

State of California



Environmental Laboratory Accreditation Program



AQUATIC TOXICITY TESTING

(Fields of Testing/Accreditation 113 and 119)

INTRODUCTION

This section covers the general accreditation requirements for aquatic toxicity testing of effluents and hazardous wastes.

Laboratory aquatic bioassay procedures will be evaluated based on comparison with protocols approved by the State Water Resources Control Board or the Department of Public Health. The approved methods are listed on our Field of Testing (FoT) worksheets that are available from our website (<http://www.dhs.ca.gov/elap>).

CERTIFICATION REQUIREMENTS

Environmental Laboratory Accreditation Program (ELAP) accreditation for aquatic toxicity testing requires the following:

1. Successful completion of on-site assessment including satisfactory response to all deficiencies noted.
2. Approval of laboratory Quality Assurance/Quality Control (QA/QC) manual and Standard Operating Procedures (SOPs) appropriate to toxicity tests performed.

Send completed FOT worksheets, all SOPs, and QA/QC Manual to ELAP before the site inspection date. Please submit documents to:

**Department of Public Health
Environmental Laboratory Accreditation Program
850 Marina Bay Parkway
Building P, 1st Floor
Richmond, CA 94804**

In addition, send the completed FOT worksheets electronically via e-mail (elapca@cdph.ca.gov), diskette, or CD to the above address

FACILITIES and EQUIPMENT

Facilities must include:

- Isolated aquatic testing facilities to avoid cross-contamination of tests and stressing test organisms, and for the safety of lab personnel.
- Test containers in sufficient number for sample and reference toxicant tests, using materials required in protocols.
- Holding facilities of appropriate size and type for each species used in testing.
- Sample mixing equipment (appropriate to protocol).
- Disposal facilities for samples, test solutions, and chemicals.
- Air supply (oil-less).
- Adequate illumination system.
- Adequate temperature control.

Equipment available to measure:

- Temperature (including a continuously recording thermometer).
- pH (accurate to 0.1 pH unit)
- Dissolved oxygen (accurate to 0.1mg/L)
- Salinity/specific conductance
- Hardness (should be measured according to EPA Method 130.2)
- Alkalinity (should be measured according to EPA Method 310.1)
- Mass (of sufficient sensitivity for type of organism tested)
- Length (to 1 mm for larger organisms)
- Miscellaneous equipment required for some methods (drying oven, microscopes, etc.)

Special Equipment may be required for:

Static renewal tests:

- Transfer of organisms or renewal of test solutions
- Feeding of test organisms.-

Flow-through tests:

- Continuous flow system constructed of material appropriate to protocols, with enough outflows and containers to run duplicates of each concentration.
- Equipment to measure flows.
- Flow-through system for control water.
- Chiller and heater unit.

DATA EVALUATION

Percent survival may be computed manually. For endpoints such as LCs, ECs, ICs, and NOEC/LOEC, computer programs are available and should be utilized. A program may be obtained from ELAP to calculate LC50s using the probit method, the moving average method and the binomial method. Send a request to the address on page one or call (916) 449-5616. Other software available to calculate toxicity endpoints includes Cetus or ToxCalc from

QUALITY CONTROL

1. Quality Assurance Plan

The QA plan developed for the laboratory must be followed and reflect actual laboratory practices. It must include current procedures and be made accessible to all analysts. Refer to the ELAP document on our website for QA/QC Document requirements.

2. Replicates

Most procedures require at least replicate test chambers, and the chronic aquatic toxicity tests require multiple replicates. Review the method manuals to determine the minimum number of replicates required.

3. Controls

At least one dilution water control must be run with each test. The control must be run under identical conditions to the test chambers. Additional controls will be required during tests when pH or salinity is adjusted or when a carrier solvent is used.

4. Food Suitability

The suitability of the food supply should be tested for EPA chronic tests and whenever feeding is required during a test.

