STATIC ACUTE BIOASSAY PROCEDURES FOR HAZARDOUS WASTE SAMPLES

Prepared by

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CALIFORNIA DEPARTMENT OF FISH AND GAME

WATER POLLUTION CONTROL LABORATORY

CHANGES IN THIS REVISION

Changes made in this revision include :

1. Temperature regime of warm water tests is changed to 20° +/- 2°C and 12° C +/- 2° C for cold water tests. The previous guidelines were 20-23° C for warm water and 14-17° C for cold water tests. This change was made to bring the temperature requirements in line with EPA aquatic toxicity testing criteria.
2. Additional types of mechanical mixing allowed under the wrist-action mixing method are listed.
3. The size of test organisms has been expanded to include the weight range of 0.2 grams to 1.0 gram per organism with the stipulation that the largest be no more than 1.5 times the weight of the smallest. This change was made to make a wider range of fish suppliers available to participating laboratories and allow laboratories raising their own fish to use those organisms on a shorter time frame.

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1.0 INTRODUCTION

The California Department of Health Services (DOHS) has adopted regulations (R-45-78) which define criteria for the identification of hazardous wastes. These criteria are codified in Chapter 30 of Title 22 of the California Code of Regulations.

Toxicity to aquatic life, specifically fish, is one of the criteria used to gauge the hazardous potential of a waste (Section 66696). The bioassay criteria used to specify a toxic waste or material are listed in Section 66696 of the Hazardous Waste Criteria Regulations (HWC) and excerpted as Appendix A of this document. An acute 96-hour bioassay is used to determine the LC50 as defined in Section 66002 (Appendix B). This 96-hour LC50 value serves as the numerical indicator of the toxicity of a waste to aquatic life.

The basic bioassay protocols to be followed in determining the LC50 value are those cited in the 17th, or most recent, edition of Standard Methods for the Examination of Water and Wastewater, Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms (Third Edition) by the U.S. Environmental Protection Agency, Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians (ASTM E 729-80), Guidelines for Performing Static Acute Toxicity Fish Bioassays in Municipal and Industrial Wastewaters by the California Department of Fish and Game and methods approved by the California Department of Health Services (DHS) with advice from the Department of Fish and Game (DFG).

Reference should be made to these sources for recommended materials, water supply specifications, and general laboratory construction and organization.

Special protocols must occasionally be developed for materials which do not readily lend themselves to standard toxicity testing. This document describes special sample preparation and procedures developed specifically for hazardous waste samples and addresses questions frequently asked by laboratories performing bioassays on hazardous waste samples. It should be used as a supplement to the general bioassay references listed above by those already familiar with basic bioassay procedures.

2.0 STANDARD BIOASSAY PROCEDURE

2.1 SAMPLE STORAGE

The sample must be in an area separate from other samples in an unopened in locked, refrigerated storage and held at 4^{o} C until the bioassay is performed. If the samples are stored in a refrigerated room common to other types of samples, they must at a minimum, be kept on a separate shelf which is labeled for hazardous waste samples only. Sample storage time prior to performing the bioassay should be kept to a minimum.

2.2 TEST CHAMBER CLEANING

Five or ten gallon glass aquaria are the most convenient and readily available bioassay containers, given the usual 10 liter volume of bioassay solution. Test chambers must be thoroughly washed and rinsed between tests. Retention of organic toxicants in the silicone sealant typically used for aquarium construction

is of particular concern. A wash procedure that includes an acid and organic solvent rinse followed by several water rinses is adequate. "Standard Methods" and EPA toxicity testing publications list specific rinse procedures. The DFG wash procedure is a modified procedure which has been tested by chemical analysis of the final rinse water :

A. Wash with warm tap water and a laboratory cleanser, such as Alconox or Haemo-Sol.

B. Rinse with warm tap water.

C. Air dry.

D. Rinse with petroleum ether or acetone.

E. Rinse with warm water.

F. Rinse with dilute (5%) hydrochloric acid.

G. Rinse 4 times with water.

H. Air dry aquaria prior to use.

Other organic solvents, such as pesticide-grade acetone or hexane, may be substituted for the petroleum ether rinse.

2.3 TEST ORGANISMS

Fish should be maintained in the laboratory for a minimum of 10 days after arrival or treatment to monitor any excessive mortality prior to testing. Some mortality due to stress associated with shipping is normal. A daily log must be maintained to record the feeding, mortalities, condition and any other information pertinent to the fish in acclimation tanks. Feeding must be withheld 48 hours prior to the start of the test and for the duration of the bioassay.

