test's initiation.
2. Order Algal cultures from: Botany Dept. of the University of Texas, Austin (512) 471-4019.
3. For additional instruction on the receipt and handling of algal test cultures, see the “**Algal Test Culture Receipt and Handling S.O.P.**”

2.3 Collection and Holding of Water Samples

Grab or composite samples should be collected into appropriately cleaned glass or plastic container(s) (e.g. cubitainers), and immediately be placed on ice (or “blue ice” type product) to bring the temperature to 4°C. The sample should be shipped or transported to the testing laboratory ASAP. Upon receipt of the sample(s) in the laboratory, sample login water quality measurements should be taken. For instruction on the login of incoming samples, see the “Test Sample(s) Log-In Procedures”. The test sample(s) used to start the test must be <36 hrs old (i.e. 36 hrs after collection). No sample > 72 hrs old should be used in any test(s). For each test, a minimum of 4 Liters of sample will be needed.

3.0 TEST INITIATION

These guidelines may be modified according to specific test conditions required by the NPDES permit of the individual client. Identify these specific requirements prior to test initiation and read the attached “**Summary of Test Conditions for *Skeletonema* and *Thalassiosira*.**”

3.1 Water Preparation

3.1.1 Diluent

1. Figure out how much water you need. Remember to have extra for rinsing the flasks.
2. Distilled water, brought to the appropriate salinity with Crystal Sea[®], is used for the salt-water algal tests unless specified otherwise.
3. Prepare 0.45 µm sterile filtered water when preparing culture media or running a ref-tox. When performing an effluent test or testing a volatile material, positive pressure filtration must be used.
4. Add nutrients to both the diluent and test waters in the following concentrations: 15mL/liter of the metal mix solution, 1 mL/liter of the minor salt solution, and 0.5 mL/liter of the vitamin stock solution (1.0 mL/liter for *Thalassiosira*). See **Table 1** for more information on the nutrient solutions.
5. Adjust the diluent pH to 8.0± 0.2 with HCl or NaOH.

3.1.2 Test Sample Water

Follow same procedure as in 3.1.1.

1. Prepare sample
2. Add nutrients

3. Adjust pH
4. Positive pressure sterile filter sample

3.2 Test Setup

1. Prepare the test flasks for each concentration. You will need 4 flasks per treatment. Label the flasks with their treatment and replicate I.D.
2. Prepare dilutions of the test material.
3. For each treatment, record the initial water quality (pH, D.O., conductivity, and temperature) onto the data sheets; check to make sure that all parameters, especially the D.O. are within acceptable levels.
4. Beginning with the Control treatment and working up through the concentration series, pour 100 mL of sample water into each of the 4 containers.

3.3 Test Inoculation

3.3.1 Determination of Inoculation Volumes:

1. Inoculation should be 2×10^4 cells/mL
2. Use 4-7 day old cultures in log growth
3. Count 1 mL of culture to find the inoculation stock algal density. Use a Sedgewick-Rafter chamber slide and count 3 or more fields on the Whipple grid. Allow algae to settle for 25 minutes on slide before counting. Use the appropriate formula calculation to determine if stock culture needs to be diluted or concentrated. Preferably, the inoculation volume should be between 0.8-1.3 mL/flask so as not to unduly dilute the test sample.
 - a. Formula: $\text{cells/mL} = N(1000) / \# \text{ fields (A)}$
 - b. N = total number of cells counted
 - c. A = area of the grid (labeled on the microscope)
 - d. $\# \text{ fields}$ = the number of fields (grids) counted
4. The formula for determining inoculation is as follows: $(\text{algal density})(x) = 2 \times 10^4$
 - a. x = mL of algal stock needed (x should be approximately 1 mL + 0.3 mL)

3.3.2 Test Inoculation

1. Randomize the flasks containing test solutions and inoculate with the calculated inoculation volume.
2. Separate out 3 flasks and pull 1 mL of each for counting, to check that the inoculation was correct. Inoculation numbers should be within 80% of each other.
3. Randomly place the test containers within the temperature-controlled water bath. Make sure that all of the necessary data are recorded upon the data sheets.

4.0 MAINTAINING THE TEST

Swirl flasks twice daily.

5.0 TEST TERMINATION

1. Take down the test after 96 hours. Keep flasks cold and in the dark to restrict any further growth. Record pH, DO and salinity in the high, low and control concentrations.
2. Count cell densities on a microscope (5 fields minimum or 400 cells in more than five fields, up to 20 fields).
3. Collect and prepare data for statistical analysis.

6.0 REFERENCE TOXICANT TESTING

To ensure that the organisms being used in the test are responding to chemical stress in a “typical” manner, a reference toxicant test is run side-by-side with the effluent test. The reference toxicant results are then compared with an in-house data base for that reference toxicant to make this determination. Once the various reference toxicant concentrations are prepared, test set-up, maintenance, and termination are identical to those above. Information regarding the algal growth reference toxicity test is presented in the “*Skeletonema & Thalassiosira* Growth Reference Toxicity Test SOP”.

7.0 DATA ANALYSIS

The cell density data for each replicate, which are recorded on the appropriate data sheets, are entered into a CETIS™ v1.023 data file labeled for identification of the specific test. Statistical analyses are performed in accordance with EPA guidelines for statistical analysis.

8.0 TEST ACCEPTABILITY CRITERIA

Test acceptability criteria for both the *Skeletonema costatum* or *Thalassiosira pseudonana* test are as follows: there must be an average of at least 1×10^5 cells/mL in the Control treatment.

9.0 QUALITY CONTROL

1. Control water (Dionized water adjusted to the appropriate salinity with Crystal Sea®) with nutrients added, is used.
2. All equipment is calibrated and operated as described in each applicable equipment SOP.
3. All staff working independently on any test shall have previously demonstrated familiarity and competency with the test, analytical equipment used, and the corresponding SOPs.

10.0 SAFETY

The *Skeletonema & Thalassiosira* chronic toxicity tests poses little risk to those performing them. Effluents can contain pathogens and appropriate safety precautions should be observed when handling these materials. Care should be taken in the preparation of the reference toxicant spiking solution. Review the MSDS for the reference toxicant. After the ref-tox spiking solution has been used, any remaining solution should be appropriately stored for hazardous waste disposal.

Table 1. Nutrient Stock Solutions for Maintaining Saltwater Algal Stock Cultures and Test Control Cultures.

Nutrient Stock Solution	Compound	Amount dissolved in 1 L Distilled Water ^A
METAL MIX SOLUTION (A):		
	FeCl ₃ •H ₂ O	0.048 g
	MnCl ₂ •4H ₂ O	0.144 g
	ZnSO ₄	0.045 g
	CuSO ₄ •5H ₂ O	0.000157 g (0.157 mg) ^B
	CoCl ₂ •6H ₂ O	0.000404 g (0.404 mg) ^C
	H ₃ BO ₃	1.140 g
	Na ₂ EDTA•2H ₂ O	1.0 g ^D
MINOR SALT MIX (B)		
	K ₂ HPO ₄	0.3 g
	NaNO ₃	5.0 g
	NaSiO ₃ •9H ₂ O	2.0 g
VITAMIN STOCK SOLUTION (C)		
	Thiamine HCl	0.5 g
	Biotin	0.001 g (1 mg) ^E
	B ₁₂	0.001 g (1 mg) ^E

- A Add salts to 900 mL of distilled water and dilute to a final volume of 1 L when all salts are dissolved.
- B CuSO₄•5H₂O - Weigh out 0.0157 g and dilute to 100 mL. Add 1 mL of this solution to Stock A.
- C CoCl₂•6H₂O - Weigh out 0.0404 g and dilute to 100 mL. Add 1 mL of this solution to Stock A.
- D Na₂EDTA•2H₂O - Use this chemical only for culturing and when specifically required by the project. EDTA will chelate metals and can alter the toxicity of the sample.
- E Biotin & B12 - Weigh out 0.1 g of each and dilute to 100 mL. Add 1 mL of this solution to Stock C.

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR ALGAL (<i>SKELETONEMA COSTATUM</i>) GROWTH TEST	
1. Test type	Static
2. Test duration	96 hours
3. Temperature	20 ± 2°C
4. Light quality	“Cool white” fluorescent lighting
5. Light intensity	60 ± 9 $\mu\text{E}/\text{m}^2/\text{s}$
6. Photoperiod	16 hours light: 8 hours darkness
7. Test chamber size	250 mL
8. Test solution volume	100 mL
9. Renewal of test solutions	None
10. Age of test organisms	4-7 days old
11. Initial cell density	20,000 cells/mL
12. # of replicates chambers per concentration	Four
13. Shaking rate	Twice daily by hand
14. Test chamber cleaning	Rinsed with dilution water
15. Dilution water	0.45 μm filtered seawater or deionized water and sea salts. Salinity: 24–35ppt
16. Dilution factor	According to NPDES permit. Effluents: ≥ 0.5 dilution series Receiving Waters: None, or ≥ 0.5 dilution series
17. Test endpoint	Growth
18. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours (no sample > 72 hours is used in test)
19. Sample volume required	2 Liters
20. Test acceptability	1 x 10 ⁵ cells/mL in controls

SUMMARY OF TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA FOR ALGAL (<i>THALASSIOSIRA PSEUDONANA</i>) GROWTH TEST	
1. Test type	Static
2. Test duration	96 hours
3. Temperature	20 ± 2°C
4. Light quality	“Cool white” fluorescent lighting
5. Light intensity	82-90 $\mu\text{E}/\text{m}^2/\text{s}$
6. Photoperiod	Continuous illumination
7. Test chamber size	250 mL
8. Test solution volume	100 mL
9. Renewal of test solutions	None
10. Age of test organisms	4-7 days old
11. Initial cell density	20,000 cells/mL
12. # of replicates chambers per concentration	Four
13. Shaking rate	Once daily by hand
14. Test chamber cleaning	Rinsed with dilution water. None during test.
15. Dilution water	0.45 μm filtered seawater or deionized water and sea salts
16. Dilution factor	According to NPDES permit. Effluents: ≥ 0.5 dilution series Receiving Waters: None, or ≥ 0.5 dilution series
17. Test endpoint	Growth
18. Sampling and holding requirements	Grab or composite samples must be used to start test within 36 hours (no sample > 72 hours is used in test)
19. Sample volume required	2 Liters
20. Test acceptability	1 x 10 ⁵ cells/mL in controls

Appendix E

Supporting Documents for Chemical Analysis

Appendix E

Attachment 1: Standard Operating Procedure for
Ammonia-N by SM 4500-NH₃ G

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Standard Operating Procedure Standard Method 4500-NH₃ BG Ammonia as N in Drinking Water, Wastewater and Solids by FIASTAR 5000, FOSS, or Flow Injection Analysis

APPROVAL: The undersigned has verified that this SOP is complete and meets requirements specified in document S0QA010.XXX:

Wet Chemistry Manager:

Date:

8-8-05

Quality Assurance Director:

Date:

8-8-05

1.0 Scope and Application:

- 1.1 This is a distillation and flow injection analysis (FIA) method applicable to the determination of Ammonia as N in drinking waters, wastewaters, and solids.
- 1.2 The specific list of Practical Quantitation Limits (PQL) for each matrix is maintained in the FGL LIMS.
- 1.3 Prior to the use of this procedure FGL employees must perform and document an initial demonstration of proficiency for the method and LIMS. For continued use, annual method proficiency must be documented. Refer to S0QA185.XXX for employee training.

2.0 Summary of Method:

2.1 Summary:

- 2.1.1 The ammonia, as ammonium hydroxide, is released from samples by means of a distillation operation under basic conditions and absorbed in a scrubber containing sulfuric acid. The ammonia in the absorbing solution is then determined colorimetrically by FIA.
- 2.1.2 The ammonia is peristaltically introduced to the flow injection analysis system. The ammonia then reacts with the sodium potassium tartrate, bleach and phenate to form a blue color.
- 2.1.2 The concentration is quantified using a five point standard curve read colorimetrically at XXX nm.

2.2 Definitions:

- 2.2.1 Definitions are listed in the FGL Quality Assurance Plan, Section 17.0.

2.3 Method Modifications:

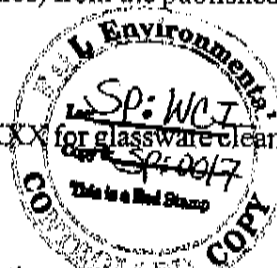
- 2.3.1 This SOP has used a reduction of sample and reagents (in the correct ratios) from the published test method.

3.0 Interferences:

- 3.1 All glassware must be properly cleaned before use. Refer to S0QA135.XXX for glassware cleaning.
- 3.2 The distillation is performed to remove interferences.

4.0 Safety:

- 4.1 A lab coat and gloves should be worn while performing analyses. Protective eyewear must be worn at all times. Refer to the individual MSDS's for safe handling of chemicals.



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5.0 Sample Containers, Preservation and Handling:

- 5.1 Samples should be collected in 1 pint plastic containers.
- 5.2 Sample preservation is 2 mL 1+1 H₂SO₄ and refrigeration at 4° C.
- 5.3 The holding time is 28 days.
- 5.4 Procedures for sampling are maintained in the FGL field services department.
- 5.5 Procedures for sample shipment are maintained in the FGL shipping department.

6.0 Equipment and Supplies:

- 6.1 FOSS FIASTAR 5000
- 6.2 Ammonia Cartridge
- 6.3 Autosampler w/120 positions for 12 mL vials

7.0 Quality Control:

- 7.1 **Preparation Quality Controls:** the batch size for extraction is 20 samples.

- 7.1.1 Method Blank: one method blank is run per batch.

- 7.1.2 Laboratory Control Sample (LCS): one LCS is run per batch.

- 7.1.3 Matrix Spike (MS): a set of duplicate MS's are run per batch.

- 7.2 **Analysis Quality Controls:** the batch size for analysis is 10 samples.

- 7.2.1 Initial/Continuing Calibration Blank (ICB/CCB): the ICB is run after the calibration and the CCB is run after every analytical batch and at the end of the analytical run.

- 7.2.2 Initial/Continuing Calibration Verification (ICV/CCV): the ICV is run after the calibration and the CCV is run after every analytical batch and at the end of the analytical run.

- 7.3 **Detection Limit Quality Controls:**

- 7.3.1 Method Detection Limit (MDL) determination: MDL's are run on an annual basis for both aqueous and solid matrices. For MDL guidance please see SOP S0QA060.XXX.

8.0 Reagent Preparation:

- 8.1 All reagents received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. Login the reagent using FGL LIMS, Laboratory, Wct Chem, Standards. In order to minimize reagent waste, an appropriate quantity of reagent should be created or purchased which will be completely used during the lifetime of the reagent. Refer to S0QA030.XXX for proper reagent preparation and documentation.

- 8.2 Record the reagent code for all reagents on the prep sheet.

- 8.3 Distillation:

- 8.3.1 0.04 N H₂SO₄: dilute 1.2 mL of conc. H₂SO₄ to 1000 mL in a volumetric flask with deionized water.

- 8.3.2 50% NaOH: dissolve 500 g of NaOH pellets and add 1000 mL of deionized water. This is an extremely exothermic reaction. This procedure should be carefully performed under a hood. Allow ample time for solution to cool; preferably, prepare the reagent a day or two in advance.

- 8.3.3 Borate Buffer: 88 mL of 0.01 in NaOH + 9.5 g Na₂B₄O₇ x 10 H₂O to 1000 mL of deionized water.

- 8.4 Analytical Reagents:

- 8.4.1 Bleach, 10%: Dilute 20 mL of bleach to 200 mL with DI H₂O.

- 8.4.2 Alkaline Tartrate Reagent: Dissolve 20 g of NaOH pellets and 15 g of potassium sodium tartrate into final volume of 500 mL with DI H₂O.

- 8.4.3 Phenol/Prusside Reagent: Dissolve 18 g of NaOH and 0.6 of sodium nitroprusside. After it is dissolved, slowly add 42 mL of liquified phenol to volumetric under the hood. Bring up to a final

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volume of 500 mL with DI H_2O . Solution is exothermic; therefore, use caution when handling. Wait for solution to cool before transferring to carrier bottle. Make a fresh solution, on a weekly basis. Store in an amber bottle.

9.0 Standard Preparation:

- 9.1 All standards received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. The LIMS then maintains the specific concentrations for each standard mix and subsequent dilutions. Login the standard using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize standard waste, an appropriate quantity of standard should be created or purchased which will be completely used during the lifetime of the standard. Refer to S0QA030.XXX for proper standard preparation and documentation.
- 9.2 Record the standard code for all standards on the prep sheet. Also record the volumes of standards used and their concentrations.
- 9.3 Primary Calibration Standard:
 - 9.3.1 Stock: (Ammonia-N 1000 mg/L): purchase from current vendor. Store in refrigerator.
 - 9.3.2 Intermediate: (Ammonia-N 50.0 mg/L): dilute 5 mL of stock standard 9.3.1 to 100 mL of 0.04 N H_2SO_4 .
 - 9.3.3 Working: undistilled standards for standard curve: independently dilute 0, 0.25, 0.5, 1.0, and 2.5 mL of stock standard 9.3.1 to 500 mL with 0.04 N H_2SO_4 to make corresponding concentrations of 0, 0.1, 0.5, 1.0, 2.0 and 5.0 mg/L.
- 9.4 Secondary Calibration Verification Standard: **this standard must be from either a different lot or supplier than the Primary calibration standard.**
 - 9.4.1 Stock: (Ammonia-N 1000 mg/L): purchase from current vendor. Store in refrigerator.
 - 9.4.2 Working: (Ammonia-N 2.0 mg/L): dilute 1.0 mL of stock standard (9.4.1) to 500 mL of 0.04 N H_2SO_4 .

10.0 Sample Preparation:

10.1 LIMS Batching:

- 10.1.1 Batch the samples for preparation using FGL LIMS, Laboratory, Wet Chem, Preparation. Completely fill in all information requested on the prep sheet. All sample observations are noted in the comments column.

10.2 Liquid QC Sample Preparation:

- 10.2.1 Method Blank: to the distillation chamber add 25 mL amount of 0.04 N H_2SO_4 .
- 10.2.2 LCS: to the distillation chamber add 25 mL of working standard (9.4.2).
- 10.2.3 MS/MSD: to the distillation chamber add a 24 mL aliquot of sample then add 1.0 mL of intermediate standard (9.3.2). This is performed in duplicate.
- 10.2.4 Continue the QC samples with step 10.3.2.9.

10.3 Solids QC Sample Preparation:

- 10.3.1 Method Blank: to a 4 oz plastic container add 40 mL of KCl.
- 10.3.2 LCS: to a 4 oz plastic container add 1 mL of working standard (9.4.2) and dilute to 40 mL with KCl.
- 10.3.3 MS/MSD: to duplicate a 4 oz plastic container add 10 g aliquots of sample then add 1 mL of working standard (9.4.2) and dilute to 40 mL with KCl.
- 10.3.4 Continue the QC samples with step 10.3.2.9.

