California Laboratory Assessment Checklist

Ceriodaphnia dubia Survival & Reproduction (USEPA Method 1002.0)

Laboratory Name: ☐

Location: ☐

ELAP Certificate ID: ☐

Assessment Date(s): ☐

Inspection Type: Renewal ☐ Amendment ☐ Initial ☐ Other ☐

Assessment Agency: ☐

Acronyms Used

ACRONYM	***FULL NAME***
DO	Dissolved Oxygen
ESP	Expert Science Panel
ELAP	Environmental Laboratory Accreditation Program
LOEC	Lowest-Observed-Effect-Concentration
NOEC	No-Observed-Effect-Concentration
OSA	On-Site Assessment
PMSD	Percent Minimum Significant Difference
PT	Proficiency Testing
QM	Quality Manual
RWC	Receiving Water Concentration
SOP	Standard Operating Procedure
V1M7	2016 TNI Standard Volume 1 Module 7
YCT_	Combined yeast-cerophyll-trout chow

DISCLAIMER

This is a guidance checklist and not a regulation. It does not change or substitute for any legal requirement. While ELAP has made every effort to ensure the accuracy of the items in the checklist, the obligations of the regulated community are determined by the relevant statutes and regulations.

Sample Collection

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
		SAMPLE REQUIREMENTS				
8.3.1		If tests are conducted on-site, are daily samples collected for renewals?				
8.3.2, 13.10.8.1		If tests are conducted at an off-site laboratory, are a minimum of three effluent samples collected (preferably on days one, three, and five)?				
8.3.3		Is sufficient sample volume collected to perform the required toxicity and chemical tests?				
8.4		Are tests conducted on receiving water samples? If yes, is the test conducted on either a single grab sample or daily grab sample of receiving water?				
8.5.3		If the effluent has been chlorinated, is total residual chlorine measured immediately following sample collection?				
8.5.4 V1M7 5.8.7.1		Does the maximum holding time of effluents (elapsed time from sample collection to first use in a test) not exceed thirty-six (36) hours?				
8.5.7.1		Are samples collected for off-site toxicity testing chilled to 0-6°C during or immediately after collection, and shipped iced to the performing laboratory?				
8.6.1		Upon arrival, are samples logged in and the temperature measured and recorded?				
8.6.1		Are samples stored at 0-6°C until used?				

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
8.8.3 13.10.1.2.6 13.10.4.1		After warming to test temperature (25 ± 1°C), is DO measured and recorded? If any test solution is supersaturated with oxygen or has a dissolved oxygen concentration below 4.0 mg/L, are test solutions gently aerated before preparing test solutions? **Caution: avoid excessive aeration.**				
8.8.5 13.10.6.1.4		Before test initiation, are (at a minimum) pH, conductivity, total residual chlorine, alkalinity and hardness measured in each new sample (undiluted effluent or receiving water)?				
8.8.6		Where toxicity may be contributed by un-ionized ammonia (i.e., total ammonia ≥5 mg/L), is total ammonia measured in undiluted effluent and receiving water samples?				
8.8.8		Is the sample pH outside the range of 6.0 – 9.0? If yes, are two parallel tests conducted (one with an adjusted pH, and one without an adjusted pH)?				
		DILUTION WATER				
5.4.2.1		Is good quality, laboratory grade deionized water, providing a resistance of 18 megaohm-cm, available and in sufficient quantity for laboratory needs?				
5.4.2.1		Does the laboratory continually or routinely (daily) measure and record the resistance of source water to confirm that it is > 18 megaohm-cm (< 0.056 µS/cm)? (ESP Recommendation)				