Bioassays designed to assess waste toxicity under the

Hazardous Waste Criteria Regulations should preferably be conducted with fathead minnows, Pimephales promelas. Alternate species are the golden shiner, Notemigonus chrysoleucas, and the rainbow trout, Salmo gairdneri. Temperature regime may dictate the most appropriate test species. The test temperature for fathead minnow and golden shiner bioassays must be maintained at 20^{o} C $\pm$ 2^{o} C. Rainbow trout bioassay temperature must be maintained at 12^{o} C $\pm$ 2^{o} C. Day-to-day temperature variation must not vary by more than 2 ^{o}C during any 24-hour period.

Both the acclimation and the test fish must be exposed to a photoperiod consisting of 8 to 16 hours of light for every 24 hour period. The fish should be exposed to the same photoperiod during the test that they were exposed to during acclimation.

Test organisms may be within the range of 0.2 grams to 1.0 grams per individual with the stipulation that the largest organism should be no more than 1.5 times the weight of smallest. The average, minimum and maximum lengths and weights must be reported from a pool of 10 organisms sacrificed at the end of each test. Loading should not exceed 1 gram biomass/L in static bioassays. A minimum of twenty fish, in two separately-prepared test chambers, must be exposed to each concentration. These duplicate test chambers must be prepared by separate subsampling, weighing and mixing of the hazardous waste sample.

2.4 DISSOLVED GASSES

Dissolved oxygen levels are generally in the range of 5.5 mg/L to 7.5 mg/L for warmwater bioassays (18 ^{o}C to 22 ^{o}C). Dissolved oxygen levels should remain above 60 percent of saturation levels for the duration of the test. Dissolved oxygen

levels in the test containers must never fall below 4 mg/L in a bioassay with fathead minnows. Bioassays with rainbow trout must never fall below 5 mg/L. Dissolved oxygen levels lower than these minima invalidate a waste bioassay.

Temperature (°C)	100% Saturation (mg/L)
15	10.2
16	9.9
17	9.7
18	9.5
19	9.3
20	9.2
21	9.0
22	8.8
23	8.7
24	8.5
25	8.4

Aeration with breathing-quality air through a narrow-bore tube is the preferred method of maintaining dissolved oxygen levels and should continue throughout the bioassay. Aeration with an airstone is less desirable and oxygenation is least preferable. An attempt should be made to raise the initial dissolved oxygen level with a short period of aeration or oxygenation with an airstone followed by aeration through a small-bore tube prior to the use of an airstone throughout the 96 hour test. Any method of aeration beyond the normal use of breathing air through a small-bore tube must be noted on the data sheet. The methods for maintaining dissolved oxygen in decreasing preference are:

1. Continual aeration with breathing air through a small-bore tube.
2. Initial short period of aeration with an airstone followed by aeration through a small-bore tube.

3. Initial short period of oxygenation with an airstone followed by aeration through a small-bore tube.
4. Initial short period of oxygenation with an airstone followed by oxygenation for entire period through a small-bore tube.
5. Aeration for entire 96 hour test period with an airstone.
6. Oxygenation for entire 96 hour test period with an airstone.

Every attempt should be made to use aeration with compressed air before moving on to less preferable methods. The period of airstone use and the rate of aeration should be kept to a minimum to minimize "stripping" any volatile toxicants from the waste sample.

3.0 HAZARDOUS WASTE CRITERIA CONSIDERATIONS

3.1 TOXICANT CONCENTRATIONS

Section 66696 specifies that the waste generator shall demonstrate that the waste does not have an LC50 less than 500 mg/L in order to qualify for a nonhazardous designation on the basis of aquatic toxicity. A bioassay with a single toxicant concentration of 500 mg/L is not deemed adequate to justify a nontoxic designation.

When screening bioassays are performed, initial screening bioassays must be conducted at two concentrations bracketing the 500 mg/L level. One of these concentrations must be 750 mg/L. The second concentration is at the discretion of the laboratory,

as long as it is below 500 mg/L. Laboratories which have historically used 250 mg/L, 500 mg/L and 750 mg/l are encouraged to retain this procedure. Screening at a minimum of two concentrations instead of the HWC regulatory level of 500 mg/L minimizes the statistical probability of assessing a waste as non-toxic which is actually toxic at 500 mg/L or less. Testing at concentrations bracketing 500 mg/L allows some estimation of the dose-response curve and a more accurate and precise determination of the LC50 of the waste sample. Standardized species tests performed at the DFG Water Pollution Control Laboratory (Hansen, et al., 1979) indicate that the 250 mg/L safety margin (250 mg/L above the 500 mg/L regulatory limit) is necessary to accommodate the 95 percent confidence limits of the LC50.