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10.4 Sample Preparation:

- 10.4.1 Refer to S0QA165.XXX for guidance on obtaining a representative sample.
- 10.4.2 **Distillation procedure:**
 - 10.4.2.1 Turn on cooling water and adjust to normal rate.
 - 10.4.2.2 Check water level in boiling flask (steam reservoir). It needs to be 3/4 full.
 - 10.4.2.3 Turn on heater (highest setting) and allow steam reservoir to come to a boil.
 - 10.4.2.4 Place a waste cup under the outlet of the condenser.
 - 10.4.2.5 Allow still to reach thermal equilibrium (4-5 mL/min).
 - 10.4.2.6 Open wasteline stopcock and let sample chamber drain completely. (Don't forget to close wasteline after draining, otherwise you will lose your sample.)
 - 10.4.2.7 Add 25 mL deionized water through the addition funnel into the sample chamber. Then add 10 mL of 50% NaOH. Let it come to a boil and continue for about 10 minutes to clean out the still out. After the still is cleaned, open the wasteline stopcock and let the sample chamber drain. Rinse the inside of the still and the outlet of the condenser about three times with deionized water.
 - 10.4.2.8 Label and add 5 mL of 0.04 N H_2SO_4 to 50 mL centrifuge tubes. These are your sample receivers.
 - 10.4.2.9 Neutralized 25 mL of sample to approximately pH 7 by adding sodium hydroxide. After neutralizing add 1.2 mL borate buffer then adjust the pH to 9.5. After adjustment transfer the sample to the addition funnel and let drain into sample chamber. pH Adjustment is not required for TKN digests - add the 25 mL digest directly to the sample chamber. Place your sample receiver under the condenser outlet so that the tip of the outlet is submerged into the 5 mL 0.04 N H_2SO_4 . Rinse addition funnel about three times with a small amount of deionized water. Close the stopcock and fill additional funnel up with deionized water. Open stopcock for a short time so that deionized water stands in the line to the sample chamber. Distill for about 3 minutes. Lower sample receiver so that the condenser tip is now above the sample level. Let distill to 25 mL total volume. If you go over 25 mL note final volume on batch sheet.
 - 10.4.2.10 Drain sample chamber completely and rinse three times with deionized water. Rinse condenser outlet with deionized water after every sample.
 - 10.4.2.11 Fill steam reservoir up to 3/4 with deionized water if necessary and wait until still reaches thermal equilibrium. The still is then ready for next sample.
- 10.4.3 **Soil Extraction:**
 - 10.4.3.1 If volatile analysis is also required for the sample, it must be returned to the volatiles refrigerator ASAP and if in a brass tube, mark an X on the end that was opened.
 - 10.4.3.2 If the sample is free of any solid (e.g. rocks or glass) particles >2 mm, simply break up any clumps, mix well, and weigh into an acid cleaned beaker. Any semi-solid particles should be crushed.
 - 10.4.3.3 Thoroughly mix the sample.
 - 10.4.3.4 Weigh 10 g of soil into a 4 oz plastic container and add 40 mL 2 N KCl.
 - 10.4.3.5 Put on shaker for one hour.
 - 10.4.3.6 The extract is then distilled as a listed in section 10.3.2.

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11.0 Calibration:

11.1 Frequency:

- 11.1.1 The calibration is performed at the beginning of each analytical run or when continuing calibration verifications indicate a new calibration is required.

11.2 Procedure and Calculation:

- 11.2.1 Prepare standards as outlined in section 9.3.3. The ammonia-N standards do not require distillation.
- 11.2.2 Analyze standards as outlined in sections 12.2.
- 11.2.3 The calibration calculation is performed by the instrument and must be reviewed by the analyst.

11.3 Acceptance Criteria:

- 11.3.1 The criteria for passing this calibration is the correlation coefficient has to be greater than 0.995.

11.4 Failure Resolution:

- 11.4.1 If the failure can be corrected by performing instrument maintenance and/or recalibration then initiate the correction. Log the instrument maintenance performed.
- 11.4.2 If you are unsure of how to handle the failure please contact your supervisor or the QA director.
- 11.4.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

12.0 Analytical Procedure:

- 12.1 **LIMS Batching:** batch the samples for analysis using FGL LIMS, Laboratory, Wet Chem, Analysis.

12.2 Sample Analysis:

- 12.2.1 Run samples on FOSS with vial positions on prep sheet.

13.0 Calculations:

- 13.1 The analysis results including analysis dilutions are automatically calculated and expressed as mg/L values by the instrument.
- 13.2 All other dilutions are entered into the LIMS and calculated by the LIMS. The LIMS also corrects for the unit changes to mg/L or mg/Kg.

14.0 Data Assessment and Failure Resolution:

- 14.1 All current Data Quality Objectives (DQO's) for quality controls listed below are maintained in the FGL LIMS and automatically checked during data assessment. Qualify the data using FGL LIMS, Laboratory, Wet Chem, Analysis or Prep.

14.2 Preparation Quality Controls:

- 14.2.1 Method Blank: the method blank must be less than the Practical Quantitation Limit used for reporting (PQL).
- 14.2.2 LCS: the percent recovery for the LCS must be within the FGL acceptance range based on the control chart.
- 14.2.3 MS/BS: the percent recovery and the relative percent difference of the duplicate spikes should be within the FGL acceptance range based on the control chart.

14.3 Analysis Quality Controls:

- 14.3.1 ICB/CCB: the ICB/CCB must be less than the instrument reporting limit (IRL).
- 14.3.2 ICV/CCV: the ICV/CCV must be within the FGL acceptance range of 90-110%.

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14.4 Detection Limit Quality Controls:

14.4.1 MDL: the MDL must be lower than the PQL.

14.5 Failure Resolution:

14.5.1 If the failure can be corrected by repreparation and/or reanalysis then initiate the correction.

14.5.2 If the failure can't be corrected by repreparation and/or reanalysis or if you are unsure of how to handle the failure please contact your supervisor or the QA director. If the data must be accepted with a failure, an explanation must accompany the failure. Refer to S0QA095.XXX for guidance on proper handling of failures.

14.5.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

15.0 Method Performance:

15.1 MDL studies are used to determine precision, accuracy and sensitivity for evaluation of performance at the reporting level. MDL's are electronically stored in the LIMS and a hard copy is maintained in the QA department.

15.2 Control charts are used for analysis and preparation quality controls to determine precision and/or accuracy on an ongoing basis for evaluating the performance of the method. Control charts are electronically maintained in the LIMS. Where applicable the control charts are used to generate the data quality objectives.

15.3 Performance Testing (PT) through interlaboratory studies is performed semi-annually (where applicable) to independently verify performance. PT Study results are maintained in the QA department.

15.4 Refer to the referenced test method(s) for published interlaboratory method performance.

16.0 Review and Reporting:

16.1 Preparation Review and Reporting:

16.1.1 Preparation Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.

16.1.2 Samples are reported in mg/L or mg/Kg units. They are printed on the Prep Summary Report of the Preparation Data Package and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

16.2 Analysis Review and Reporting:

16.2.1 Analysis Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.

16.2.2 Samples are analyzed in mg/L units. They are printed on the Raw Data for instrumental methods or the Analysis Summary Report for manual methods and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

17.0 Record Storage and Archiving:

17.1 Preparation Records:

17.1.1 The records generated during preparation are Preparation Package Narrative, Prep Confirmation Report, Quality Control Reports, Prep Summary Report and Prep Worksheet. These all form the Prep/Extraction Data Package.

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- 17.1.2 After the Preparation Data Package has been reviewed, it is filed in the Wet Chemistry department filing cabinet. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

17.2 Analysis Records:

- 17.2.1 The records generated during analysis are Analysis Package Narrative, Analysis Confirmation Report, Quality Control Reports, Analysis Runlog and Raw Data or Analysis Summary Report. These all form the Analysis Data Package.
- 17.2.2 After the Analysis Data Package has been reviewed, it is filed in the Wet Chemistry department filing cabinet. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

18.0 Maintenance:

- 18.1 A maintenance log book is utilized to document repair on the instrument. It is imperative that these logs are kept up to date. Log books will be checked for completeness during the annual QA audit. Refer to SOP S0QA140.XXX for instrument maintenance.
- 18.2 Specific instrument maintenance information is located in the manufacturers instructions.
- 18.3 The instrument identification information is maintained in the FGL Quality Assurance Plan.

19.0 Pollution Prevention and Waste Disposal:

- 19.1 Where feasible, techniques have been used to reduce the amount of standards and reagents in order to minimize the amount of waste generated by this procedure. This is primarily through the proper management of reagents and standards (see sections 8.1 and 9.1) and the reduction of sample and reagents (in the proper ratios) used to perform the procedure.
- 19.2 Waste from this analysis meets standard sewage discharge requirements. Waste from the analysis may be disposed of down the sink while rinsing with tap water. Samples are kept for one month prior to disposal.

20.0 References:

- 20.1 "Standard Methods for the Examination of Water and Wastewater," AWWA, 18th ed, 1992, Method No. 4500-NH₃ BG.

21.0 Associated Documents:

- 21.1 S0QA030.XXX - SOP for Standard/Reagent Preparation and Documentation
- 21.2 S0QA035.XXX - SOP for Non-conformance/Corrective Action Program
- 21.3 S0QA060.XXX - SOP for Performing MDL/IDL Studies
- 21.4 S0QA090.XXX - SOP for Data Reduction and Recording
- 21.5 S0QA095.XXX - SOP for Qualifying Data
- 21.6 S0QA105.XXX - SOP for Review of Data Packages by Analysts and Manager
- 21.7 S0QA135.XXX - SOP for Glassware and Plasticware Cleaning
- 21.8 S0QA140.XXX - SOP for Instrument Maintenance
- 21.9 S0QA165.XXX - SOP for Spiking, Diluting and Homogenizing
- 21.10 S0QA185.XXX - SOP for Analyst Demonstration of Proficiency
- 21.11 S0QA215.XXX - SOP for Archiving Records.

Appendix E

Attachment 2: Standard Operating Procedure for
Anions (chloride, nitrate-N, sulfate) by EPA 300.0

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Standard Operating Procedure EPA Method 300.0 and EPA 9056 Anions in Drinking Water, Wastewater and Solids by Dionex DX-500 Ion Chromatography

APPROVAL: The undersigned has verified that this SOP is complete and meets requirements specified in document S0QA010.XXX:

Wet Chemistry Manager:

Date: 2-14-06

Quality Assurance Director:

Date: 2-14-06

1.0 Scope and Application:

- 1.1 This is an ion-chromatography method applicable to the determination of anions in drinking water, wastewater and solids.
- 1.2 The specific list of analytes and their associated Practical Quantitation Limits (PQL) for each matrix is maintained in the FGL LIMS.
- 1.3 Prior to the use of this procedure FGL employees must perform and document an initial demonstration of proficiency for the method and LIMS. For continued use, annual method proficiency must be documented. Refer to S0QA185.XXX for employee training.

2.0 Summary of Method:

2.1 Summary:

- 2.1.1 An autosampler picks up a fixed quantity of sample and delivers it to a valve to mix with a carrier eluent. The carbonate/bicarbonate eluent elutes the sample across a guard column and an analytical column made of aminated polystyrene. Anions are separated based on their affinities for the basic exchange sites on the column, eluting at different retention times. To suppress the background conductivity and to heighten sensitivity, the solution then passes through a micromembrane suppressor unit prior to entering the detector. There, ions are protonated into their weak acid forms with hydrogen ions that have been electrolytically split from water molecules. The detector measures the electrical conductivity of the solution, which is directly proportional to the conductive species present, and adjusts for small temperature variations. This information is stored as digital information and then is simultaneously plotted and saved onto a hard drive. The plots depict for each of the analytes peaks with area counts that are quantified by comparison against a stored six point standard curve.
- 2.1.2 Drinking water and wastewater samples are directly injected into the instrument. If the sample is turbid it is filtered through a 0.45um filter prior to injection.
- 2.1.3 Soil samples are extracted according to method 300.0, section 11.7. This is a passive extraction using deionized water and the results are considered to be on a soluble basis. The samples are extracted in a 1:10 ratio (sample to extraction fluid).

2.2 Definitions:

- 2.2.1 Definitions are listed in the FGL Quality Assurance Plan, Section 17.0.

2.3 Method Modifications:

- 2.3.1 This SOP contains no known modifications from the published test method.

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3.0 Interferences:

- 3.1 All glassware must be properly cleaned before use. Refer to S0QA135.XXX for glassware cleaning.
- 3.2 It is possible to overload the detector cell, causing an erroneously low result (the fraction of available charge carried by each ion is reduced when those ions are present in high concentrations). It is also possible for some organic acids to coelute with an anion and for an anion to shift retention time forward (causing it to be misidentified) when present in high concentrations and consequently masking another analyte. All these problems may be handled by diluting the sample to eliminate such interferences. If interferences are such that an extremely high dilution renders the result meaningless, report ND at the detection limit or analyze the anion in question by another method.

4.0 Safety:

- 4.1 A lab coat and gloves should be worn while performing analyses. Protective eyewear must be worn at all times. Refer to the individual MSDS's for safe handling of chemicals.

5.0 Sample Containers, Preservation and Handling:

- 5.1 Liquid samples should be collected in 1 pint plastic containers.
- 5.2 Soil samples should be collected in 8 oz widemouth jars, brass tubes or ziplock bags. Other containers may be suitable.
- 5.3 Sample preservation is refrigeration at 4°C.
- 5.4 The liquid holding time is 28 days for all anions except nitrate, nitrite, or phosphate. These anions are 48 hours. The solid sample holding time for preparation is 28 days. Once extracted, the soluble samples begin a 48 hr. holding time for nitrate, nitrite, and phosphate.
- 5.5 Procedures for sampling are maintained in the FGL field services department.
- 5.6 Procedures for sample shipment are maintained in the FGL shipping department.

6.0 Equipment and Supplies:

- 6.1 DX-500 Dionex ion chromatograph
Dionex AS autosampler with prep module and solution bottles.
IONPAC AS14 analytical column, IONPAC AG14 guard column
ASRS-Ultra 4mm suppressor
Eluent degas module with carboys
Tech-grade helium.
shell vials (1.5ml)

7.0 Quality Control:

- 7.1 **Preparation Quality Controls:** the batch size for preparation is 20 samples.
 - 7.1.1 Method Blank: one method blank is run per batch.
 - 7.1.2 Laboratory Control Sample (LCS): one LCS is run per batch.
 - 7.1.3 Matrix Spike (MS/MSD): a set of duplicate MS's are run per batch.
 - 7.1.4 Blank Spikes (BS): a set of duplicate BS's are run per batch when sample quantity is insufficient for duplicate MS's to be run.
- 7.2 **Analysis Quality Controls:**
 - 7.2.1 Initial/Continuing Calibration Blank (ICB/CCB): the ICB is analyzed after the calibration beginning an analytical run. The CCB is analyzed after every 10 injections, and at the end of the analytical run.
 - 7.2.2 Initial/Continuing Calibration Verification (ICV/CCV): the ICV is run after the calibration at the

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beginning of each analytical run. The CCVs are analyzed every 10 injections and at the end of the analytical run. The concentration level of the ICV and CCV's must be at two levels. (ie: ICV = 10X, CCV = 20X)

7.3 Detection Limit Quality Controls:

7.3.1 Method Detection Limit (MDL) determination: MDL's are run initially for each analyte and matrix as part of the initial demonstration of performance. MDL's are then performed each time there is a change in the test method that affects how the test is performed or when a change in instrumentation occurs that affects the sensitivity of the analysis. Also, MDL's may be required by project or to support new lower regulatory limits. For MDL guidance please see SOP S0QA060.XXX.

8.0 Reagent Preparation:

8.1 All reagents received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. Login the reagent using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize reagent waste, an appropriate quantity of reagent should be created or purchased which will be completely used during the lifetime of the reagent. Refer to S0QA030.XXX for proper reagent preparation and documentation.

8.2 Record the reagent code for all reagents on the prep sheet.

8.3 AS14 500 mM Na_2CO_3 Eluent Concentrate: add 26.49g of sodium carbonate to a 0.5 liter volumetric flask and dilute to mark with deionized water. This concentrate preparation must be recorded in the LIMS electronic reagent/standard preparation log.

8.4 AS14 500 mM NaHCO_3 Eluent Concentrate: add 21.00g of sodium bicarbonate to a 0.5 liter volumetric flask and dilute to mark with deionized water. This concentrate preparation must be recorded in the LIMS electronic reagent/standard preparation log.

8.5 AS14, 3.5 mM Na_2CO_3 /1.0 mM NaHCO_3 Eluent: pipet 14.0 mL of carbonate concentrate (8.3) and 4.0 mL of bicarbonate concentrate (8.4) into a 2 L volumetric flask and dilute to mark with deionized water. Pour eluent into carboy #1.

9.0 Standard Preparation:

9.1 All standards received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. The LIMS then maintains the specific concentrations for each standard mix and subsequent dilutions. Login the standard using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize standard waste, an appropriate quantity of standard should be created or purchased which will be completely used during the lifetime of the standard. Refer to S0QA030.XXX for proper standard preparation and documentation.

9.2 Record the standard code for all standards on the prep sheet. Also record the volumes of standards used and their concentrations.

9.3 Primary Calibration Standard:

9.3.1 Stock: purchased from Inorganic Ventures, Part # *FGLANION-2*

9.3.1.1 **Note:** the stock standards are stable for at least 1 month. After 1 month the standards may still be used until the manufacturer expiration date but the standard stabilities must be verified upon use. The independent reference standard (9.4) from Inorganic Ventures has been verified to maintain stability through its expiration date and may be used for this purpose.

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Stock	F	Cl	NO ₂	Br	NO ₃	PO ₄	SO ₄
mg/L	50	500	300	100	400	300	1000

9.3.2 The following guide is for making the custom calibration standards:

Cal #	Vol. Init. (mL)	Vol. final (mL)	Dil. Factor
1	1	500	500x
2	2	500	250x
3	1	100	100x
4	2	100	50x
5	10	100	10x
6	20	100	5x

9.3.3 The following table is a guide for Standard Concentration amounts.

Cal #	F	Cl	NO ₂	Br	NO ₃	PO ₄	SO ₄
1	0.1	1	0.6	200	0.8	0.6	2
2	0.2	2	1.2	400	1.6	1.2	4
3	0.5	5	3	1000	4	3	10
4	1	10	6	2000	8	6	20
5	5	50	30	10,000	40	30	100
6	10	100	60	20,000	80	60	200

Note: Br is reported in parts per billion.