5. Reference Toxicants

It is a laboratory's responsibility to demonstrate its ability to obtain consistent, precise results with reference toxicants before it performs toxicity tests with effluents for permit compliance purposes. To meet this requirement, the intra-laboratory precision of each type of test to be used in a laboratory must be determined by performing five or more tests with different batches of test organisms, using the same reference toxicant, at the same concentrations, with the same test conditions, and the same data analysis methods. In addition, most protocols require reference toxicant testing to evaluate the sensitivity of each batch of test organisms. The type of reference toxicant that should be used is dependent on the type of organism being tested. The table on page 9 lists the recommended toxicants for most species. Selection of a toxicant should be based on safety to analysts, ease of disposal, and ability to generate precise endpoint.

6. Performance Evaluation Samples

At the present time, ELAP does not require annual performance evaluation samples for ELAP laboratories certified or seeking certification to perform aquatic bioassays. Third-party sample providers are now approved by NVLAP for NELAP laboratories.

TEST ORGANISMS

Test organisms must be humanely destroyed before disposal. Any surviving organisms, including controls, may not be released or used in future tests.

A permit must be obtained from the Department of Fish and Game if organisms are collected from wild stocks.

A list of registered aqua culturists is available from the Department of Fish and Game Regional offices or at:

<http://www.dfg.ca.gov/fishing/assets/publications/AquaCultureReport.pdf>

Pages 5 through 8 provide information on suppliers of commonly used aquatic toxicity test organisms. ELAP does not endorse any particular supplier nor is this list inclusive.

FRESHWATER FISH SUPPLIERS

ORGANISM	SUPPLIER	LOCATION	PHONE
Juvenile Fathead Minnows (1-90 day old)	Aquatic Biosystems	Fort Collins, CO	(800) 331-5916
	Aquatox	Hot Springs, AR	(501) 520-0560
	Cosper Environmental	Bohemia, NY	(800) 428-7733
	Environmental Consulting & Testing	Superior, WI	(800) 377-3657
	Sticklebacks Unlimited	Vallejo, CA	(707) 644-6997
	Thomas Fish Co.	Anderson, CA	(530) 378-1006
Larval and Juvenile Rainbow Trout	Block Environmental	Pleasant Hill, CA	(925) 682-7200
	Lost River Trout Hatchery	Mackay, ID	(208) 588-2866
	Spring Creek Hatchery	Lewiston, MT	(406) 538-3538
	Sticklebacks Unlimited	Vallejo, CA	(707) 644-6997
	Thomas Fish Co.	Anderson, CA	(530) 378-1006
Fathead Minnow Larvae (1-14 day old)	Aquatic Biosystems	Fort Collins, CO	(800) 331-5916
	Aquatox	Hot Springs, AR	(501) 767-9120
	Cosper Environmental	Bohemia, NY	(800) 428-7733
	Environmental Consulting & Testing	Superior, WI	(800) 377-3657
	Sachs System Aquaculture	St. Augustine, FL	(904) 824-6308

FRESHWATER TEST ORGANISM SUPPLIERS

ORGANISM	SUPPLIER	LOCATION	PHONE
Water Fleas	Aquatic Biosystems	Fort Collins, CO	(800) 331-5916
	Aquastar	Mobile, AL	(800) 831-9279
	Cosper Environmental	Bohemia, NY	(800) 428-7733
	Environmental Consulting & Testing	Superior WI	(800) 377-3657
Green Algae	Aquatic Biosystems	Fort Collins, CO	(800) 331-5916
	American Type Culture Collection (Culture No. ATCC 22662)	Manassas, VA	(703) 365-2700
	Aquatic Biology, Quality Assurance Research Division, EPA	EMSL-Cincinnati Newtown Facility	(513) 533-8114
	Enviro Sciences	Carrollton, TX	(214) 241-8952
	Culture Collection of Algae, Botany Dept. University of Texas	Austin, TX	(512) 471-4019