Serial dilution bioassays to determine the LC50 and associated confidence limits are conducted on those samples which cause mortality of 40 percent or greater at 750 mg/L in the screening bioassay. Test organisms are exposed to a minimum of five concentrations of the waste material over a 96-hour period in a definitive bioassay. Definitive test concentrations are most frequently constructed as a geometric progression, although best scientific judgment may dictate a different concentration range. An approved method of calculating the LC50 which produces 95 percent confidence limits must be used to report the bioassay data. The binomial test, probit analysis and moving average (angle) are three commonly-used methods considered appropriate.

3.2 SAMPLE PREPARATION

Samples should be mixed, if possible, prior to opening the sample container. A wrist-action shaker, a three-dimensional shaker-mixer, such as a Turbula, or any other appropriate mechanical device may be used for mixing. Viscous samples should be stirred after opening to make the sample as homogeneous as possible.

At a minimum, subsamples must be mixed using a mechanical method. Two methods of standard sample preparation are presently used on each sample for both the screening and the serial dilution bioassays at the Water Pollution Control Laboratory. Samples are mixed using a wrist-action shaker and an ultrasonic probe. The wrist-action shaker method is the preferred method for powders and other samples which may require some time to dissolve prior to introduction to the test aquaria. The ultrasonic method can produce suspensions of relatively insoluble material which are stable for the 96-hour period of most aquatic toxicity tests. Both methods are detailed here.

3.2.1 Wrist-action shaker method:

While this method was developed for a wrist-action shaker some other methods of mechanical agitation are also allowed. Orbital shaker tables, roller mixers (similar to those used for the Waste Extraction Test) and sieve shakers modified to hold the erlenmeyer flasks can be used in this method. The goals is to provide sufficiently active mixing over the six hour mixing period.

3.2.2 Ultrasonic method:

The ultrasonic probe that is approved for these procedures should be similar to a HEAT SYSTEMS Model W-375, allowing variable power output and variable "active" cycle during which the ultrasonic horn is actively mixing the sample. Ultrasonic baths do not provide adequate mixing and are not appropriate for this procedure.

A. An appropriate amount of the well-mixed sample is weighed on a top-loading balance into a 50 mL disposable polyethylene beaker.

B. Approximately 30 mL of water is added to the beaker.

C. The disposable beaker is packed in ice and the tip of a Heat Systems W-375 Sonicator, or equivalent, is placed approximately 2 cm into the sample. The sonicator is set to pulse at 50 percent duty cycle and the output is adjusted for efficient mixing. Output is usually at half power. The sample is sonicated for 1 minute.

D. The disposable beaker and its contents are removed from the sonication apparatus and placed into a prepared test chamber with 10 liters (minus the amount added to the disposable beaker) of water. The aerating pipet is placed into the disposable beaker to hold it below the surface.

E. The initial dissolved oxygen, pH and temperature are measured.

F. 10 fish, less than 1 gram each, are added to each test container.

G. Dissolved oxygen, pH and temperature are measured daily. Dead fish are counted and removed every 24 hours.

A control of at least 10 fish in the same dilution water is run during each bioassay to assure that the fish are healthy and that treatment mortalities are the result of toxicant stress.

A. An appropriate amount of the well-mixed sample is weighed on a top-loading balance into a 50 mL disposable polyethylene beaker.

B. Approximately 30 mL of water is added to the beaker.

C. The disposable beaker is packed in ice and the tip of a Heat Systems W-375 Sonicator, or equivalent, is placed approximately 2 cm into the sample. The sonicator is set to pulse at 50 percent duty cycle and the output is adjusted for efficient mixing. Output is usually at half power. The sample is sonicated for 1 minute.

D. The disposable beaker and its contents are removed from the sonication apparatus and placed into a prepared test chamber with 10 liters (minus the amount added to the disposable beaker) of water. The aerating pipet is placed into the disposable beaker to hold it below the surface.

E. The initial dissolved oxygen, pH and temperature are measured.

F. 10 fish, less than 1 gram each, are added to each test container.

G. Dissolved oxygen, pH and temperature are measured daily. Dead fish are counted and removed every 24 hours.

A control of at least 10 fish in the same dilution water is run during each bioassay to assure that the fish are healthy and that treatment mortalities are the result of toxicant stress.