9.4 Secondary Calibration Verification Standard: **This standard must be from either a different lot or supplier than the Primary calibration standard.**

9.4.1 Stock: purchased from Inorganic Ventures, Part # *FGL-ANION-QC2*

9.4.2 Working: dilute stock 9.4.1 x10 with deionized water for the beginning CCV and x20 for subsequent and ending CCVs. Make sure to thoroughly mix CCV before running. **Note:** these standards must be made on a daily basis.

10.0 Sample Preparation for Drinking or Waste Water and Soils:

10.1 Water Samples

10.1.1 Refer to S0QA165.XXX for guidance on obtaining a representative sample

10.1.2 Filter turbid samples with a 0.45 micron syringe filter directly into auto sampler vial or a clean container for dilution when necessary.

10.2 Water Sample QC Preparation:

10.2.1 LCS: 20x. Dilute standard 9.4.1 directly into auto-sampler vial.

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- 10.2.2 MS/MSD: 10x. To duplicate autosampler vials add 0.15 mL aliquots of sample then add 0.15 mL of stock standard (9.4.1) and dilute to 1.5 mL with deionized water.
- 10.2.3 The QC samples are ready for analysis.
- 10.3 **Solid Sample Preparation:**
 - 10.3.1 Weigh 5 gram into a labeled centrifuge tube. Record the weights on the batch sheet.
 - 10.3.2 Add 50 mL of deionized water to each sample.
 - 10.3.3 Samples are ready for extraction, 10.6.
- 10.4 **Solids Sample Quality Control Checks:**
 - 10.4.1 Method Blank: to a centrifuge tube add 50 mL of deionized water.
 - 10.4.2 LCS: to a centrifuge tube add 5.0 mL of stock standard (9.4.1) and dilute to 50 mL with deionized water.
 - 10.4.3 MS/MSD: to duplicate centrifuge tubes add 5 g aliquots of sample then add 50 mL of stock standard (9.4.1) and dilute to 50 mL with deionized water.
 - 10.4.4 Continue the QC samples with extraction, 10.6.
- 10.5 **Solid Sample Extraction:** The samples and quality control checks are extracted on a shaker for 2 hours. Filter through a 0.45um filter using a syringe and lure lock filter.
 - 10.5.1 Remember there is a 48 hr. holding time for nitrate, nitrite, and phosphate analyses that begins after the samples are removed from the shaker.
- 10.6 **LIMS Batching:**
 - 10.6.1 Batch the samples for IC Water or IC Soil preparation using FGL LIMS, Laboratory, Preparation. Completely fill in all information requested in the LIMS prep. record. All sample dilutions and observations are noted by hand in the comments column.
- 11.0 **Calibration:**
 - 11.1 **Frequency:**
 - 11.1.1 The calibration is performed a minimum of every 6 months or when continuing calibration verifications indicate a new calibration is required.
 - 11.2 **Procedure and Calculation:**
 - 11.2.1 Open Chromeleon - Browser. The calibration data is stored as part of the Method file. Upon calibration, the date will be reflected as last update. The curve is a six point curve plotted by quadratic fit with a forced origin point.
 - 11.2.2 Follow the start-up procedure outlined in section 12.1.
 - 11.2.3 Method and Program files in upper window. FGL IC\$ Anion Sequence Data on left.
 - 11.2.4 Label the CALS in the sequence using the format SMMDD##. (example: if analysis date is Jan. 4: 1st level = S010401A, 2nd level= S010402A, 3rd= S010403A, etc...
 - 11.2.5 Change the "Type" for each cal to "Standard".
 - 11.2.6 Assure that the Program is 4A0808AS.pgm, and Method is 4C0804-QNT throughout the entire sequence.
 - 11.2.7 Fill vials with respective standards and insert vials into correct position in auto-sampler tray.
 - 11.2.8 Place tray in auto-sampler, making sure it is level. .
 - 11.2.9 Once it has stabilized back to its normal operating value, click on "Batch." Click on "START." Instrument analysis will begin and highlight the current injection.

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11.3 Acceptance Criteria:

- 11.3.1 The criteria for a acceptable calibration is that the correlation coefficient (r^2) for each analyte of interest must be equal to or greater than 0.995.

11.4 Failure Resolution:

- 11.4.1 If the failure can be corrected by performing instrument maintenance and/or re-calibration then initiate the correction. Log the instrument maintenance performed.
- 11.4.2 If you are unsure of how to handle the failure please contact your supervisor or the QA director.
- 11.4.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

12.0 Analytical Procedure:

12.1 Instrument Start-up:

- 12.1.1 Check 5 gal. waste container and empty if necessary.
- 12.1.2 Check settings and pressure levels of the helium tank.
- 12.1.3 Check the levels of eluent in carboy. It should be at least half full. Top off and degas.
- 12.1.4 Check the pressure on the eluent degas module. It should be set at 10 psi.
- 12.1.5 Open Chromeleon Software, Conductivity Detector Window. Various settings for Pump and Conductivity Detector may be controlled through the software.

12.2 Sample Procedure:

- 12.2.1 Follow the start-up procedure outlined above.
- 12.2.2 **LIMS Batching:** batch the samples for analysis using FGL LIMS.
- 12.2.3 Fill out the prep sheet with vial positions and dilutions.

13.0 Calculations:

- 13.1 Analytes are identified by comparing retention times of peaks in samples with those in standards.
- 13.2 The analysis results are automatically calculated and expressed as mg/L values by the Dionex software. Analysis dilutions are automatically calculated by the Dionex software.
- 13.3 The LIMS calculates preparation extractions and corrects for the unit changes to mg/Kg.

14.0 Data Assessment and Failure Resolution:

- 14.1 All current Data Quality Objectives (DQO's) for quality controls listed below are maintained in the FGL LIMS and automatically checked during data assessment. Qualify the data using FGL LIMS..

14.2 Preparation Quality Controls:

- 14.2.1 Method Blank: the method blank must be less than the Practical Quantitation Limit used for reporting (PQL).
- 14.2.2 LCS: the percent recovery for the LCS must be within the EPA Method 300.0 limits of 90-110%.
- 14.2.3 MS/BS: the percent recovery and the relative percent difference of the duplicate spikes should be within the FGL acceptance range based on the control chart.

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14.3 Analysis Quality Controls:

- 14.3.1 ICB/CCB: the ICB/CCB must be less than the instrument reporting limit (IRL).
- 14.3.2 ICV/CCV: for 300.0 the ICV/CCV must be within the EPA Limits of 90-110%.
- 14.3.3 Retention times are monitored by LIMS. The RT window will be based on three times the standard deviation of the RT data collected in LIMS.

14.4 Detection Limit Quality Controls:

- 14.4.1 MDL: the MDL must be lower than the PQL.
- 14.4.2 Refer to S0QA060.xxx for guidance.

14.5 Failure Resolution:

- 14.5.1 If the failure can be corrected by reparation and/or reanalysis then initiate the correction.
- 14.5.2 If the failure can't be corrected by reparation and/or reanalysis or if you are unsure of how to handle the failure please contact your supervisor or the QA director. If the data must be accepted with a failure, an explanation must accompany the failure. Refer to S0QA095.XXX for guidance on proper handling of failures.
- 14.5.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

15.0 Method Performance:

- 15.1 MDL studies are used to determine precision, accuracy and sensitivity for evaluation of performance at the reporting level. MDL's are electronically stored in the LIMS
- 15.2 Control charts are used for analysis and preparation quality controls to determine precision and/or accuracy on an ongoing basis for evaluating the performance of the method. Control charts are electronically maintained in the LIMS. The control charts are used to generate the data quality objectives for the MS and MSD.
- 15.3 Performance Testing (PT) through interlaboratory studies is performed semi-annually (where applicable) to independently verify performance. PT Study results are maintained in the QA department.
- 15.4 Refer to the referenced test method(s) for published interlaboratory method performance.

16.0 Review and Reporting:

16.1 Preparation Review and Reporting:

- 16.1.1 Data is reviewed electronically in FGL LIMS. No hard copy is generated.
- 16.1.2 Samples are reported in mg/L or mg/Kg units. They are electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

16.2 Analysis Review and Reporting:

- 16.2.1 Data is reviewed electronically in FGL LIMS. No hard copy are generated. Refer to S0QA105.XXX for data package review.
- 16.2.2 Samples are analyzed in mg/Kg units. They are printed on the Raw Data for instrumental methods or the Analysis Summary Report for manual methods and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

17.0 Record Storage and Archiving:

17.1 Preparation Records:

- 17.1.1 The electronic records generated during preparation are stored electronically in LIMS.

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17.2 Analysis Records:

- 17.2.1 The raw data is generated as a PDF file which is referenced in LIMS when data is uploaded and copied to a secure location on the network.
- 17.2.2 The LIMS LRD text file is stored in a secure location on the network.

18.0 Maintenance:

- 18.1 A maintenance log book is utilized to document repair on the instrument. It is imperative that these logs are kept up to date. Log books will be checked for completeness during the annual QA audit. Refer to SOP S0QA140.XXX for instrument maintenance.
- 18.2 Specific instrument maintenance information is located in the manufacturers instructions. For spare parts please refer to F2WCI070.XXX. This list is posted where the spare parts are stored.
- 18.3 The instrument identification information is maintained in the FGL Quality Assurance Plan.
- 18.4 Column Cleaning:
 - 18.4.1 Disconnect the column from the AMMS-1 and the detector.
 - 18.4.2 Connect a container of 1M HCl+0.1M KCl directly into the bottom of the pump's priming block.
 - 18.4.3 Set a flow rate of 1 mL/min and pump 60 mL of the cleaning solution through the column, followed by 30 mL of deionized water.
 - 18.4.4 After cleaning the column, install it in the system and let it equilibrate with the eluent for 30-60 minutes. The column is equilibrated when consecutive injections of the standard give reproducible retention times.

19.0 Pollution Prevention and Waste Disposal:

- 19.1 Where feasible, techniques have been used to reduce the amount of standards and reagents in order to minimize the amount of waste generated by this procedure. This is primarily through the proper management of reagents and standards (see sections 8.1 and 9.1) and the reduction of sample and reagents (in the proper ratios) used to perform the procedure.
- 19.2 The waste container from the IC contains sulfuric acid. The waste must be neutralized prior to flushing down sink with tap water. Fill out neutralization log with estimated volumes.
- 19.3 Any samples found to contain hazardous waste must be given to the Hazardous Waste Officer for proper segregation and disposal.

20.0 References:

- 20.1 *"Methods for the Determination of Inorganic Substances in Environmental Samples,"* USEPA, EPA/600/R-93/100, 1993, Method No. 300.0.

21.0 Associated Documents:

- 21.1 S0QA030.XXX - SOP for Standard/Reagent Preparation and Documentation
- 21.2 S0QA035.XXX - SOP for Non-conformance/Corrective Action Program
- 21.3 S0QA060.XXX - SOP for Performing MDL/IDL Studies
- 21.4 S0QA090.XXX - SOP for Data Reduction and Recording
- 21.5 S0QA095.XXX - SOP for Qualifying Data
- 21.6 S0QA105.XXX - SOP for Review of Data Packages by Analysts and Manager
- 21.7 S0QA135.XXX - SOP for Glassware and Plasticware Cleaning
- 21.8 S0QA140.XXX - SOP for Instrument Maintenance
- 21.9 S0QA165.XXX - SOP for Spiking, Diluting and Homogenizing

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- 21.10 S0QA185.XXX - SOP for Analyst Demonstration of Proficiency
- 21.11 S0QA215.XXX - SOP for Archiving Records.

Appendix E

Attachment 3: Standard Operating Procedure for Phosphate by SM 4500-P E

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Standard Operating Procedure Method SM 4500-P E Ortho-phosphate in Aqueous Matrix by Perkin-Elmer Lambda 20 UV/VIS Spectrophotometer

APPROVAL: The undersigned has verified that this SOP is complete and meets requirements specified in document S0QA010.XXX:

Wet Chemistry Manager: _____

Date: _____

Quality Assurance Director:  _____

Date: 7-28-06

1.0 Scope and Application:

- 1.1 This is a colorimetric method applicable to the determination of Ortho-phosphate in drinking water, wastewater, and surface water.
- 1.2 The specific Practical Quantitation Limits (PQL) for each matrix is maintained in the FGL LIMS.
- 1.3 Prior to the use of this procedure FGL employees must perform and document an initial demonstration of proficiency for the method and LIMS. For continued use, annual method proficiency must be documented. Refer to S0QA185.XXX for employee training.

2.0 Summary of Method:

2.1 Summary:

- 2.1.1 An acidic solution of ammonium molybdate and potassium antimonyl tartrate is added to an aliquot of sample. After 2 minutes, a blue color develops proportional to the phosphate concentration.
- 2.1.2 The concentration is quantified using a five point standard curve prepared using a spectrophotometer set at 890 nm.

2.2 Definitions:

- 2.2.1 Definitions are listed in the FGL Quality Assurance Plan, Section 17.0.

2.3 Method Modifications:

- 2.3.1 This SOP has used a reduction of sample and reagents (in the correct ratios) from the published test method.

3.0 Interferences:

- 3.1 All glassware must be properly cleaned before use. Refer to S0QA135.XXX for glassware cleaning.
- 3.2 0.1 mg/L arsenate may produce false positives.
- 3.3 Cr⁺⁶ and NO₂ at concentrations of 1 mg/L and higher may produce low results.

4.0 Safety:

- 4.1 A lab coat and gloves should be worn while performing analyses. Protective eyewear must be worn at all times. Refer to the individual MSDS's for safe handling of chemicals.

5.0 Sample Containers, Preservation and Handling:

- 5.1 Samples should be collected in 1 pint plastic containers.
- 5.2 Sample preservation is refrigeration at 4° C.

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- 5.3 The holding time 48 hours.
- 5.4 Procedures for sampling are maintained in the FGL field services department.
- 5.5 Procedures for sample shipment are maintained in the FGL shipping department.

6.0 Equipment and Supplies:

- 6.1 MBAS glass tubes
- 6.2 HACH PhosVer³ phosphate reagent pillows
- 6.3 Phenolic caps
- 6.4 Samco transfer pipets
- 6.5 Perkin Elmer Lambda 20 Spectrophotometer

7.0 Quality Control:

- 7.1 **Preparation Quality Controls:** the batch size for extraction is 20 samples.
 - 7.1.1 Method Blank: one method blank is run per batch.
 - 7.1.2 Matrix Spike (MS): a set of duplicate MS's are run per batch.
- 7.2 **Analysis Quality Controls:** the batch size for analysis is 10 samples.
 - 7.2.1 Initial/Continuing Calibration Blank (ICB/CCB): the ICB is run after the calibration and the CCB is run after every analytical batch and at the end of the analytical run.
 - 7.2.2 Initial/Continuing Calibration Verification (ICV/CCV): the ICV is run after the calibration and the CCV is run after every analytical batch and at the end of the analytical run.
- 7.3 **Detection Limit Quality Controls:**
 - 7.3.1 Method Detection Limit (MDL) determination: MDL's are analyzed as part of the initial demonstration and whenever major changes are made to the method (i.e. sample volume changed). For MDL guidance, please see SOP S0QA060.XXX.

8.0 Reagent Preparation:

- 8.1 All reagents received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. Login the reagent using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize reagent waste, an appropriate quantity of reagent should be created or purchased which will be completely used during the lifetime of the reagent. Refer to S0QA030.XXX for proper reagent preparation and documentation.
- 8.2 Record the reagent code for all reagents on the prep sheet.
- 8.3 Color reagent: HACH phosphate color reagent (PhosVer³)

9.0 Standard Preparation:

- 9.1 All standards received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. The LIMS then maintains the specific concentrations for each standard mix and subsequent dilutions. Login the standard using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize standard waste, an appropriate quantity of standard should be created or purchased which will be completely used during the lifetime of the standard. Refer to S0QA030.XXX for proper standard preparation and documentation.
- 9.2 Record the standard code for all standards on the prep sheet. Also record the volumes of standards used and their concentrations.
- 9.3 **Primary Calibration Standard:**
 - 9.3.1 Stock: Phosphate-P 1000 mg/L; purchase from current vendor.

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9.3.2 Intermediate : Phosphate-P 50 mg/L: dilute 5 mL of standard stock to 100 mL with deionized water.

9.4 Secondary Calibration Verification Standard: **this standard must be from either a different lot or supplier than the Primary calibration standard.** Use 0.5mL of intermediate standard to 50mL of DI H₂O for CCV standard.

9.4.1 CCV: Use 10mL per glass tube of CCV.

10.0 Sample Preparation:

10.1 LIMS Batching:

10.1.1 Batch the samples for preparation using FGL LIMS, Preparation. All sample observations are noted in the comments column.

10.2 QC Sample Preparation:

10.2.1 Method Blank: to glass tubes add 10 mL of deionized water.

10.2.2 MS/MSD: to duplicate 5mL of sample, add 5mLs of CCV standard (9.3.2).

10.2.3 Continue the QC samples with step 12.2.2.

10.3 Sample Preparation:

10.3.1 Refer to S0QA165.XXX for guidance on obtaining a representative sample.

10.3.2 Turbid samples should be filtered prior to analysis.

11.0 Calibration:

11.1 Frequency:

11.1.1 The calibration is performed a minimum of every six months or when continuing calibration verifications indicate a new calibration is required.

11.2 Procedure and Calculation:

11.2.1 Calibration standards - 0, 0.0125, 0.05, 0.125, 0.25, 0.5, 0.75, 1.0 mg/L: dilute 0, 0.0125, 0.05, 0.125, 25, 0.5, 0.75, 1.0 mL of the intermediate standard (9.3.2) up to 50 mL with deionized water.

11.2.2 Analyze standards as outlined in section 12.2.

11.2.3 Please refer to section 11.0 of SOP SXWCI176.XXX for the appropriate UV/VIS spectrophotometer calibration.

11.2.4 The calibration calculation is performed by the FGL LIMS.

11.3 Acceptance Criteria:

11.3.1 The correlation coefficient must be greater than 0.995.

11.4 Failure Resolution:

11.4.1 If the failure can be corrected by performing instrument maintenance and/or recalibration then initiate the correction. Log the instrument maintenance performed.

11.4.2 If you are unsure of how to handle the failure please contact your supervisor or the QA director.

11.4.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

12.0 Analytical Procedure:

12.1 LIMS Batching: batch the samples for analysis using FGL LIMS, Analysis.

12.2 Sample Analysis:

12.2.1 Set the spectrophotometer wavelength to 890 as outlined in section 12.0 of SOP SXWCI176.XXX for using the spectrophotometer.

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- 12.2.2 Measure 10 mL of each sample into a glass tube.
- 12.2.3 Add 1 pillow of color reagent to each sample. Mix and wait 2 minutes for the color to develop.
- 12.2.4 Rinse the cuvette once with the blank then fill. Place in the instrument cuvette holder making sure that there are no air bubbles or smears present. Autozero the instrument.
- 12.2.5 Continue in the same manner for check standards, method blanks and samples but do not autozero.
- 12.2.6 Record the absorbance on the prep sheet, following the procedure outlined in section 12.0 in SXWC1176.XXX.