MARINE TEST ORGANISM SUPPLIERS

ORGANISM	SUPPLIER	LOCATION	PHONE
Abalone	Abalone Farm	Cayucos, CA	(805) 995-2495
	Abalone International	Crescent City, CA	(707) 464-6913
	The Cultured Abalone	Santa Barbara, CA	(805) 685-1956
	McCormick and Associates	Ojai, CA	(805) 488-1041
	US Abalone	Davenport, CA	(831) 457-2700
	Brezina and Associates	Dillon Beach, CA	(707) 878-2853
Bivalves	Carlsbad Aquafarm	Carlsbad, CA	(760) 438-2444
	Dave Guttoff	San Diego, CA	(858) 453-2741
	Botany Department University of Texas	Austin, TX	(512) 471-4019
Red Algae and Diatoms	Dave Guttoff	San Diego, CA	(619) 685-7647
Giant Kelp	McCormick and Associates	Ojai, CA	(805) 488-1041
	Dave Guttoff	San Diego, CA	(619) 685-7647

MARINE TEST ORGANISM SUPPLIERS

ORGANISM	SUPPLIER	LOCATION	PHONE
Silversides and Topsmelt	Aquatic Biosystems	Fort Collins, CO	(800) 331-5196
	Aquatic Indicators	St. Augustine, FL	(904) 829-2780
	Aquatox	Hot Springs, AR	(501) 520-0560
<u>Holmesimysis costata</u>	Dave Gutoff	San Diego, CA	(858) 453-2741
<u>Mysidopsis bahia</u>	Aquatic Biosystems	Fort Collins, CO	(800) 331-5196
	Aquatic Indicators	St. Augustine, FL	(904) 829-2780
	Aquatox	Hot Springs, AR	(501) 520-0560
<u>Neomysis Mercedis</u>	Brezina and Associates	Dillon Beach, CA	(707) 878-2853
Sand Dollars and Sea Urchins	Dave Gutoff	San Diego, CA	(858) 453-2741
	Santa Barbara Marine Bio.	Santa Barbara, CA	(805) 963-9531
	McCormick and Associates	Ojai, CA	(805) 488-1041
	Marinus, Inc.	Long Beach, CA	(562) 435-6522
Sanddabs	Aquatic Biosystems	Fort Collins, CO	(800) 331-5196
	Brezina and Associates	Dillon Beach, CA	(707) 878-2853

RECOMMENDED REFERENCE TOXICANTS

SPECIES	REFERENCE TOXICANT	PROTOCOL
Red alga <u>Champia parvula</u>	Sodium dodecyl sulfate (SDS), cadmium chloride, copper sulfate	EPA-821-R-02-014
Alga <u>Selenastrum capricornutum</u>	SDS, copper sulfate, sodium chloride, cadmium chloride, etc.	EPA-821-R-02-013
Shrimp <u>Mysidopsis bahia</u>	SDS, cadmium chloride, copper sulfate	EPA-821-R-02-014
<u>Holmesimysis costata</u>	zinc sulfate	EPA/600/R-95/136
Water flea <u>Ceriodaphnia dubia</u>	SDS, copper sulfate, sodium chloride, cadmium chloride, etc.	EPA-821-R-02-013
Giant kelp <u>Macrocystis pyrifera</u>	Copper chloride	EPA/600/R-95/136
Red abalone <u>Haliotis rufescens</u>	Zinc sulfate	EPA/600/R-95/136
Oyster <u>Crassostrea gigas</u>	Copper sulfate, sodium azide, copper chloride	EPA/600/R-95/136
Mussel <u>Mytilis edulis</u>	Copper sulfate, sodium azide, copper chloride	EPA/600/R-95/136
Urchins <u>Strongylocentrotus purpuratus</u> <u>Strongylocentrotus franciscanus</u> <u>Dendraster excentricus</u>	Silver nitrate, SDS, copper sulfate Copper chloride	EPA/600/R-95/136
Silversides <u>Menidia beryllina</u>	SDS, cadmium chloride, copper	EPA-821-R-02-014
Fathead minnows <u>Pimephales promelas</u>	SDS, copper sulfate, sodium chloride, cadmium chloride, etc.	EPA-821-R-02-013