3.3 REPLICATE CONCENTRATIONS

The minimum number of organisms per test concentration is twenty. More test organisms per concentration may be required, in some instances, to narrow the 95 percent confidence limit around the LC50. Replicate tests at each test concentration are required. More replicates of a given concentration or a greater number of fish per test container, will increase the precision of the results.

3.4 SPECIAL SAMPLE PREPARATION PROCEDURES

The inability to demonstrate a dose-response relationship, where the mortality increases with increasing concentration, is an indicator that some altered sample preparation technique may be required.

3.4.1 SUBSAMPLING

Hazardous waste samples which cannot be subsampled directly from the sample container frequently require the subsampling device be washed in some manner to include any adhered sample in the test container. A pipette is often used to subsample a liquid. A polystyrene pipette used to subsample a hazardous waste can be broken into sections and sonicated with an ultrasonic probe in bioassay dilution water to remove any adhering sample. Alternatively, any adhering material can be washed from the the subsampling device into the test container with an organic solvent. This methodology would require a solvent control in addition to the standard control (ASTM E 729, 11.1.2).

3.4.2 SEDIMENT-CONTAINING HAZARDOUS WASTE SAMPLES

A potential exists for physical damage to fish gills from any solid material introduced into an aquarium. Bioassays on salmon performed with Mt. St. Helens volcanic ash and mudflow sediments produced 96-hour static LC50s ranging from 18,672 mg/L to 28,134 mg/L in volcanic ash and 11,000 mg/L to 29580 mg/L in mudflow sediments (Stober, et al., 1981). The author's concluded

that "in the absence of high water velocities, gill damage is mild even at very high sediment concentrations" (p. 76). Test concentrations of 750 mg/L or less should generally not be of concern over a 96 hour period. Test fish may be separated from settled ash material by an inert screen supported just above the bottom of the test aquaria where this is considered necessary. Teflon screen is the preferred material and is available from large scientific supply houses. Nylon or plastic screen may be substituted in emergency situations. The control tank should also contain a screen if screens are placed in the test aquaria.

3.4.3 pH ADJUSTMENT

Aquatic organisms are stressed by pH values deviating markedly from a relatively neutral level. The species recommended as test organisms are greatly stressed by pH values outside the range of 5.0 to 9.0 . Values falling above or below this range may significantly influence the results of the bioassay. Acidic samples which produce a pH less than 5.5 in any test concentration may be adjusted with sodium hydroxide to a pH of 5.5 prior to introduction of the test organisms. Alkaline samples producing a pH greater than 8.5 may be adjusted with hydrochloric acid to a pH of 8.5. Periodic pH adjustment or the addition of a minor amount of sodium bicarbonate buffer may be necessary under certain conditions. A second pH-adjusted control must accompany any bioassay in which the treatment pH has been adjusted. The pH of this second control should be adjusted to the pH of the treatment tanks. A detailed description of the measures taken must be provided with the test data if pH control is required.

Waste ash from waste-to-energy generation facilities is a type of waste frequently requiring pH adjustment. These ashes tend to be either relatively neutral or highly alkaline due to the addition of lime at some point in the process. Hydrochloric acid may be added to decrease the pH to 8.5 and a small amount of sodium bicarbonate may be introduced to maintain the pH near that of the holding tank. A dilution water only control tank and a pH-adjusted control of 8.5 would be required for this ash bioassay. Sodium hydroxide should be used to raise the pH.

3.4.4 USE OF CARRIER SOLVENTS

Solvents other than water may be necessary to dissolve a waste sample containing organic materials. An organic solvent used as a carrier for waste samples should be chosen for its low toxicity to animals, low volatility and ability to dissolve organic compounds. Dimethylformamide, triethylene glycol, acetone, ethanol and methanol are frequently used as carriers in toxicity testing. The carrier concentration must not exceed 0.5 mL/L in any test container (ASTM E729, 9.1). A solvent control is required, in addition to the standard control, when a carrier solvent is used. The solvent control should contain the highest concentration of solvent used in any treatment (ASTM E729, 11.1.2).

3.4.5 GENERATORS FOR SOLUTIONS OF HYDROPHOBIC MATERIALS

Methods developed to prepare solutions of hydrophobic materials for continuous-flow bioassays may be of use for hazardous waste samples containing hydrophobic substances (Gingerich, et al., 1979; Phipps, et al., 1982 and Veith and Comstock, 1975).