13.0 Calculations:

- 13.1 The absorbances and dilutions are entered into the LIMS and calculated by the LIMS. The LIMS also converts from $\text{PO}_4\text{-P}$ to PO_4 if needed.

14.0 Data Assessment and Failure Resolution:

- 14.1 All current Data Quality Objectives (DQO's) for quality controls listed below are maintained in the FGL LIMS and automatically checked during data assessment. Qualify the data using FGL LIMS, Laboratory, Wet Chem, Analysis or Prep.

14.2 Preparation Quality Controls:

- 14.2.1 Method Blank: the method blank must be less than the Practical Quantitation Limit used for reporting (PQL).
- 14.2.2 MS/BS: the percent recovery and the relative percent difference of the duplicate spikes should be within the FGL acceptance range based on the control chart.

14.3 Analysis Quality Controls:

- 14.3.1 ICB/CCB: the ICB/CCB must be less than the instrument reporting limit (IRL).
- 14.3.2 ICV/CCV: the ICV/CCV must be within the FGL acceptance range based on the control charts.

14.4 Detection Limit Quality Controls:

- 14.4.1 MDL: Refer to SOP S0QA060.XXX

14.5 Failure Resolution:

- 14.5.1 If the failure can be corrected by reparation and/or reanalysis then initiate the correction.
- 14.5.2 If the failure can't be corrected by reparation and/or reanalysis or if you are unsure of how to handle the failure please contact your supervisor or the QA director. If the data must be accepted with a failure, an explanation must accompany the failure. Refer to S0QA095.XXX for guidance on proper handling of failures.
- 14.5.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

15.0 Method Performance:

- 15.1 MDL studies are used to determine precision, accuracy and sensitivity for evaluation of performance at the reporting level. MDL's are electronically stored in the LIMS.
- 15.2 Control charts are used for analysis and preparation quality controls to determine precision and/or accuracy on an ongoing basis for evaluating the performance of the method. Control charts are electronically maintained in the LIMS. Where applicable the control charts are used to generate the data quality objectives.
- 15.3 Performance Testing (PT) through interlaboratory studies is performed semi-annually (where applicable) to independently verify performance. PT Study results are maintained in the QA

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department.

15.4 Refer to the referenced test method(s) for published interlaboratory method performance.

16.0 Record Storage and Archiving:

16.1 Preparation Records:

16.1.1 Hard copy records generated during the sample preparation and analysis consist of only the preparation batch sheet.

16.1.2 Batch sheet records are stored in the QA department and periodically archived.
Refer to SOP S0QA215.XXX.

17.0 Maintenance:

17.1 A maintenance log book is utilized to document repair on the instrument. It is imperative that these logs are kept up to date. Log books will be checked for completeness during the annual QA audit.
Refer to SOP S0QA140.XXX for instrument maintenance.

17.2 The instrument identification information is maintained in the FGL Quality Assurance Plan.

18.0 Pollution Prevention and Waste Disposal:

18.1 Where feasible, techniques have been used to reduce the amount of standards and reagents in order to minimize the amount of waste generated by this procedure. This is primarily through the proper management of reagents and standards (see sections 8.1 and 9.1) and the reduction of sample and reagents (in the proper ratios) used to perform the procedure.

18.2 Waste from this analysis meets standard sewage discharge requirements. Waste from the analysis may be disposed of down the sink while rinsing with tap water. Samples are kept for one month prior to disposal.

19.0 References:

19.1 "Standard Methods for the Examination of Water and Wastewater," 20th ed., AWWA, 1998, Method No. 4500-P E.

20.0 Associated Documents:

- 20.1 S0QA030.XXX - SOP for Standard/Reagent Preparation and Documentation
- 20.2 S0QA035.XXX - SOP for Non-conformance/Corrective Action Program
- 20.3 S0QA060.XXX - SOP for Performing MDL/IDL Studies
- 20.4 S0QA090.XXX - SOP for Data Reduction and Recording
- 20.5 S0QA095.XXX - SOP for Qualifying Data
- 20.6 S0QA105.XXX - SOP for Review of Data Packages by Analysts and Manager
- 20.7 S0QA135.XXX - SOP for Glassware and Plasticware Cleaning
- 20.8 S0QA140.XXX - SOP for Instrument Maintenance
- 20.9 S0QA165.XXX - SOP for Spiking, Diluting and Homogenizing
- 20.10 S0QA185.XXX - SOP for Analyst Demonstration of Proficiency
- 20.11 S0QA215.XXX - SOP for Records Archiving, Retrieving and Disposal

Appendix E

Attachment 4: Standard Operating Procedure for
Total Dissolved Solids by SM 2540 C

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Standard Operating Procedure Standard Method 2540 C Total, Fixed and Volatile Dissolved Solids Dried at 180°C in Drinking Water and Wastewater by Gravimetric Analysis

APPROVAL: The undersigned has verified that this SOP is complete and meets requirements specified in document S0QA010.XXX.

Wet Chemistry Manager:

Date: 2-23-06

Quality Assurance Director:

Date: 2-23-06

1.0 Scope and Application:

- 1.1 This is a gravimetric method applicable to the determination of Total Dissolved Solids (TDS, total filterable residue) Fixed Dissolved Solids (FDS, Inorganic Dissolved Solids) or Volatile Dissolved Solids (VDS, Organic Dissolved Solids) in drinking water and wastewaters.
- 1.2 The specific list of analytes and their associated Practical Quantitation Limits (PQL) for each matrix is maintained in the FGL LIMS.
- 1.3 Prior to the use of this procedure FGL employees must perform and document an initial demonstration of proficiency for the method and LIMS. For continued use, annual method proficiency must be documented. Refer to S0QA185.XXX for employee training.

2.0 Summary of Method:

2.1 Summary:

- 2.1.1 Drinking waters and wastewaters are filtered through a glass fiber filter, Whatman Grade 934AH or equivalent. The filtrate is evaporated and the weight of residue is determined. The result is calculated from the volume of sample used and weight of residue found.
- 2.1.2 For fixed or volatile dissolved solids the TDS residue is heated at 500°C and the remaining residue (FDS) or loss (VDS) is calculated.

2.2 Definitions:

- 2.2.1 Definitions are listed in the FGL Quality Assurance Plan, Section 17.0.

2.3 Method Modifications:

- 2.3.1 This SOP has used a reduction of sample and reagents (in the correct ratios) from the published test method.

3.0 Interferences:

- 3.1 All glassware must be properly cleaned before use. Refer to S0QA135.XXX for glassware cleaning.
- 3.2 Samples with high amounts of calcium, magnesium, chloride and sulfate may be hygroscopic and may require longer drying times. For these samples use a smaller aliquot of sample for analysis. Samples high in undissolved solids should be filtered by vacuum for better efficiency.
- 3.3 Samples high in organics can not be compared with the TDS to EC ratio.

4.0 Safety:

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- 4.1 A lab coat and gloves should be worn while performing analyses. Protective eyewear must be worn at all times. Refer to the individual MSDS's for safe handling of chemicals.

5.0 Sample Containers, Preservation and Handling:

- 5.1 Samples should be collected in 1 pint containers.
- 5.2 Sample preservation is refrigeration at 4° C.
- 5.3 The holding time is 7 days.
- 5.4 Procedures for sampling are maintained in the FGL field services department.
- 5.5 Procedures for sample shipment are maintained in the FGL shipping department.

6.0 Equipment and Supplies:

- 6.1 Filtration manifold using 60 mL disposable syringes
- 6.2 Whatman 934AH syringe filters, 1.5 u, 13cm (or equivalent)
- 6.3 Pretared glass 100 mL beakers
- 6.4 Pretared crucibles (for FDS or VDS only)
- 6.5 Oven, set at 90°C for initial evaporation
- 6.6 Oven, set at 180°C for final drying
- 6.7 Muffle furnace set at 500°C (for FDS or VDS only)
- 6.8 Desiccator
- 6.9 Analytical balance

7.0 Quality Control:

- 7.1 **Preparation Quality Controls:** the batch size for extraction is 20 samples.
 - 7.1.1 Method Blank: one method blank is run per batch.
 - 7.1.2 Laboratory Control Sample/Continuing Calibration Verification (LCS/CCV): one LCS and one CCV are run per batch. The CCV is grouped with the prep information and essentially used as a duplicate LCS.
 - 7.1.3 Duplicate: One duplicate is analyzed per batch.
- 7.2 **Detection Limit Quality Controls:**
 - 7.2.1 Method Detection Limit (MDL) determination: The TDS MDL is run on an annual basis. Because of method similarities between TDS, FDS and VDS analyses the MDL from the TDS procedure is applied to FDS and VDS. For MDL guidance please see SOP S0QA060.XXX.

8.0 Reagent Preparation: N/A

9.0 Standard Preparation:

- 9.1 All standards received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. The LIMS then maintains the specific concentrations for each standard mix and subsequent dilutions. Login the standard using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize standard waste, an appropriate quantity of standard should be created or purchased which will be completely used during the lifetime of the standard. Refer to S0QA030.XXX for proper standard preparation and documentation.
- 9.2 Record the standard code for all standards on the prep sheet. Also record the volumes of standards used and their concentrations.
- 9.3 Primary Calibration Standard:

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9.3.1 Stock: reagent grade sodium chloride. Purchase from current vendor.

9.3.2 Working Standard (approx. 1000 mg/L): Weigh 1g of NaCl, record exact weight, and add to 1L volumetric partially filled with deionized water. Dissolve and adjust volume to 1 L with deionized water. The final concentration in mg/L is the weight recorded x 1000 x purity of .

10.0 Sample Preparation:

10.1 LIMS Batching:

10.1.1 Batch the samples for preparation using FGL LIMS, Laboratory, Wet Chemistry, Preparation. Completely fill in all information requested on the prep sheet. All sample observations are noted in the comments column.

10.2 QC Sample Preparation:

10.2.1 Method Blank: to the 60 syringe add 50 mL of deionized water.

10.2.2 LCS and CCV: to the 60 mL syringe add 50.0 mL of working standard (9.3.2).

10.2.3 Duplicates: to duplicate 60 mL syringes add 50.0 mL aliquots of sample.

10.2.4 Continue the QC samples with step 10.3.2.5.

10.3 Sample Preparation:

10.3.1 Refer to S0QA165.XXX for guidance on obtaining a representative sample.

10.3.2 Sample filtering:

10.3.2.1 For obtaining pretared beakers refer to SOP S2WCI180.XXX sections 12.1, 12.2 and 12.3.

10.3.2.2 Place 6 beakers inside the vacuum manifold below each syringe mount.

10.3.2.3 Put the 934AH filter on a clean dry syringe, twist to lock and mount onto the vacuum manifold.

10.3.2.4 Pour at least 50 mL of sample into the syringe and tap out any air bubbles. Adjust the volume to the 50 mL mark with a pipet. This equates to 51 mL.

10.3.2.5 Initiate vacuum to pass the sample through the filter and into the beaker.

10.3.2.6 When filtering is complete, rinse the syringe 2 times with approximately 10 mL of deionized water each time.

10.3.2.7 When a filter clogs perform the following steps:

10.3.2.7.1 Disconnect the filter and syringe from the manifold. Dispense the remaining sample into a new filter and syringe. Keep the clogged filter and syringe.

10.3.2.7.2 The new filter and syringe is then mounted on the manifold and the vacuum initiated. When the filtering is complete the new filter and syringe are rinsed with deionized water. After rinsing the vacuum is turned off and the new filter and syringe are removed from the manifold.

10.3.2.7.3 The clogged filter is then reattached to the manifold and the vacuum initiated. The filter is then rinsed with deionized water to rinse remaining sample which was left in the clogged filter.

10.3.2.8 Carefully transfer the samples to oven set at 90°C for initial evaporation. When dry, set oven to 180 ± 2°C for at least one hour.

10.3.2.9 After drying, immediately place pans with filters in desiccator until cool. This is approximately one hour.

10.3.2.10 For postweighing procedures refer to SOP S2WCI180.XXX section 12.4.

10.3.2.11 Repeat steps 10.3.2.8 through 10.3.2.10.

10.3.2.12 Reweigh the dishes to verify the weights are within 0.0005g or 4% of each other (after subtracting the tare weight).

10.3.2.13 Repeat this drying and weighing cycle until the criteria in 10.3.2.12 are met.

10.3.2.14 For fixed or volatile solids place the sample in muffle furnace set at 500°C overnight, then cool sample in desiccator.

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- 10.3.2.15 After ashing, immediately place pans with filters in desiccator until cool. This is approximately one hour.
- 10.3.2.16 For postweighing procedures refer to SOP S2WCI180.XXX section 12.4.
- 10.3.2.17 Repeat steps 10.3.2.14 through 10.3.2.16.
- 10.3.2.18 Reweigh the dishes to verify the weights are within 0.0005g or 4% of each other (after subtracting the tare weight). Record the second weight.
- 10.3.2.19 Repeat this drying and weighing cycle until the criteria in 10.3.2.18 are met.

11.0 Calibration:

- 11.1 Refer to SOP S0QA155.XXX

12.0 Analytical Procedure:

- 12.1 Refer to S2WCI180.XXX

13.0 Calculations:

- 13.1 The LIMS System calculates the concentration after the final weight, tare weight (already entered from PREWT), and sample volume are entered.
- 13.2 Total Dissolved Solids mg/L =
$$\frac{(\text{final beaker wt g} - \text{tare wt g}) \times 1000000}{\text{mL sample}}$$
- 13.3 Fixed Dissolved Solids mg/L =
$$\frac{(\text{final beaker wt g @ } 500^{\circ}\text{C} - \text{tare wt g}) \times 1000000}{\text{mL sample}}$$
- 13.4 Volatile Dissolved Solids mg/L =
$$\frac{(\text{final beaker wt g @ } 105^{\circ}\text{C} - \text{final beaker wt g @ } 500^{\circ}\text{C}) \times 1000000}{\text{mL sample}}$$

14.0 Data Assessment and Failure Resolution:

- 14.1 All current Data Quality Objectives (DQO's) for quality controls listed below are maintained in the FGL LIMS and automatically checked during data assessment. Qualify the data using FGL LIMS, Laboratory, Wet Chem, Analysis or Prep.
- 14.2 **Preparation Quality Controls:**
 - 14.2.1 Method Blank: the method blank must be less than the Practical Quantitation Limit used for reporting (PQL).
 - 14.2.2 LCS/CCV: the percent recoveries for the LCS and CCV must be within the FGL acceptance range based on the control chart.
 - 14.2.3 Duplicates: the relative percent difference (RPD) of the duplicates should be within the FGL acceptance range based on the control chart.
 - 14.2.4 TDS to EC Check: the TDS to EC ratio should be within the 0.55 to 0.7. If it is not, the result is suspect and should be verified unless sample site historically has a different ratio or the sample is known to contain organics.
- 14.3 **Detection Limit Quality Controls:**
 - 14.3.1 MDL: the MDL must be lower than the PQL.
- 14.4 **Failure Resolution:**
 - 14.4.1 If the failure can be corrected by repreparation and/or reanalysis then initiate the correction.
 - 14.4.2 If the failure can't be corrected by repreparation and/or reanalysis or if you are unsure of how to handle the failure please contact your supervisor or the QA director. If the data must be

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accepted with a failure, an explanation must accompany the failure. Refer to S0QA095.XXX for guidance on proper handling of failures.

- 14.4.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

15.0 Method Performance:

- 15.1 MDL studies are used to determine precision, accuracy and sensitivity for evaluation of performance at the reporting level. MDL's are electronically stored in the LIMS and a hard copy is maintained in the QA department.
- 15.2 Control charts are used for analysis and preparation quality controls to determine precision and/or accuracy on an ongoing basis for evaluating the performance of the method. Control charts are electronically maintained in the LIMS. Where applicable the control charts are used to generate the data quality objectives.
- 15.3 Performance Testing (PT) through interlaboratory studies is performed semi-annually (where applicable) to independently verify performance. PT Study results are maintained in the QA department.
- 15.4 Refer to the referenced test method(s) for published interlaboratory method performance.

16.0 Review and Reporting:

16.1 Preparation Review and Reporting:

- 16.1.1 Preparation Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.
- 16.1.2 Samples are reported in mg/L units. They are printed on the Prep Summary Report of the Preparation Data Package and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

16.2 Analysis Review and Reporting:

- 16.2.1 Analysis Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.
- 16.2.2 Samples are analyzed in g units. They are printed on the Raw Data for instrumental methods or the Analysis Summary Report for manual methods and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

17.0 Record Storage and Archiving:

17.1 Preparation Records:

- 17.1.1 The records generated during preparation are Preparation Package Narrative, Prep Confirmation Report, Quality Control Reports, Prep Result Summary and Prep Worksheet. These all form the Prep/Extraction Data Package.
- 17.1.2 After the Preparation Data Package has been reviewed, it is filed in the QA department filing boxes. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

17.2 Analysis Records:

- 17.2.1 The records generated during analysis are Analysis Package Narrative, Analysis Confirmation Report, Quality Control Reports, Analysis Runlog and Raw Data or Result Summary Report.

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These all form the Analysis Data Package.

- 17.2.2 After the Analysis Data Package has been reviewed, it is filed in the QA department filing boxes. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

18.0 Maintenance: N/A

19.0 Pollution Prevention and Waste Disposal:

- 19.1 Where feasible, techniques have been used to reduce the amount of standards and reagents in order to minimize the amount of waste generated by this procedure. This is primarily through the proper management of reagents and standards (see sections 8.1 and 9.1) and the reduction of sample and reagents (in the proper ratios) used to perform the procedure.
- 19.2 Waste from this analysis meets standard sewage discharge requirements. Waste from the analysis may be disposed of down the sink while rinsing with tap water. Samples are kept for one month prior to disposal.

20.0 References:

- 20.1 *"Standard Methods for the Examination of Water and Wastewater,"* AWWA, 18th ed, 1992, Method No. 2540 C.

21.0 Associated Documents:

- 21.1 S0QA030.XXX - SOP for Standard/Reagent Preparation and Documentation
- 21.2 S0QA035.XXX - SOP for Non-conformance/Corrective Action Program
- 21.3 S0QA060.XXX - SOP for Performing MDL/IDL Studies
- 21.4 S0QA090.XXX - SOP for Data Reduction and Recording
- 21.5 S0QA095.XXX - SOP for Qualifying Data
- 21.6 S0QA105.XXX - SOP for Review of Data Packages by Analysts and Manager
- 21.7 S0QA135.XXX - SOP for Glassware and Plasticware Cleaning
- 21.8 S0QA140.XXX - SOP for Instrument Maintenance
- 21.9 S0QA165.XXX - SOP for Spiking, Diluting and Homogenizing
- 21.10 S0QA185.XXX - SOP for Analyst Demonstration of Proficiency
- 21.11 S0QA215.XXX - SOP for Records Archiving, Retrieving and Disposal

Appendix E

Attachment 5: Standard Operating Procedure for Total Suspended Solids by SM 2540 D

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Standard Operating Procedure Standard Method 2540 D Total, Fixed and Volatile Suspended Solids Dried at 103-105°C in Drinking Water and Wastewater by Gravimetric Analysis

APPROVAL: The undersigned has verified that this SOP is complete and meets requirements specified in document S0QA010.XXX:

Wet Chemistry Manager: _____

Date: 8-25-05

Quality Assurance Director: Donna E. [Signature]

Date: 3-23-05

1.0 Scope and Application:

- 1.1 This is a gravimetric method applicable to the determination of Total Suspended Solids (TSS, total non-filterable residue) Fixed Suspended Solids (FSS, Inorganic Suspended Solids) or Volatile Suspended Solids (VSS, Organic Suspended Solids) in drinking water and wastewaters.
- 1.2 The specific list of analytes and their associated Practical Quantitation Limits (PQL) for each matrix is maintained in the FGL LIMS.
- 1.3 Prior to the use of this procedure FGL employees must perform and document an initial demonstration of proficiency for the method and LIMS. For continued use, annual method proficiency must be documented. Refer to S0QA185.XXX for employee training.

2.0 Summary of Method:

2.1 Summary:

- 2.1.1 An aliquot of a well mixed sample is poured through a glass fiber filter. The residue left behind on the filter is then dried in an oven at $104 \pm 1^\circ\text{C}$. The total suspended solid amount can be calculated from the sample volume and residue weight information. The detection limit, based on 100 mL, is 10 mg/L.
- 2.1.2 For fixed or volatile suspended solids the TSS residue is heated at 500°C and the remaining residue (FSS) or loss (VSS) is calculated.

2.2 Definitions:

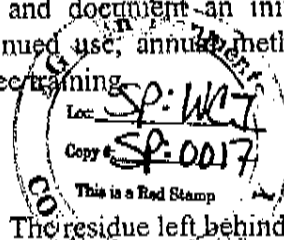
- 2.2.1 Definitions are listed in the FGL Quality Assurance Plan, Section 17.0.

2.3 Method Modifications:

- 2.3.1 This SOP has used a reduction of sample and reagents (in the correct ratios) from the published test method.

3.0 Interferences:

- 3.1 All glassware must be properly cleaned before use. Refer to S0QA135.XXX for glassware cleaning.
- 3.2 Particles that are not considered representative of the sample should be removed (i.e. leaves, sticks, bugs, etc.).
- 3.3 Positive interferences may arise from samples high in dissolved solids such as saline waters. This interference is removed by proper rinsing of the filter.



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4.0 Safety:

- 4.1 A lab coat and gloves should be worn while performing analyses. Protective eyewear must be worn at all times. Refer to the individual MSDS's for safe handling of chemicals.

5.0 Sample Containers, Preservation and Handling:

- 5.1 Samples should be collected in 1 pint containers.
- 5.2 Sample preservation is refrigeration at 4° C.
- 5.3 The holding time is 7 days.
- 5.4 Procedures for sampling are maintained in the FGL field services department.
- 5.5 Procedures for sample shipment are maintained in the FGL shipping department.

6.0 Equipment and Supplies:

- 6.1 Filtration manifold using filter funnels with magnetic bases and vacuum flasks
 - Graduated cylinders or pipettes
 - Pretared 934AH filters in aluminum pans, 1.5 u, (or equivalent)
 - Pretared crucibles with glass fiber filter (for FDS or VDS only)
 - Oven, set at 104°C
 - Muffle furnace set at 500°C (for FDS or VDS only)
 - Dessicator
 - Analytical balance

7.0 Quality Control:

- 7.1 **Preparation Quality Controls:** the batch size for extraction is 20 samples.
 - 7.1.1 Method Blank: one method blank is run per batch.
 - 7.1.2 Laboratory Control Sample/Continuing Calibration Verification (LCS/CCV): one LCS and one CCV are run per batch. The CCV is grouped with the prep information and essentially used as a duplicate LCS.
 - 7.1.3 Duplicate: One duplicate is analyzed per batch.
- 7.2 **Detection Limit Quality Controls:**
 - 7.2.1 Method Detection Limit (MDL) determination: The TSS MDL is run on an annual basis. Because of method similarities between TSS, FSS and VSS analyses the MDL from the TSS procedure is applied to FSS and VSS. For MDL guidance please see SOP S0QA060.XXX.

8.0 Reagent Preparation: N/A

9.0 Standard Preparation:

- 9.1 All standards received and prepared must be properly labeled and recorded in the LIMS electronic reagent/standard preparation log. The LIMS then maintains the specific concentrations for each standard mix and subsequent dilutions. Login the standard using FGL LIMS, Laboratory, Wet Chem, Standards. In order to minimize standard waste, an appropriate quantity of standard should be created or purchased which will be completely used during the lifetime of the standard. Refer to S0QA030.XXX for proper standard preparation and documentation.
- 9.2 Record the standard code for all standards on the prep sheet. Also record the volumes of standards used and their concentrations.
- 9.3 Primary Calibration Standard:

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9.3.1 Stock: (500 mg/L): dilute 0.25g of diatomaceous earth to 500 mL with deionized water. This is stored in a 1 L amber glass container. A large container is used to allow thorough mixing prior to taking an aliquot.

10.0 Sample Preparation:

10.1 LIMS Batching:

10.1.1 Batch the samples for preparation using FGL LIMS, Laboratory, Wet Chem, Preparation. Completely fill in all information requested on the prep sheet. All sample observations are noted in the comments column.

10.2 QC Sample Preparation:

- 10.2.1 Method Blank: pass 100 mL of deionized water through a pretared filter.
- 10.2.2 LCS and CCV: pass 50 mL of stock standard (9.3.1) through a pretared filter.
- 10.2.3 Duplicates: pass duplicate aliquots of sample through a pretared filter.
- 10.2.4 Continue the QC samples with step 10.3.2.8.

10.3 Sample Preparation:

10.3.1 Refer to S0QA165.XXX for guidance on obtaining a representative sample.

10.3.2 Sample filtering:

- 10.3.2.1 For obtaining pretared filters refer to SOP S2WCI180.XXX sections 12.1.
- 10.3.2.2 Set up the filtration apparatus by attaching the filter funnel base to the vacuum flask, placing a glass fiber filter on the filter funnel base then attach the filter funnel top.
- 10.3.2.3 Shake sample and pour 100 ml aliquot into a graduated cylinder. **Proper mixing just prior to taking an aliquot is critical. If the sample is being performed in duplicate or for the LCS and CCV you must mix between each aliquot.**
- 10.3.2.4 For samples high in suspended solids a smaller sample size may be used. However, if the sample passes readily through the filter add more sample up to 100 mL.
- 10.3.2.5 If the aliquot used clogs the filter then the sample must be restarted with a smaller aliquot.
- 10.3.2.6 Pour the aliquot into the filter funnel then rinse the graduated cylinder into the funnel three times.
- 10.3.2.7 After the sample has passed through the filter rinse down the funnel three times. Allow all water to drain. After rinsing is complete remove the filter and place it back into the same aluminum pan it was in.
- 10.3.2.8 Carefully transfer the pans with filters to oven set at 104°C. Dry overnight.
- 10.3.2.9 After drying, immediately place pans with filters in desiccator until cool. This is approximately one hour.
- 10.3.2.10 For postweighing procedures refer to SOP S2WCI180.XXX section 12.4.
- 10.3.2.11 Repeat steps 10.3.2.8 through 10.3.2.10.
- 10.3.2.12 Reweigh the dishes to verify the weights are within 0.0005g or 4% of each other (after subtracting the tare weight).
- 10.3.2.13 Repeat this drying and weighing cycle until the criteria in 10.3.2.12 are met.
- 10.3.2.14 For fixed or volatile solids place the sample in muffle furnace set at 500°C overnight, then cool sample in desiccator.
- 10.3.2.15 After ashing, immediately place pans with filters in desiccator until cool. This is approximately one hour.
- 10.3.2.16 For postweighing procedures refer to SOP S2WCI180.XXX section 12.4.
- 10.3.2.17 Repeat steps 10.3.2.14 through 10.3.2.16.

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10.3.2.18 Reweigh the dishes to verify the weights are within 0.0005g or 4% of each other (after subtracting the tare weight). Record the second weight.

10.3.2.19 Repeat this drying and weighing cycle until the criteria in 10.3.2.18 are met.

11.0 Calibration:

11.1 Refer to SOP S0QA155.XXX

12.0 Analytical Procedure:

12.1 Refer to S2WCI180.XXX

13.0 Calculations:

13.1 The LIMS System calculates the concentration after the final weight, tare weight (already entered from PREWT), and sample volume are entered.

13.2 Total Suspended Solids mg/L =
$$\frac{(\text{final filter wt g} - \text{tare wt g}) \times 1000000}{\text{mL sample}}$$

13.3 Fixed Suspended Solids mg/L =
$$\frac{(\text{final filter wt g @ } 500^{\circ}\text{C} - \text{tare wt g}) \times 1000000}{\text{mL sample}}$$

13.4 Volatile Suspended Solids mg/L =
$$\frac{(\text{final filter wt g @ } 105^{\circ}\text{C} - \text{final filter wt g @ } 500^{\circ}\text{C}) \times 1000000}{\text{mL sample}}$$

14.0 Data Assessment and Failure Resolution:

14.1 All current Data Quality Objectives (DQO's) for quality controls listed below are maintained in the FGL LIMS and automatically checked during data assessment. Qualify the data using FGL LIMS, Laboratory, Wet Chem, Analysis or Prep.

14.2 Preparation Quality Controls:

14.2.1 Method Blank: the method blank must be less than the Practical Quantitation Limit used for reporting (PQL).

14.2.2 LCS/CCV: the percent recoveries for the LCS and CCV must be within the FGL acceptance range based on the control chart.

14.2.3 Duplicates: the relative percent difference (RPD) of the duplicates should be within the FGL acceptance range based on the control chart.

14.3 Analysis Quality Controls:

14.3.1 ICV/CCV: the ICV/CCV must be within the FGL acceptance range based on the control charts.

14.4 Detection Limit Quality Controls:

14.4.1 MDL: the MDL must be lower than the PQL.

14.5 Failure Resolution:

14.5.1 If the failure can be corrected by reparation and/or reanalysis then initiate the correction.

14.5.2 If the failure can't be corrected by reparation and/or reanalysis or if you are unsure of how to handle the failure please contact your supervisor or the QA director. If the data must be accepted with a failure, an explanation must accompany the failure. Refer to S0QA095.XXX for guidance on proper handling of failures.

14.5.3 Refer to S0QA035.XXX if it is determined that a nonconformance and/or corrective action are required.

15.0 Method Performance:

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- 15.1 MDL studies are used to determine precision, accuracy and sensitivity for evaluation of performance at the reporting level. MDL's are electronically stored in the LIMS and a hard copy is maintained in the QA department.
- 15.2 Control charts are used for analysis and preparation quality controls to determine precision and/or accuracy on an ongoing basis for evaluating the performance of the method. Control charts are electronically maintained in the LIMS. Where applicable the control charts are used to generate the data quality objectives.
- 15.3 Performance Testing (PT) through interlaboratory studies is performed semi-annually (where applicable) to independently verify performance. PT Study results are maintained in the QA department.
- 15.4 Refer to the referenced test method(s) for published interlaboratory method performance.

16.0 Review and Reporting:

16.1 Preparation Review and Reporting:

- 16.1.1 Preparation Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.
- 16.1.2 Samples are reported in mg/L units. They are printed on the Prep Summary Report of the Preparation Data Package and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

16.2 Analysis Review and Reporting:

- 16.2.1 Analysis Data Packages are compiled, reviewed, signed and dated by the analyst. They are then turned in to the supervisor or peer for review. Refer to S0QA105.XXX for data package review.
- 16.2.2 Samples are analyzed in g units. They are printed on the Raw Data for instrumental methods or the Analysis Summary Report for manual methods and electronically maintained in the FGL LIMS. Refer to S0QA090.XXX for data reduction and recording.

17.0 Record Storage and Archiving:

17.1 Preparation Records:

- 17.1.1 The records generated during preparation are Preparation Package Narrative, Prep Confirmation Report, Quality Control Reports, Prep Result Summary and Prep Worksheet. These all form the Prep/Extraction Data Package.
- 17.1.2 After the Preparation Data Package has been reviewed, it is filed in the QA department filing boxes. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

17.2 Analysis Records:

- 17.2.1 The records generated during analysis are Analysis Package Narrative, Analysis Confirmation Report, Quality Control Reports, Analysis Runlog and Raw Data or Result Summary Report. These all form the Analysis Data Package.
- 17.2.2 After the Analysis Data Package has been reviewed, it is filed in the QA department filing boxes. Periodically the files are removed for archival. Refer to S0QA215.XXX for archiving records.

18.0 Maintenance: N/A

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19.0 Pollution Prevention and Waste Disposal:

- 19.1 Where feasible, techniques have been used to reduce the amount of standards and reagents in order to minimize the amount of waste generated by this procedure. This is primarily through the proper management of reagents and standards (see sections 8.1 and 9.1) and the reduction of sample and reagents (in the proper ratios) used to perform the procedure.
- 19.2 Waste from this analysis meets standard sewage discharge requirements. Waste from the analysis may be disposed of down the sink while rinsing with tap water. Samples are kept for one month prior to disposal.
- 19.3 Any samples found to contain hazardous waste must be given to the Hazardous Waste Officer for proper segregation and disposal.

20.0 References:

- 20.1 *"Standard Methods for the Examination of Water and Wastewater,"* AWWA, 18th ed, 1992, Method No. 2540 D.

21.0 Associated Documents:

- 21.1 S0QA030.XXX - SOP for Standard/Reagent Preparation and Documentation
- 21.2 S0QA035.XXX - SOP for Non-conformance/Corrective Action Program
- 21.3 S0QA060.XXX - SOP for Performing MDL/IDL Studies
- 21.4 S0QA090.XXX - SOP for Data Reduction and Recording
- 21.5 S0QA095.XXX - SOP for Qualifying Data
- 21.6 S0QA105.XXX - SOP for Review of Data Packages by Analysts and Manager
- 21.7 S0QA135.XXX - SOP for Glassware and Plasticware Cleaning
- 21.8 S0QA140.XXX - SOP for Instrument Maintenance
- 21.9 S0QA165.XXX - SOP for Spiking, Diluting and Homogenizing
- 21.10 S0QA185.XXX - SOP for Analyst Demonstration of Proficiency
- 21.11 S0QA215.XXX - SOP for Records Archiving, Retrieving and Disposal

Appendix E

Attachment 6: Standard Operating Procedure for
Organochlorine, Organophosphorus, and
Pyrethroid Pesticides by GC/MS

CRG MARINE LABORATORIES

2020 Del Amo Blvd., Suite 200, Torrance, CA 90501 (310) 533-5190

SEPARATORY FUNNEL LIQUID-LIQUID EXTRACTION AND ANALYSIS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Approved by:

Richard Gossett, Laboratory Manager

Date

METHOD 625:

SEPARATORY FUNNEL LIQUID-LIQUID EXTRACTION AND ANALYSIS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

REFERENCES: U.S. EPA 40CFR Part 136

1.0 SCOPE AND APPLICATION

- 1.1 This method covers the extraction and concentration procedures required for the determination of chlorinated pesticides and PCBs and semi-volatile base/neutral and acid-extractable compounds in Laboratory Operating Procedure Methods (LOPM) 625. It is applicable to liquid samples.
- 1.2 The glassware cleaning procedure for extraction glassware is listed in this method.

Table 1. Target Compound List

COMPOUND	RETENTION TIME DB-5 COLUMN	METHOD DETECTION LIMIT (ng/L)
D5-Phenol (Recovery Surrogate)	8.00	*
Naphthalene-d8 (Recovery Surrogate)	13.34	*
2-Fluorophenol (Recovery Surrogate)	15.00	*
Acenaphthene-d10 (Recovery Surrogate)	21.49	*
2,4,5,6-Tetrachloro-m-xylene (Recovery Surrogate)	26.45	*
2,4,6-Tribromophenol (Recovery Surrogate)	27.17	*
PCB030 (Recovery Surrogate)	31.66	*
Phenanthrene-d10 (Recovery Surrogate)	32.29	*
Anthracene-d10 (Internal Standard)	32.84	*
PCB112 (Recovery Surrogate)	46.29	*
2,2'-5,5'-Tetrabromobiphenyl (Internal Standard)	52.07	*

Chrysene-d12 (Recovery Surrogate)	56.25	*
PCB198 (Recovery Surrogate)	60.57	*
Perylene-d12 (Recovery Surrogate)	68.49	*
Phenol	8.21	100
2-Chlorophenol	10.56	50
Aniline	11.37	100
2,4-Dimethylphenol	11.11	100
bis(2-Chloroethoxy) methane	11.44	50
1,3-Dichlorobenzene	11.98	10
1,4-Dichlorobenzene	12.13	10
1,2-Dichlorobenzene	12.39	10
Benzyl Alcohol	12.23	100
2-Nitrophenol	12.41	100
bis(2-Chloroisopropyl) ethane	12.61	50
2,4-Dichlorophenol	12.83	50
N-nitrosodi-n-propylamine	12.95	50
N-nitrosodimethylamine	12.97	50
Hexachloroethane	13.15	50
Nitrobenzene	13.32	50
Naphthalene	13.39	1.0
Isophorone	13.99	50
Dichlorvos	14.52	10
bis(2-Chloroethyl) ether	14.59	50
1,2,4-Trichlorobenzene	14.81	10
Benzidine	15.31	50
4-Chloro-3-methylphenol	15.59	100
4-Chloroaniline	15.74	50
2-Methylnaphthalene	15.98	1.0
Hexachlorobutadiene	16.24	50
1-Methylnaphthalene	16.39	1.0
2,4,6-Trichlorophenol	17.42	50
Benzoic Acid	17.70	100
Biphenyl	18.30	1.0
2,6-Dimethylnaphthalene	19.05	1.0
Mevinphos	19.55	10
Hexachlorocyclopentadiene	19.83	50
Acenaphthylene	20.47	1.0
Dimethyl Phthalate	20.56	5
Acenaphthene	21.74	1.0
4-Nitroaniline	21.82	50