4.0 PHYSICAL AND CHEMICAL MEASUREMENTS

A log book recording the standardization schedule and type of standardization performed must be kept for all instruments used to determine physical or chemical parameters. Minimally, this will include the dissolved oxygen meter, the temperature sensor and pH meter.

The following measurements comprise the minimum set of parameters to be measured during the course of the bioassay.

PARAMETER	MINIMUM FREQUENCY
Control and treatment:	
temperature	Initial and every 24 hours
dissolved oxygen	Initial and every 24 hours
pH	Initial and every 24 hours
mortalities	Every 24 hours
Control and highest treatment (definitive only):	
hardness	Initial and Final
alkalinity	Initial and Final
Control (screening only):	
hardness	Initial
alkalinity	Initial

Other parameters which may be appropriate, depending on the nature of the waste and test conditions, may also require measurement. These might include:

Total and Unionized Ammonia

Total and Dissolved Metals

Total and Suspended Solids

Electrical Conductivity
Chloride

5.0 DATA REPORTING

Measurements should be recorded on an appropriate data sheet which indicates the calculated LC50, 95 percent confidence limits and method used to calculate them. A sample data sheet is included as Appendix C.

Univariate and multivariate statistical tests based on different models have been applied to bioassay data (Carter and Hubert, 1984; Chew and Hamilton, 1985; McClave, et al., 1981; Volund, 1982) . A microcomputer program, developed by the United States Environmental Protection Agency, to calculate the LC50 and 95 percent confidence limits by the binomial, moving average and probit method is included on an IBM format floppy disk. Some other formats are available on request. Computer programs included in the third edition of Methods for Measuring the Acute Toxicity of Effluents to Aquatic Organisms (U.S. EPA, 1985) should be used with extreme caution. Additional aquatic toxicity programs are available for downloading from the EPA electronic bulletin board in Cincinnati (513) 527-8359. Current hours are:

Monday, Tuesday & Thursday	4:30 PM - 4:30 AM
Wednesday	2:30 PM - 4:30 AM
Friday to Monday	4:30 PM - 4:30 AM

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APPENDIX A. Title 22 Section 66696 of California Code of Regulations defining the toxicity criteria for a hazardous waste.

Article 10. Extremely Hazardous Wastes and Extremely Hazardous Materials

66665. List of Extremely Hazardous Wastes.

NOTE: Authority cited: Sections 208, 25141 and 25150, Health and Safety Code. Reference: Section 25141, Health and Safety Code.

HISTORY:

1. Repealer of Article 10 (Section 66665) filed 9-27-84; effective thirtieth day thereafter (Register 84, No. 41).

Article 11. Criteria for Identification of Hazardous and Extremely Hazardous Wastes

66693. Applicability of Hazardous Waste Criteria.

Any waste which is hazardous pursuant to any of the criteria set forth in this article is a hazardous waste and shall be managed in accordance with the provisions of this chapter.

NOTE: Authority cited: Sections 208, 25141 and 25150, Health and Safety Code. Reference: Section 25141, Health and Safety Code.

HISTORY:

1. New Article 11 (Sections 66693–66721, not consecutive) filed 9-27-84; effective thirtieth day thereafter (Register 84, No. 41).

66694. Sampling and Sample Management.

Sampling and sample management of the wastes and other materials for analysis and testing pursuant to the criteria of this article shall be in accord with the sampling planning, methodology and equipment, and the sample processing, documentation and custody procedures specified in Section One of "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods", SW-846, 2nd edition, U.S. Environmental Protection Agency, 1982.

NOTE: Authority cited: Sections 208, 25141 and 25150, Health and Safety Code. Reference: Section 25141, Health and Safety Code.

HISTORY:

1. Editorial correction filed 10-3-84; designated effective 10-27-84 (Register 84, No. 41).

66696. Toxicity Criteria.

(a) A waste, or a material, is toxic and hazardous if it:

(1) Has an acute oral LD_{50} less than 5,000 milligrams per kilogram; or

(2) Has an acute dermal LD_{50} less than 4,300 milligrams per kilogram; or

(3) Has an acute inhalation LC_{50} less than 10,000 parts per million as a gas or vapor; or

(4) Has an acute aquatic 96-hour LC_{50} less than 500 milligrams per liter when measured in soft water (total hardness 40 to 48 milligrams per liter of calcium carbonate) with fathead minnows (*Pimephales promelas*), rainbow trout (*Salmo gairdneri*) or golden shiners (*Notemigonus crysoleucas*) according to procedures described in Part 800 of "Standard Methods for the Examination of Water and Wastewater (15th Edition)", American Public Health Association, 1981, or by other test methods or test fish approved by the Department, using test samples prepared or meeting the conditions for testing as prescribed in Section 66700(c) and (d), and solubilized, suspended, dispersed or emulsified by the procedures recommended in Part 800 of the cited text or by other methods approved by the Department; or

APPENDIX B. Title 22 Section 66002 of California Code of Regulations defining the acute aquatic 96-hour LC50.