2,4-Dinitrophenol	22.27	100
4-Nitrophenol	23.30	100
2,3,5-Trimethylnaphthalene	24.42	1.0
3-Nitroaniline	24.57	50
Fluorene	25.18	1.0
Diethyl Phthalate	25.45	5
2,6-Dinitrotoluene	25.89	50
2-Methyl-4,6-dinitrophenol	26.04	100
Dibenzofuran	26.12	50
Demeton	26.21	10
2,4-Dinitrotoluene	26.55	10
Ethoprop	26.83	10
4-Chlorophenyl phenyl ether	28.97	50
2-Nitroaniline	29.13	50
Phorate	29.17	10
N-nitrosodiphenylamine	30.04	50
Azobenzene	30.21	50
Dimethoate	30.60	5
Pentachlorophenol	31.70	50
Phenanthrene	32.67	1.0
4-Bromophenyl phenyl ether	32.96	50
Anthracene	32.96	1.0
alpha-BHC	33.26	1.0
Diazinon	33.48	5
Disulfoton	33.65	10
Hexachlorobenzene	33.90	1.0
beta-BHC	35.29	1.0
gamma-BHC	35.79	1.0
Methyl Parathion	36.69	10
delta-BHC	37.56	1.0
Fenchlorophos	37.91	10
1-Methylphenanthrene	38.01	1.0
Dibutyl Phthalate	39.46	5
Malathion	39.91	5
Fenthion	40.38	10
Chlorpyrifos	40.53	5
Heptachlor	41.36	1.0
Trichloronate	41.44	10
Fluoranthene	42.98	1.0
Aldrin	43.99	1.0
Pyrene	44.85	1.0
Tetrachlorvinphos	45.61	10
Tokuthion	46.91	10
Heptachlor Epoxide	47.00	1.0

Bolstar	47.40	10
gamma-Chlordane	48.77	1.0
2,4'-DDE	49.18	1.0
Endosulfan I	49.69	1.0
alpha-Chlordane	49.91	1.0
Fensulfothion	50.10	10
trans-Nonachlor	50.29	1.0
4,4'-DDE	51.50	1.0
Merphos	51.55	10
Dieldrin	51.61	1.0
2,4'-DDD	52.11	1.0
Butylbenzyl Phthalate	53.13	5
Endrin	53.19	1.0
Endosulfan II	53.84	1.0
4,4'-DDD	54.53	1.0
2,4'-DDT	54.79	1.0
Endrin Aldehyde	55.20	1.0
Benz[a]anthracene	56.25	1.0
Chrysene	56.52	1.0
Endosulfan Sulfate	56.93	1.0
4,4'-DDT	57.24	1.0
bis-(2-ethylhexyl) Phthalate	59.24	5
Endrin Ketone	60.23	1.0
Guthion	60.47	10
Coumaphos	60.52	10
Methoxychlor	61.36	1.0
Mirex	64.27	1.0
Di-n-octyl Phthalate	64.71	5
Benzo[b]fluoranthene	65.47	1.0
Benzo[k]fluoranthene	65.87	1.0
Benzo[e]pyrene	67.71	1.0
Benzo[a]pyrene	68.07	1.0
Perylene	68.77	1.0
3,3'-Dichlorobenzidine	72.45	50
Indeno[1,2,3-c,d]pyrene	76.29	1.0
Dibenz[a,h]anthracene	76.68	1.0
Benzo[g,h,i]perylene	77.87	1.0
Esfenvalerate/Fenvalerate ^a		5
Toxaphene ^a		10
PCBs By Congener ^a		1
PCBs By Aroclor ^a		10

^aThese compounds are mixtures of various isomers. Fenvalerate/Esfenvalerate contain 4, Toxaphene contains 116 camphenes, CRG analyzes for 48 PCB Congeners and 7 PCB Aroclors.

2.0 SUMMARY OF METHOD

A measured volume of sample, usually 2 liters, is serially extracted with methylene chloride at pH >11 and again at pH <2 using a separatory funnel. The methylene chloride extract is concentrated in preparation for instrumental analysis. Samples are to be stored at 4 °C, extracted within 7 days of collection, and analyzed within 40 days of extraction.

A 1-3 µL sample is injected into a gas chromatograph (GC) equipped with a mass selective detector. The GC is temperature programmed to separate the compounds and confirmation is achieved for the single component peaks using ions specific to each target compound. Compounds eluting from the GC are identified by matching the retention times of the unknown peaks with those from a known calibration standard and the concentration of each identified component is measured by comparison of the responses.

3.0 PREVENTION OF INTERFERENCES

- 3.1 Solvents, glassware, and other processing apparatus are to be free of any interferences. A procedural blank is to be analyzed with each sample batch to demonstrate the absence of any method interferences.
- 3.2 High purity solvents are to be used to minimize interferences.
- 3.3 Phthalate esters and PCBs are contaminants found in many types of products commonly used in the laboratory. Care should be taken to avoid or eliminate the use of plastic products during sample processing and handling.
- 3.4 Impurities in the carrier and makeup gases may be avoided by using Ultra-High purity gases and/or gas purifying cartridges. See the instrument manufacturer for guidelines.
- 3.5 Contamination by carryover may occur whenever high level and low-level samples are sequentially analyzed. To reduce carryover, the syringe used for sample injection shall be rinsed a minimum of 5 times between samples using n-hexane. Whenever possible, samples shall be analyzed from low to high concentrations.
- 3.6 A procedural blank shall be analyzed with each batch of 20 or less samples to check for contamination during sample processing.

4.0 SAFETY

- 4.1 It is mandatory to wear a laboratory coat, closed-toe shoes and safety glasses in the laboratory. Gloves are to be worn when working with samples.
- 4.2 All glassware cleaning and extraction procedures involving any solvent exposure shall take place in a fume hood. Use of a respirator and appropriate safety gloves are recommended for working with solvents.
- 4.3 Material Safety Data Sheets (MSDS) are on file in the laboratory and are available to all personnel involved in the use of hazardous materials during any procedure.
- 4.4 Extreme caution and the proper use of safety equipment are required during the handling of any hazardous material. If the analyst has any questions regarding safety, he or she should contact a supervisor or the laboratory director prior to the start of this procedure.

5.0 APPARATUS AND MATERIALS

- 5.1 Glassware
 - A. Separatory funnel: 2 L, with Teflon stopcock
 - B. Round-bottom flasks: 250 mL
 - C. Pear-shaped flasks: 25 mL
 - D. Graduated cylinder: 100 mL
 - E. Erlenmeyer flask: 1 L
 - F. Glass filter funnel
 - G. Pasteur pipettes
 - H. Gastight volumetric syringes: 100, 500 μ L
 - I. Autosampler vials with Teflon-lined screw caps: 2 mL
- 5.2 Glass wool
- 5.3 pH indicator paper: pH 0-6
- 5.4 Graduated cylinder: 2 L

- 5.5 Heavy duty aluminum foil
- 5.6 Roto-evaporator system with aspirator pump and water bath set at $30 \pm 5^{\circ}\text{C}$
- 5.7 Chiller unit set at $10 \pm 5^{\circ}\text{C}$ or cool tap water
- 5.8 High-temperature oven set at $1000 \pm 50^{\circ}\text{F}$
- 5.9 Non-ionic detergent
- 5.10 Hewlett Packard 6890 GC equipped with a Mass Selective Detector, an HP7673 Low-volume Autosampler and an on-column injector
- 5.11 J&W Scientific XLB Column (or equivalent), 30 meters in length, 0.25 μm film thickness, and 0.25 mm I.D
- 5.12 10 μL syringe for the HP7673 Autosampler
- 5.13 Ultra-high purity helium
- 5.14 Fused Silica Retention Gap, 5 meters in length, 0.53 mm I.D.

6.0 REAGENTS

- 6.1 Deionized water
- 6.2 Pesticide grade hexane and methylene chloride
- 6.3 Method 625 spike solutions prepared from stock solutions obtained from an accredited supplier. The solution used is dependent on the clients target analyte list.
 - Chlorinated Pesticides
 - PCB Congeners
 - Base/Neutral Extractables
 - Acid Extractables
- 6.4 Anhydrous granular sodium sulfate
- 6.5 Concentrated sulfuric acid
- 6.6 Stock solutions. All stock solutions are purchased from NIST traceable commercial suppliers. Store at or below 4°C and protect

from light. Stock standards shall be replaced after one year or sooner if comparison with check standards indicates a problem.

6.7 Calibration Standards. Prepare a minimum of five concentration levels for each parameter of interest. One of the concentration levels shall be near the method detection limit. The remaining concentration levels shall bracket the expected concentrations found in the samples. Calibration solutions shall be replaced after 6 months or sooner if a problem is indicated.

6.8 Internal Standards. Select one or more internal standards that are similar in analytical behavior to the compounds of interest. The analyst shall demonstrate that the selected compound(s) is not affected by the method or matrix interference.

6.8.1 Just prior to analysis, add a known constant amount of internal standard to all calibration solutions, blanks, and samples.

6.9 Recovery Surrogates. Select one or more internal standards that are similar in analytical behavior to the compounds of interest.

6.9.1 Prior to the extraction of the samples, add a known amount of recovery surrogate to all blanks and samples. See Methods 3510 and 3545 for additional information about this procedure.

6.10 Sodium Hydroxide

7.0 CALIBRATION AND MAINTENANCE

7.1 Shimadzu QP2010 or HP5972 GC/MS

7.1.1 GC Oven Operating Conditions:

Initial Oven Temperature = 45 °C

Initial Hold = 5 min

Ramp 1 = 2.5 °C/min to 285 °C

Hold Time = 15 min

7.1.2 Injector Operating Conditions:

Injector = Splitless or On-Column

Mode = Track Oven Temperature (On-Column Only)

Nominal Initial Pressure = 35.0 psi (on)

7.1.3 Column Operating Conditions:

Max Temp = 325 °C

Mode = Constant Flow

Initial Flow = 1.5 mL/min
Average Carrier Velocity = 40 cm/sec
Carrier Gas = Helium

7.1.4 Detector Operating Conditions:

Transfer Line Temperature = 285 °C
Ionization Voltage = 70 eV
Gain = +100 to +300 volts over standard sensitivity target
tune

7.1.5 Autosampler Operating Conditions (Back Injector):

Sample Washes = 2
Sample Pumps = 2
Injection Volume = 2.0 µL
Syringe Size = 10 µL
Post Injection Washes Solvent A = 3
Post Injection Washes Solvent B = 3
Plunger Speed = Fast

7.1.6 System Maintenance

Prior to each set of samples, remove ca. 30 cm of the retention gap or column, replace injector septum if needed, and refill the solvent wash bottles.

Replace the gas cartridges every 6 months.

Replace the GC columns as needed.

Enter all maintenance actions into the instrument maintenance logbook.

8.0 QUALITY CONTROL

- 8.1 With each batch of samples (maximum 20 samples per batch), a procedural blank is extracted and analyzed to demonstrate that procedural interferences are under control. Deionized water is used as the blank matrix.
- 8.2 With each batch of samples, a duplicate sample and/or matrix spike/matrix spike duplicate (MS/MSD) set of samples is analyzed with the appropriate extraction procedure to measure the precision of the extraction procedure. A non-spiked sample of an MS/MSD set is analyzed to determine background concentrations for each parameter of interest. The MS/MSD samples are spiked with specific

parameters at a concentration greater than ten times the method detection limit and analyzed to determine the percent recovery of the spiked compounds. For concentrations at ten times the method detection limit, a precision factor between the duplicate samples or MS/MSD samples is calculated and compared to the corresponding QC acceptance criteria.

- 8.3 Every sample, spike set, and blank is spiked with an appropriate surrogate spike solution consisting of 1 to 6 surrogate compounds. The surrogate spike is used to demonstrate the efficiency of the extraction and analytical procedure by allowing calculation of the percent recovery of each surrogate compound.

Control charts and control limits are generated by measuring the mean and standard deviation of the surrogate percent recovery for the previous 20 samples. Upper and lower *warning limits* are calculated at two times the standard deviation from the mean. Upper and lower *control limits* are calculated at three times the standard deviation from the mean. Surrogate control limits and results are presented with the analytical results. When surrogate results indicate atypical method performance, a quality control check sample is analyzed and an evaluation of the procedure and instrumentation is made.

- 8.4 If any individual parameter falls outside of the designated range for percent recovery, that parameter has failed the acceptance criteria. An evaluation of the method procedure and instrumentation shall be made to uncover evidence of any atypical performance. If there is atypical performance of the method procedure and/or instrumentation, the problem shall be immediately identified and corrected prior to the analysis of any further samples. A re-spike and/or quality control check sample shall be analyzed and evaluated. If possible, all samples from the suspect batch shall be re-analyzed under corrected method conditions. If samples can not be re-analyzed, the analytical results for the non-spiked samples are suspect and shall be reported with the result flagged and followed by an explanation of the problem.
- 8.5 QA/QC records are maintained to document the quality of data generated. If any constituent falls outside the designated range, that compound has failed the acceptance criteria. Failure to meet the stated requirement shall require that corrective action be taken to eliminate the problem prior to the analysis of any samples. Samples from the batch being analyzed at the time the failure is detected shall be reanalyzed after the corrective action has been taken. A batch is defined as 20 or less samples. If any sample cannot be reanalyzed,

the result for that element shall be flagged and a detailed report is included with the result.

- 8.5.1 **Initial Calibration Check-** Prior to analyzing any samples, using a second-source calibration standard an initial calibration of the instrument is performed with each batch of samples. This calibration shall be within 15% of the initial calibration curve.
- 8.5.2 **Calibration Check-** Using a second-source calibration standard, a calibration check will be performed every 12 hours and at the end of every batch of samples. The calibration check shall be within 15% of the initial calibration curve.
- 8.5.3 **Matrix Spikes-** Matrix spike and matrix spike duplicates as well as duplicate samples shall be analyzed with each batch of samples to determine the precision for each compound. A control chart is generated to document the precision. Control limits are established by using the mean and standard deviation from 20 results. Upper and lower warning limits are two times the standard deviation and upper and lower “out of control” limits are three times the standard deviation for those compounds that are greater than 10 times the method detection limit.
- 8.5.4 **CRM/LCM-** Certified reference materials and/or lab control materials shall be analyzed with each batch of samples. The reported value shall be within the limits set forth by the agency providing the material.
- 8.5.5 **Blanks-** Lab reagent blanks shall be analyzed with each batch of samples. No compound shall be detected at greater than 3 times the method detection limit.
- 8.5.6 **QCS-** A method standard is extracted along with each batch of samples. Prepare the QC check standard to 1L of reagent water.
- 8.5.6 **Internal Standards-** Internal standards shall be added in known amounts to blanks, calibration standards, continuing calibration check solutions, and samples to compensate for instrumental drift.
- 8.5.7 **Recovery Surrogates-** Recovery surrogates shall be added in known amounts to all blanks and samples to indicate

sample processing efficiency. Sample results shall not be adjusted for surrogate recovery efficiency unless specifically requested by the client.

- 8.5.8 Daily GCMS Performance Test- At the beginning of each batch of samples, the GCMS system must be checked to see if acceptable performance criteria are achieved for DFTPP. The criteria are presented in the following table.

Mass	m/z Abundance Criteria
51	30-60 percent of Mass 198
68	< 2 percent of Mass 69
70	< 2 percent of Mass 69
127	40-60 percent of Mass 198
197	< 1 percent of Mass 198
198	Base peak, 100 percent relative abundance
199	5-9 percent of Mass 198
275	10-30 percent of Mass 198
365	> 1 percent of Mass 198
441	Present but < Mass 443
442	> 40 percent of Mass 198
443	17-23 percent of Mass 442

9.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

- 9.1 All samples are collected in amber glass jars with Teflon-lined screw caps. All samples are kept at 4 ± 2 °C from the time of collection until extraction.
- 9.2 If Residual Chlorine is present, add 80mg of sodium thiosulfate per liter of sample and mix well. Please refer to the SOP for Residual Chlorine determination.

10.0 PROCEDURE

- 10.1 Glassware cleaning procedure: High-temperature oven option

Wash glassware with non-ionic detergent and water. Rinse glassware thoroughly with tap water, then rinse once with deionized water. Place glassware in high temperature oven and bake at a minimum of 1000 ± 50 °F for 2 hours according to the following conditions:

Set initial temperature ramp to 536°C over 1 hours then hold for 3 hours. Once the oven program shuts off, the oven begins

to cool back down to 30 °F. Consecutive oven runs can be done once the oven has cooled to less than 150 °F.

CAUTION: Do not open oven door or turn off blower until oven temperature is below 570 °F.

Once the glassware has cooled, cover all exposed areas that will touch the sample with aluminum foil or place it upside down onto foil until use.

10.2 Glassware cleaning procedure: Solvent rinse option

Wash glassware with non-ionic detergent and water. Rinse glassware thoroughly with tap water, then rinse once with deionized water. Let dry, then use Teflon squeeze bottles to rinse three times with methylene chloride and three times with hexane.

10.3 Sodium sulfate cleaning procedure

Clean sodium sulfate either by heating in the high-temperature oven using the same program as the glassware cleaning procedure or by rinsing with several mLs of methylene chloride.

10.4 Glass wool cleaning procedure

Clean glass wool either by heating in the high-temperature oven using the same program as the glassware cleaning procedure or by rinsing with several mLs of methylene chloride.

10.5 Sample extraction

10.5.1 Remove sample from the refrigerator and bring to room temperature.

10.5.2 Decant some of the sample into the sink, if necessary, to allow for the addition of solvent.

10.5.3 Use a gas-tight volumetric syringe to pipette the appropriate QC surrogates into the sample. The surrogate solution(s) should be at room temperature prior to use. Record the sample ID, name and volume of surrogate used, standard solutions logbook page number containing details of solution preparation, and analyst initials in the laboratory notebook.

- 10.5.4 For matrix spike/matrix spike duplicate samples, use a gas-tight volumetric syringe to pipette the appropriate QA/QC spikes into the sample. The spike solution(s) should be at room temperature prior to use. Record the sample ID, name and volume of spike used, standard solutions logbook page number containing details of solution preparation, and analyst initials in the laboratory notebook.
- 10.5.5 Adjust the pH to >11 using NaOH solution. Then use the 100 mL graduated cylinder to measure 100mL of methylene chloride and add it directly to the sample bottle. Recap the bottle tightly and shake it continuously and vigorously for at least 2 minutes.
- 10.5.6 Allow the sample bottle to sit untouched for 5 minutes so that the organic solvent and aqueous layers can separate.
- 10.5.7 Decant approximately half of the sample into the 2 L Erlenmeyer flask and the remainder, including the solvent layer, into the separatory funnel. Allow the organic and aqueous layers in the separatory funnel to separate for 5 minutes.
- 10.5.8 Prepare the collection flask as follows:
- Place a small amount of glass wool into the bottom of a glass filter funnel, then add approximately 50 g of anhydrous sodium sulfate. Place the funnel into the neck of a 250 mL round bottom flask.
- 10.5.9 Filter the solvent extract through the sodium sulfate and collect it in the 250 mL round bottom flask.
- 10.5.10 Add 75 mL methylene chloride to the empty sample bottle and swirl it around thoroughly to wash down the walls of the bottle. Pour the sample portions from the Erlenmeyer flask and the separatory funnel back into the sample bottle and repeat the shaking step in 10.4.5. Allow for layer separation, decant, and collect the extract in the same 250 mL flask.
- 10.5.11 For samples being analyzed only for Base/Neutral compounds only, the third extraction is identical to the second. For samples being analyzed for acid extractable compounds, adjust the sample pH to less than 2 by adding a small amount of concentrated sulfuric acid prior to the

shaking step of the third extraction. The third extraction is otherwise identical to the second.

- 10.5.12 After the third extraction is complete, measure the total volume of the sample using the 2 L graduated cylinder and record it in the laboratory notebook.

10.6 Sample concentration

- 10.6.1 Prepare the roto-evaporator for use according to the following parameters:

water bath temperature at 30 ± 5 °C

chiller temperature at 10 ± 5 °C or cool tap water

- 10.6.2 Attach the 250 mL round bottom flask to the distillation trap and secure it with a plastic spring clip.
- 10.6.3 Close the stopcock and lower the flask into the water bath. Turn on the roto-evaporator motor and adjust the rotation to a medium speed. Adjust the vacuum so that no solvent flashes up into the distillation trap.
- 10.6.4 Concentrate the sample to approximately 10 mL. Break the internal vacuum by opening the stopcock. Stop the motor, raise the arm, and remove the flask from the trap.
- 10.6.5 Use a Pasteur pipette to transfer the sample to a 25 mL pear-shaped flask. Rinse the 250 mL flask three times with approximately 1 mL methylene chloride and transfer each rinse to the 25 mL flask.
- 10.6.6 Attach the 25 mL pear-shaped flask to the distillation trap using the adaptor and concentrate the sample to approximately 500 µL. Take care not to let the sample go to dryness.
- 10.6.7 Transfer the sample to an autosampler vial using a Pasteur pipette. Rinse the 25 mL flask three times with approximately 250 µL methylene chloride, transferring each rinse to the autosampler vial.
- 10.6.8 The sample extract is now ready for instrumental analysis.

- 10.7 Using the Hewlett Packard data system, load the appropriate method for the parameters of choice.

- 10.8 Using the Hewlett Packard data system, set up a sequence table for the analysis of the samples. The sequence table should include all calibrations necessary for five calibration levels of each parameter of interest, the recovery surrogate solution, a calibration check solution for every 12 hours of operation, and all blanks and samples.
- 10.9 Place the vials in the autosampler tray insuring that they are in the same order as the sequence table.
- 10.10 Load and run the sequence file and insure that the autosampler operates correctly.
- 10.11 From the results of the calibrations, build a calibration table.
- 10.12 Once the calibration table is completed, load the result file for each sample and print the appropriate report.

11.0 CALCULATIONS

- 11.1 The qualitative identification of compounds determined by this method is based on retention time matching. Results are confirmed by quantification using a specific mass for each compound and comparison with the retention times.
- 11.2 An internal standard calibration procedure is used by calculating the relative response factor (RRF) for each analyte using the following formula:

$$\text{RRF} = \frac{(A_x)(C_{IS})}{(A_{IS})(C_x)}$$

Where:

A_x	=	Area of the Target Analyte Peak
C_{IS}	=	Mass of the Internal Standard
A_{IS}	=	Area of the Internal Standard Peak
C_x	=	Concentration of the Target Analyte

- 11.3 The quantitation of each analyte of interest shall be based on the area of the peak of each ion at the retention corresponding to the calibration standard. The concentration is calculated using the following formula:

$$\text{Concentration} = \frac{(A_{UNK})(C_{IS})}{(A_{IS})(C_x)}$$

$$(A_{IS})(RRF_{TA})(SW)$$

Where:

A_{UNK}	=	Peak area of the sample
C_{IS}	=	Mass of the Internal Standard
A_{IS}	=	Area of the Internal Standard Peak
RRF_{TA}	=	Relative Response Factor for the Target Analyte
SW	=	Weight of sample extracted

11.4 The Method Detection Limit (MDL) is defined as the minimum concentration of a compound that can be measured and reported with 99% confidence that the value is greater than zero. The MDLs listed in Table 1 were determined using a clean marine sediment sample following US EPA guidelines in 40CFR.

Appendix F

Example Field Log Sheet and Chain-of-Custody Form

Qualitative Measures

1) Dominant Substrate:

Record the dominant substrate in the upstream reach of the sample location using one of the following categories:

Boulder (B), cobble (C), gravel (G), sand (S), fines (F) or cement (K).

2) Algae:

a) Filamentous: Record the percent of the flowing water surface, up-stream from your sample location, that you estimate is occupied by filamentous algae.

b) Other Periphyton: Record the percent of substrate in the wetted channel, looking up stream from your sample location, that you estimate is covered in periphyton. Other periphyton is defined here as the living community attached to the substrate, including algae that is not the green filamentous type, aquatic mosses, fungi, diatoms and sessile invertebrates. To make this estimate feel the surface of the rocks and other substrate materials and estimate the percent of the substrate that is covered with a slimy organic community.

3) Shading:

Record the percent of the stream's surface (water surface), up-stream from your sample location, that you estimate would be shaded if the sun was directly over the creek.

4) Plants:

a) Bank: Record the percent of the surface of both banks, up-stream from your sample location that you estimate to be covered by vegetation. This estimate refers only to plants and roots at the water's edge.

b) In-Stream: Record the percent of the flowing water's surface, up-stream from your sample location that you estimate to be occupied by aquatic vegetation. This is a percent of the total water surface that is occupied by aquatic vegetation.

Other Notes

Visually assess the stream corridor and comment on anything that you feel may directly affect or contribute to changes in water quality. Some standard comments and categories of observations follow.

a) Recent/Current Weather Events: heavy rains, cold front or heat spells

b) Water Color: black, brown, yellow, white, green, etc.

c) Site Odors: sulfides, sewage, petroleum, unidentifiable odor or none.

d) In stream Activities: construction, major erosion events, recent scour or other

e) Other Foreign Matter: suspended matter, oily sheen, foam or other debris.

f) Biological Activity: Note the presence of fish, birds, mammals or invertebrates observed and record one of the following categories: True count up to 25, then estimate > 25, > 50 or >100.

g) Trash : Bank and in stream debris such as fertilizer bags, aerosol cans, batteries human wastes, homeless encampments, dumping of furniture or appliances. Record the true number of paper and recyclable trash items up to 10 items (count 1-10 items of trash), greater than 10 items should be recorded as >10 items of trash.

h) Tidal Influences: evidence of recent tidal surge (i.e. kelp or driftwood) or of possible salt-water influence.

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CHAIN-OF-CUSTODY RECORD

[illegible]

MATRIX CODES: (SW = Surface Water); (WW = Wastewater); (STRMW = Stormwater); (SED = Sediment); (TISS = Tissue)

Appendix G

Calculations for Data Quality Assessments

Calculations for Data Quality Assessments

This appendix documents the calculations used to assess precision, accuracy, and completeness of the data.

Precision

Precision is a measure of the degree to which replicate measurements differ from one another. Precision assessed through calculation of field and laboratory duplicates and matrix spike duplicates is expressed as the Relative Percent Difference (RPD).

RPD for laboratory and field duplicates is calculated as follows:

$$RPD = 100 \times \left(\frac{|\text{replicate 1} - \text{replicate 2}|}{(\text{replicate 1} + \text{replicate 2}) \div 2} \right)$$

RPD for matrix spike duplicates is calculated as follows:

$$RPD = 100 \times \left(\frac{|\text{recovery 1} - \text{recovery 2}|}{(\text{recovery 1} + \text{recovery 2}) \div 2} \right)$$

where *Recovery* is calculated as described for matrix spikes, below.

If assessed with three or more replicate measurements, precision should be expressed as Relative Standard Deviation (RSD). RSD is calculated as:

$$RSD = 100 \times \left(\frac{\text{standard deviation of replicated measurements}}{\text{average of replicate measurements}} \right)$$

Accuracy

Accuracy is the degree to which a measured value agrees with a true or expected value for a parameter. Accuracy is typically assessed using standard reference materials, laboratory control samples, and matrix spikes. Recovery of laboratory control samples and standard reference materials is calculated as:

$$\% \text{ Recovery} = 100 \times \left(\frac{\text{recovered concentration}}{\text{true spike concentration}} \right)$$

Recovery of matrix spikes is calculated as:

$$\% \text{ Recovery} = 100 \times \left(\frac{\text{total recovered concentration} - \text{sample concentration}}{\text{true spike concentration}} \right)$$

When sample concentrations are less than the method detection limit, a value of "0" (zero) will be used as the sample result concentration for purposes of calculating spike recoveries.

Completeness

Completeness may be defined as the number of valid measurements compared to the total number of measurements collected. Completeness is calculated as:

$$\% \text{ Completeness} = 100 \times \left(\frac{\text{number of valid measurements}}{\text{total number of measurements}} \right)$$

Appendix H

Caltrans Stormwater Monitoring Protocols, Chapter 13

SECTION 13

QA/QC DATA EVALUATION

All data reported by the analytical laboratory must be carefully reviewed to determine whether the project's data quality acceptability limits or objectives (DQOs) have been met. This section describes a process for evaluation of all laboratory data, including the results of all QA/QC sample analysis.

Before any results are reported by the laboratory, the deliverable requirements should be clearly communicated to the laboratory, as described in the "Laboratory Data Package Deliverables" discussion in *Section 12*.

The current section discusses QA/QC data evaluation in the following two parts:

KEY TOPICS

- Initial Data Quality Screening
- Data Quality Evaluation

The initial data quality screening identifies problems with laboratory reporting while they may still be corrected. When the data reports are received, they should be immediately checked for conformity to chain of custody requests to ensure that all requested analyses have been reported. The data are then evaluated for conformity to holding time requirements, conformity to reporting limit requests, analytical precision, analytical accuracy, and possible contamination during sampling and analysis. The data evaluation results in rejection, qualification, and narrative discussion of data points or the data as a whole. Qualification of data, other than rejection, does not necessarily exclude use of the data for all applications. It is the decision of the data user, based on specifics of the data application, whether or not to include qualified data points.

➤ INITIAL DATA QUALITY SCREENING

The initial screening process identifies and corrects, when possible, inadvertent documentation or process errors introduced by the field crew or the laboratory. The initial data quality control screening should be applied using the following three-step process:

1. *Verification check between sampling and analysis plan (SAP), chain of custody forms, and laboratory data reports:* Chain of custody records should be compared with field logbooks and laboratory data reports to verify the accuracy of all sample identification and to ensure that all samples submitted for analysis have a value reported for each parameter requested. Any deviation from the SAP that has not yet

been documented in the field notes or project records should be recorded and corrected if possible.

Sample representativeness should also be assessed in this step. The minimum acceptable storm capture parameters (number of aliquots and percent storm capture) per amount of rainfall are specified in **Section 10**. Samples not meeting these criteria are generally not analyzed; however, selected analyses can be run at the Caltrans task manager's discretion. If samples not meeting the minimum sample representativeness criteria are analyzed, the resulting data should be rejected ("R") or qualified as estimated ("J"), depending upon whether the analyses were approved by Caltrans. Grab samples should be taken according to the timing protocols specified in the SAP. Deviations from the protocols will result in the rejection of the data for these samples or qualification of the data as estimated. The decision to reject a sample based on sample representativeness should be made prior to the submission of the sample to the laboratory, to avoid unnecessary analytical costs.

2. *Check of laboratory data report completeness:* As discussed in **Section 12**, the end product of the laboratory analysis is a data report that should include a number of QA/QC results along with the environmental results. QA/QC sample results reported by the lab should include both analyses requested by the field crew (field blanks, field duplicates, lab duplicates and MS/MSD analysis), as well as internal laboratory QA/QC results (method blanks and laboratory control samples).

There are often differences among laboratories in terms of style and format of reporting. Therefore, it is prudent to request in advance that the laboratory conform to the style and format approved by Caltrans as shown in **Section 14**. The Caltrans data reviewer should verify that the laboratory data package includes the following items:

- ✓ A narrative which outlines any problems, corrections, anomalies, and conclusions.
- ✓ Sample identification numbers.
- ✓ Sample extraction and analysis dates.
- ✓ Reporting limits for all analyses reported.
- ✓ Results of method blanks.
- ✓ Results of matrix spike and matrix spike duplicate analyses, including calculation of percent recovered and relative percent differences.
- ✓ Results of laboratory control sample analyses.
- ✓ Results of external reference standard analyses.
- ✓ Surrogate spike and blank spike analysis results for organic constituents.

- ✓ A summary of acceptable QA/QC criteria (RPD, spike recovery) used by the laboratory.

Items missing from this list should be requested from the laboratory.

3. *Check for typographical errors and apparent incongruities:* The laboratory reports should be reviewed to identify results that are outside the range of normally observed values. Any type of suspect result or apparent typographical error should be verified with the laboratory. An example of a unique value would be if a dissolved iron concentration has been reported lower than 500 µg/L for every storm event monitored at one location and then a value of 2500 µg/L is reported in a later event. This reported concentration of 2500 µg/L should be verified with the laboratory for correctness.

Besides apparent out-of-range values, the indicators of potential laboratory reporting problems include:

- Significant lack of agreement between analytical results reported for laboratory duplicates or field duplicates.
- Consistent reporting of dissolved metals results higher than total or total recoverable metals.
- Unusual numbers of detected values reported for blank sample analyses.
- Inconsistency in sample identification/labeling.

If the laboratory confirms a problem with the reported concentration, the corrected or recalculated result should be issued in an amended report, or if necessary the sample should be re-analyzed. If laboratory results are changed or other corrections are made by the laboratory, an amended laboratory report should be issued to update the project records.

➤ DATA QUALITY EVALUATION

The data quality evaluation process is structured to provide systematic checks to ensure that the reported data accurately represent the concentrations of constituents actually present in stormwater. Data evaluation can often identify sources of contamination in the sampling and analytical processes, as well as detect deficiencies in the laboratory analyses or errors in data reporting. Data quality evaluation allows monitoring data to be used in the proper context with the appropriate level of confidence.

QA/QC parameters that should be reviewed are classified into the following categories:

- ✓ Reporting limits

- ✓ Holding times
- ✓ Contamination check results (method, field, trip, and equipment blanks)
- ✓ Precision analysis results (laboratory, field, and matrix spike duplicates)
- ✓ Accuracy analysis results (matrix spikes, surrogate spikes, laboratory control samples, and external reference standards)

Each of these QA/QC parameters should be compared to data quality acceptability criteria, inalso known as the project’s data quality objectives (DQOs). The key steps that should be adhered to in the analysis of each of these QA/QC parameters are:

1. Compile a complete set of the QA/QC results for the parameter being analyzed.
2. Compare the laboratory QA/QC results to accepted criteria (DQOs).
3. Compile any out-of-range values and report them to the laboratory for verification.
4. Prepare a report that tabulates the success rate for each QA/QC parameter analyzed.

This process should be applied to each of the QA/QC parameters as discussed below.

Reporting Limits

Stormwater quality monitoring program DQOs should contain a list of acceptable reporting limits that the lab is contractually obligated to adhere to, except in special cases of insufficient sample volume or matrix interference problems. The reporting limits used should ensure a high probability of detection. , Table 12-1 provides recommended reporting limits for selected parameters.

Holding Times

Holding time represents the elapsed time between sample collection time and sample analysis time. Calculate the elapsed time between the sampling time and start of analysis, and compare this to the required holding time. For composite samples that are collected within 24-hours or less, the time of the final sample aliquot is considered the “sample collection time” for determining sample holding time. For analytes with critical holding times (48 hours), composite samples lasting longer than 24-hours require multiple bottle composite samples. Each of these composite samples should represent less than 24 hours of monitored flow, and subsamples from the composites should have been poured off and analyzed by the laboratory for those constituents with critical holding times (*see Section 12*). It is important to review sample holding times to ensure that analyses occurred within the time period that is generally accepted to maintain stable parameter concentrations. Table 12-1 contains the holding times for selected parameters. If holding times are exceeded, inaccurate concentrations or false negative results may be reported.

Samples that exceed their holding time prior to analysis are qualified as “estimated”, or may be rejected depending on the circumstances.

Contamination

Blank samples are used to identify the presence and potential source of sample contamination and are typically one of four types:

1. **Method blanks** are prepared and analyzed by the laboratory to identify laboratory contamination.
2. **Field blanks** are prepared by the field crew during sampling events and submitted to the laboratory to identify contamination occurring during the collection or the transport of environmental samples.
3. **Equipment blanks** are prepared by the field crew or laboratory prior to the monitoring season and used to identify contamination coming from sampling equipment (tubing, pumps, bailers, etc.).
4. **Trip blanks** are prepared by the laboratory, carried in the field, and then submitted to the laboratory to identify contamination in the transport and handling of volatile organics samples.
5. **Filter blanks** are prepared by field crew or lab technicians performing the sample filtration. Blank water is filtered in the same manner and at the same time as other environmental samples. Filter blanks are used to identify contamination from the filter or filtering process.

If no contamination is present, all blanks should be reported as “not detected” or “non-detect” (e.g., constituent concentrations should not be detected above the reporting limit). Blanks reporting detected concentrations (“hits”) should be noted in the written QA/QC data summary prepared by the data reviewer. In the case that the laboratory reports hits on method blanks, a detailed review of raw laboratory data and procedures should be requested from the laboratory to identify any data reporting errors or contamination sources. When other types of blanks are reported above the reporting limit, a similar review should be requested along with a complete review of field procedures and sample handling. Often times it will also be necessary to refer to historical equipment blank results, corresponding method blank results, and field notes to identify contamination sources. This is a corrective and documentative step that should be done as soon as the hits are reported.

If the blank concentration exceeds the laboratory reporting limit, values reported for each associated environmental sample must be evaluated according to USEPA guidelines for data evaluations of organics and metals (USEPA, 1991; USEPA, 1995) as indicated in Table 13-1.

Table 13-1. USEPA Guidelines for Data Evaluation

<i>Step</i>	<i>Environmental Sample</i>	<i>Phthalates and other common contaminants</i>	<i>Other Organics</i>	<i>Metals</i>
1.	Sample > 10X blank concentration	No action	No action	No action
2.	Sample < 10X blank concentration	Report associated environmental results as “non-detect” at the reported environmental concentration.	No action	Results considered an “upper limit” of the true concentration (note contamination in data quality evaluation narrative).
3.	Sample < 5X blank concentration	Report associated environmental results as “non-detect” at the reported environmental concentration.	Report associated environmental results as “non-detect” at the reported environmental concentration.	Report associated environmental results as “non-detect” at the reported environmental concentration.