Article 1. Definitions

66002. Acute Aquatic 96-Hour LC_{50}.

"Acute aquatic 96-hour LC_{50}" means the concentration of a substance or mixture of substances in water, in milligrams per liter, which produces death within 96 hours in half of a group of at least 10 test fish.

NOTE: Authority cited: Sections 208, 25141 and 25150, Health and Safety Code. Reference: Section 25141, Health and Safety Code.

HISTORY:

1. New section filed 9-27-84; effective thirtieth day thereafter (Register 84, No. 41). For history of Chapter 30, see Register 79, No. 19.

66004. Acute Dermal LD_{50}.

"Acute dermal LD_{50}" means the dose of a substance or mixture of substances, in milligrams per kilogram of test animal body weight, which, when applied continuously to the bare skin for 24 hours, produces death within 14 days in half of a group of 10 or more rabbits.

NOTE: Authority cited: Sections 208, 25141 and 25150, Health and Safety Code. Reference: Section 25141, Health and Safety Code.

HISTORY:

1. New section filed 9-27-84; effective thirtieth day thereafter (Register 84, No. 41).

66005. Acute Inhalation LC_{LO}.

"Acute inhalation LC_{LO}" means the lowest concentration of a substance or mixture of substances in air, other than acute inhalation LC_{50}, in parts per million by volume if the substance or mixture of substances is a gas or vapor, reported to have caused death in humans or animals.

NOTE: Authority cited: Sections 208, 25141 and 25150, Health and Safety Code. Reference: Section 25141, Health and Safety Code.

HISTORY:

1. New section filed 9-27-84; effective thirtieth day thereafter (Register 84, No. 41).

66006. Acute Inhalation LC_{50}.

"Acute inhalation LC_{50}" means the concentration of a substance or mixture of substances in air, in parts per million by volume if the substance or mixture of substances is a gas or vapor, which when inhaled continuously for 8 hours by a group of 10 or more laboratory white rats, each weighing between 200 and 300 grams, produces death in half the group within 14 days.

NOTE: Authority cited: Sections 208, 25141 and 25150, Health and Safety Code. Reference: Section 25141, Health and Safety Code.

HISTORY:

1. New section filed 9-27-84; effective thirtieth day thereafter (Register 84, No. 41).

66007. Acute LD_{LO}.

"Acute LD_{LO}" means the lowest dose, other than an acute LD_{50}, of a substance or mixture of substances, in milligrams per kilogram body weight introduced orally or dermally over any given period of time in one or more divided portions and reported to have caused death in humans or animals.

NOTE: Authority cited: Sections 208, 25141 and 25150, Health and Safety Code. Reference: Section 25141, Health and Safety Code.

HISTORY:

1. New section filed 9-27-84; effective thirtieth day thereafter (Register 84, No. 41).

APPENDIX C. Sample bioassay data reporting sheet.

State of California
DEPARTMENT OF FISH AND GAME
Fish and Wildlife Water Pollution Control Laboratory
Rancho Cordova, CA 95670

SAMPLE AND BIOASSAY INFORMATION

Static: _____ Cont. flow: _____ Species: _____ WPCL No._____

Screening: _____ Definitive: _____ Common Name: _____ Additional No.: _____

Dilution Water: _____ Length ____mm Weight ____g Collectors No.: _____

Hardness: _____ mg/1 Number per tank: _____ Del'd by: _____

Alkalinity: _____ mg/1 Supplier: _____ Date Rc'd: _____

Tank Volume: _____ liters Acclimation Temp.: _____°C Project: _____

Date	Initial	24-hour	48-hour	72-hour	96-hour
Time					

	DO	°C	pH	DO	°C	pH	#m	DO	°C	pH	#m	DO	°C	pH	#m	DO	°C	pH	#m	MO. Dead
Control																				

Remarks:_____
_____
_____

LCSO: _____ 95% Confidence Limits:_____

LCSO Method: _____ Non-Lineal interpolation: _____ Probit:_____ Moving Average:_____

Analyst: _____ Date/Time: ____/____/_____