Specifically, if the concentration in the environmental sample is less than five times the concentration in the associated blank, the environmental sample result is considered, for reporting purposes, “not-detected” *at the environmental sample result concentration* (phthalate and other common contaminant results are considered non-detect if the environmental sample result is less than ten times the blank concentration). The laboratory reports are not altered in any way. The qualifications resulting from the data evaluation are made to the evaluator’s data set for reporting and analysis purposes to account for the apparent contamination problem. For example, if dissolved copper is reported by the laboratory at 4 µg/L and an associated blank concentration for dissolved copper is reported at 1 µg/L, data qualification would be necessary. In the data reporting field of the database (see **Section 14**), the dissolved copper result would be reported as 4 µg/L, the numerical qualifier would be reported as “<”, the reporting limit would be left as reported by the laboratory, and the value qualifier would be reported as “U” (“not detected above the reported environmental concentration”).

When reported environmental concentrations are greater than five times (ten times for phthalates) the reported blank “hit” concentration, the environmental result is reported unqualified at the laboratory-reported concentration. For example, if dissolved copper is reported at 11 µg/L and an associated blank concentration for dissolved copper is reported at 1 µg/L, the dissolved copper result would still be reported as 11 µg/L.

Precision

Duplicate samples provide a measure of the data precision (reproducibility) attributable to sampling and analytical procedures. Precision can be calculated as the relative percent difference (RPD) in the following manner:

$$RPD_i = \frac{2 * |O_i - D_i|}{(O_i + D_i)} * 100\%$$

where:

RPD_i = Relative percent difference for compound i

O_i = Value of compound i in original sample

D_i = Value of compound i in duplicate sample

The resultant RPDs should be compared to the criteria specified in the project's DQOs. The DQO criteria shown in Table 13-2 below are based on the analytical method specifications and laboratory-supplied values. Project-specific DQOs should be developed with consideration to the analytical laboratory, the analytical method specifications, and the project objective. Table 13-2 should be used as a reference point as the least stringent set of DQO criteria for Caltrans monitoring projects.

Laboratory and Field Duplicates

Laboratory duplicates are samples that are split by the laboratory. Each half of the split sample is then analyzed and reported by the laboratory. A pair of field duplicates is two samples taken at the same time, in the same manner into two unique containers. Subsampling duplicates are two unique, ostensibly identical, samples taken from one composite bottle (see **Section 10**). Laboratory duplicate results provide information regarding the variability inherent in the analytical process, and the reproducibility of analytical results. Field duplicate analysis measures both field and laboratory precision, therefore, it is expected that field duplicate results would exhibit greater variability than lab duplicate results. Subsampling duplicates are used as a substitute for field duplicates in some situations and are also an indicator of the variability introduced by the splitting process.

The RPDs resulting from analysis of both laboratory and field duplicates should be reviewed during data evaluation. Deviations from the specified limits, and the effect on reported data, should be noted and commented upon by the data reviewer. Laboratories typically have their own set of maximum allowable RPDs for laboratory duplicates based on their analytical history. In most cases these values are more stringent than those listed in Table 13-2. Note that the laboratory will only apply these maximum allowable RPDs to laboratory duplicates. In most cases field duplicates are submitted "blind" (with pseudonyms) to the laboratory.

Environmental samples associated with laboratory duplicate results greater than the maximum allowable RPD (when the numerical difference is greater than the reporting limit) are qualified as “J” (estimated). When the numerical difference is less than the RL, no qualification is necessary. Field duplicate RPDs are compared against the maximum allowable RPDs used for laboratory duplicates to identify any pattern of problems with reproducibility of results. Any significant pattern of RPD exceedances for field duplicates should be noted in the data report narrative.

Corrective action should be taken to address field or laboratory procedures that are introducing the imprecision of results. The data reviewer can apply “J” (estimated) qualifiers to any data points if there is clear evidence of a field or laboratory bias issue that is not related to contamination. (Qualification based on contamination is assessed with blank samples.)

Laboratories should provide justification for any laboratory duplicate samples with RPDs greater than the maximum allowable value. In some cases, the laboratory will track and document such exceedances, however; in most cases it is the job of the data reviewer to locate these out-of-range RPDs. When asked to justify excessive RPD values for field duplicates, laboratories most often will cite sample splitting problems in the field. Irregularities should be included in the data reviewer’s summary, and the laboratory’s response should be retained to document laboratory performance, and to track potential chronic problems with laboratory analysis and reporting.

Accuracy

Accuracy is defined as the degree of agreement of a measurement to an accepted reference or true value. Accuracy is measured as the percent recovery (%R) of spike compound(s). Percent recovery of spikes is calculated in the following manner:

$$\%R = 100\% * [(C_s - C) / S]$$

where:

- %R = percent recovery
- C_s = spiked sample concentration
- C = sample concentration for spiked matrices
- S = concentration equivalent of spike added

Accuracy (%R) criteria for spike recoveries should be compared with the limits specified in the project DQOs. A list of typical acceptable recoveries is shown in Table 13-2. As in the case of maximum allowable RPDs, laboratories develop acceptable criteria for an allowable range of recovery percentages that may differ from the values listed in Table 13-2.

Percent recoveries should be reviewed during data evaluation, and deviations from the specified limits should be noted in the data reviewer's summary. Justification for out of range recoveries should be provided by the laboratory along with the laboratory reports, or in response to the data reviewer's summary.

Laboratory Matrix Spike and Matrix Spike Duplicate Samples

Evaluation of analytical accuracy and precision in environmental sample matrices is obtained through the analysis of laboratory matrix spike (MS) and matrix spike duplicate (MSD) samples. A matrix spike is an environmental sample that is spiked with a known amount of the constituent being analyzed. A percent recovery can be calculated from the results of the spike analysis. A MSD is a duplicate of this analysis that is performed as a check on matrix recovery precision. MS and MSD results are used together to calculate RPD as with the duplicate samples. When MS/MSD results (%R and RPD) are outside the project specifications, as listed in Table 13-2, the associated environmental samples are qualified as "estimates due to matrix interference". Surrogate standards are added to all environmental and QC samples tested by gas chromatography (GC) or gas chromatography-mass spectroscopy (GC-MS). Surrogates are non-target compounds that are analytically similar to the analytes of interest. The surrogate compounds are spiked into the sample prior to the extraction or analysis. Surrogate recoveries are evaluated with respect to the laboratory acceptance criteria to provide information on the extraction efficiency of every sample.

External Reference Standards

External reference standards (ERS) are artificial certified standards prepared by an external agency and added to a batch of samples. ERS's are not required for every batch of samples, and are often only run quarterly by laboratories. Some laboratories use ERS's in place of laboratory control spikes with every batch of samples. ERS results are assessed the same as laboratory control spikes for qualification purposes (see below). The external reference standards are evaluated in terms of accuracy, expressed as the percent recovery (comparison of the laboratory results with the certified concentrations). The laboratory should report all out-of-range values along with the environmental sample results. ERS values are qualified as "biased high" when the ERS recovery exceeds the acceptable recovery range and "biased low" when the ERS recovery is smaller than the recovery range.

Laboratory Control Samples

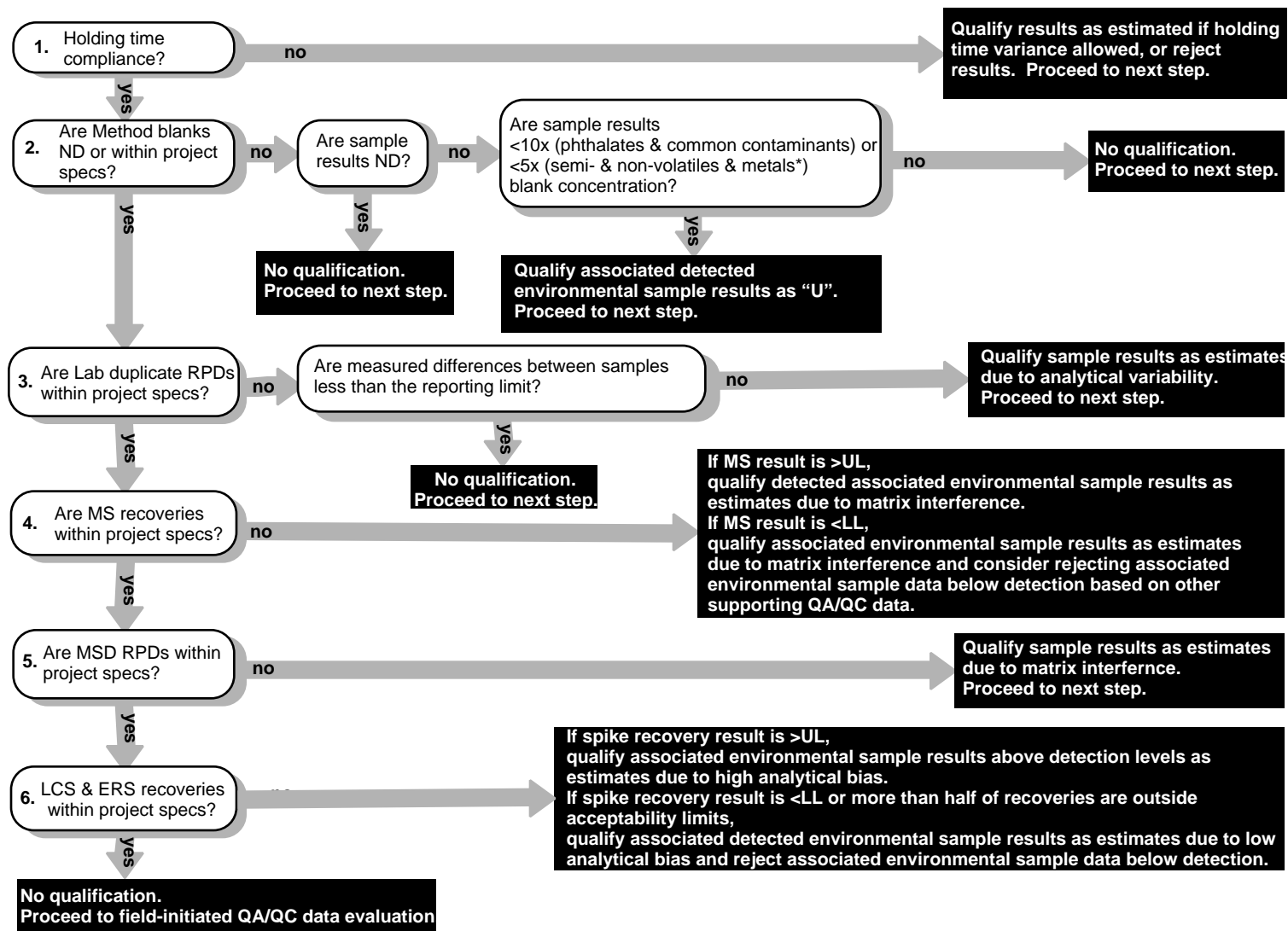
LCS analysis is another batch check of recovery of a known standard solution that is used to assess the accuracy of the entire recovery process. LCSs are much like ERS's except that a certified standard is not necessarily used with LCSs, and the sample is prepared internally by the laboratory so the cost associated with preparing a LCS sample is much lower than the cost of ERS preparation. LCSs are reviewed for percent recovery within

control limits provided by the laboratory. LCS out-of-range values are treated in the same manner as ERS out-of-range values. Because LCS and ERS analysis both check the entire recovery process, any irregularity in these results supersedes other accuracy-related qualification. Data are rejected due to low LCS recoveries when the associated environmental result is below the reporting limit.

A flow chart of the data evaluation process, presented on the following pages as Figures 13-1 (lab-initiated QA/QC samples) and 13-2 (field-initiated QA/QC), can be used as a general guideline for data evaluation. Boxes shaded black in Figures 13-1 and 13-2 designate final results of the QA/QC evaluation.

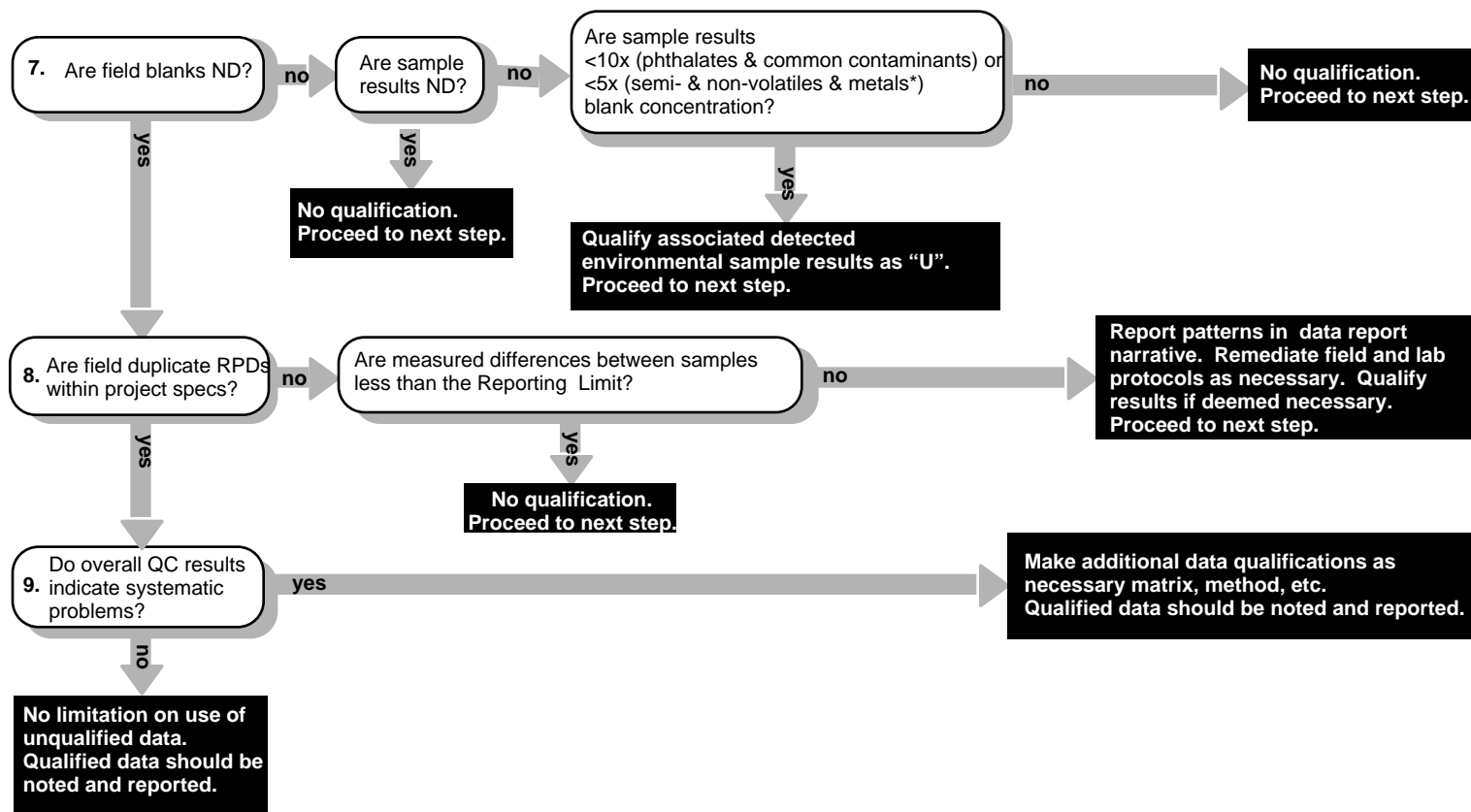
Table 13-2. Typical Control Limits for Precision and Accuracy for Analytical Constituents

Analyte	EPA Method Number or Standard Method	Maximum Allowable RPD	Recovery Upper Limit	Recovery Lower Limit
Conventionals				
BOD	405.1; SM 5210B	20%	80%	120%
COD	410.1; 410.4; SM 5220C; SM 5220D	20%	80%	120%
Hardness	130.2; 130.1; SM 2340B	20%	80%	120%
pH	150.1	20%	NA	NA
TOC/DOC	415.1	15%	85%	115%
TDS	160.1	20%	80%	120%
TSS	160.2	20%	80%	120%
Turbidity	180.1	20%	NA	NA
Nutrients				
NH3-N	350.2; 350.3	20%	80%	120%
NO3-N	300.0	20%	80%	120%
NO2-N	300.0	20%	80%	120%
NO3/NO2-N	353.2	20%	80%	120%
P	365.2	20%	80%	120%
Ortho-P	365.2; 365.3	20%	80%	120%
TKN	351.3	20%	80%	120%
Metals				
Ag	272.2; 200.8	20%	75%	125%
Al	200.9; 200.8	20%	75%	125%
Cd	213.2; 200.8	20%	75%	125%
Cr	218.2; 200.8	20%	75%	125%
Cu	220.2; 200.8	20%	75%	125%
Ni	249.2; 200.8	20%	75%	125%
Pb	239.2; 200.8	20%	75%	125%
Zn	289.2; 200.8	20%	75%	125%
As	206.3; 200.8	20%	75%	125%
Fe	200.9; SM 3500-Fe B	20%	75%	125%
Se	200.9; 270.3; 200.8	20%	75%	125%
Hg	1631	21%	79%	121%
Total Petroleum Hydrocarbons				
TPH (gasoline)	8015b	21%	45%	129%
TPH (diesel)		21%	45%	129%
TPH (motor oil)		21%	45%	129%
Oil & Grease	1664	18%	79%	114%
Pesticides and Herbicides				
Glyphosate	547	30%	70%	130%
OP Pesticides (esp. diazinon and chlorpyrifos)	8141; ELISA	25%	see method for constituent specific	
OC Pesticides	8081	25%		
Chlorinated Herbicides	8150; 8151	25%		
Carbamate Pesticides	8321	25%		
Miscellaneous Organic Constituents				
Base/Neutrals and Acids	625; 8270	30% to 50% (analyte dependent)	see method for constituent specific	
PAHs	8310			
Purgeables	624; 8260	20%		
Purgeable Halocarbons	601	30%	see method, Table 2	
Purgeable Aromatics	602	20%	see method for constituent specific	
Miscellaneous Constituents				
Cyanide	335.2	20%	75	125
Bacteriological				
Fecal Coliform	SM 9221E	-	-	-
Total Coliform	SM 9221B	-	-	-



*Environmental results between 5x and 10x the blank concentration are qualified as "an upper limit on the true concentration" and the data user should be cautioned.

Figure 13-1. Technical Data Evaluation for Lab-Initiated QA/QC Samples



*Environmental results between 5x and 10x the blank concentration are qualified as "an upper limit on the true concentration" and the data user should be cautioned.

Figure 13-2. Technical Data Evaluation for Field-Initiated QA/QC Samples