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
7.2.1		Opt. 1: Is standard, synthetic dilution water prepared using deionized water and reagent grade chemicals?				
7.2.3.3		Opt. 2: Is the dilution water prepared using 80% deionized water and 20% mineral water such as PERRIER® Water, or equivalent?				
7.3.2		Opt. 3: If receiving water is used for dilution water, is water collected within 96 h of test start and chilled to 0-6°C during or immediately following collection, and maintained at that temperature prior to use in the test (unless the sample is used within 24 h)?				
8.8.5 13.10.6.1.4		Are pH, conductivity, alkalinity and hardness measured after preparation of standard, synthetic dilution water?				
7.5.1		Is the dilution water not held for more than 14 days? (USEPA Method Recommendation)				
		***CERIODAPHNIA DUBIA* FOOD**				
13.6.14.4.3		Is thawed YCT stored in the refrigerator and used for a maximum of two weeks? Is frozen YCT not stored over three months?				
13.6.14.4.4		Is the dry weight of solids in each batch of YCT measured and recorded before use? Should contain 1.7-1.9 g solids/L (USEPA and ESP Recommendation)				
13.6.15.2.3		Does the algae concentrate contain 3.0 to 3.5 X 10^7 cells/mL? Density measured by (select one): o Electronic particle counter o Microscope and hemocytometer o Fluorometer o Spectrophotometer				

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
		***CERIODAPHNIA DUBIA* CULTURES**				
13.6.16 13.6.16.5.1		Does the laboratory maintain mass cultures as a "backup" reservoir of organisms?				
13.6.16.11.1		Does the laboratory document the source of organisms used to start cultures?				
13.6.16.9.4.3		Are mass cultures fed daily at the rate of 7 mL YCT and 7 mL algae concentrate/L culture?				
13.6.16.2 13.6.16.6.1		Does the laboratory maintain individual organisms cultured in 15 mL of culture medium in 30-mL (1 oz) plastic cups or 30-mL glass beakers, with one neonate placed in each cup?				
13.6.16.11.1		Does the laboratory record the rate of reproduction and daily observations of the condition and behavior of the organisms in individual cultures?				
13.6.16.6.3 13.6.16.9.4.4		Are individual organisms fed daily at the rate of 0.1 mL YCT and 0.1 mL algae concentrate per 15 mL culture (either before or after transfer) and transferred to fresh medium a minimum of three times a week?				
13.6.16.6.4		Are new brood boards started weekly, using neonates from adults (< 14 days old) which produce at least eight young in their third or fourth brood?				
13.6.16.8.3		Are clear, double-strength safety glass or 6 mm plastic panels placed on the culture vessels to exclude dust and dirt, and reduce evaporation?				
13.6.16.8.4		Are organisms transferred with a pipet of approximately 2-mm bore, and carefully released under the surface of the water?				

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
		TEST PROCEDURE				
8.10.2 and 13.10.2.1		Are effluent tests conducted with a minimum of 5 effluent concentrations and a control? (USEPA recommends a 0.5 dilution factor). Does each treatment (including the control) have 10 replicates?				
13.10.2.2		Are test chambers randomly assigned to the test board using a template or by using random numbers? Does the laboratory ensure the same template is not used for every test?				
13.10.2.3		Are all neonates used to start each test less than 24 h old and within 8 h of the same age?				
13.6.16.6.1 13.10.2.3		Are all neonates used to start the test obtained from individual cultures (see also 3.6.16.2 above)?				
13.10.2.3		Are the following recorded on test data sheets? ○ Age range of test neonates ○ Source of test neonates ○ Feeding of neonates				
13.10.2.3 13.10.2.4		Does the lab randomly select 10 brood cups, containing adults that have had 8 or more young in their third or subsequent brood, to start the tests?				

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
13.10.2.4		Are the test organisms (neonates) from these 10 brood cups assigned to test chambers using a block randomization procedure, such that offspring from a single female are distributed evenly among the treatments, appearing once in every test concentration?				
13.10.8.2		Are the test organisms transferred to the freshly prepared solutions daily, using a small-bore (2 mm) glass or polyethylene dropper or pipet? Are the animals released under the surface of the water so that air is not trapped under the carapace?				
13.10.3.2		Is the test water temperature maintained at 25 ± 1°C?				
13.10.8.1		Does the lab prepare new test solutions each day for daily test solution renewal?				
8.5.4		Are samples for renewal solutions used no later than 72 h after first use unless a variance has been granted by the NPDES permitting authority?				

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
13.10.5.1		Are organisms fed when the test is initiated, and daily thereafter? Is food added to the new test solutions either immediately before or immediately after the adults are transferred? Does each feeding consist of 0.1 mL YCT and 0.1 mL algae (*S. capricornutum*) concentrate/15 mL test solution? Does the laboratory record the volume dispensed to confirm the targeted food concentrations in the test cups? (ESP Recommendation)				
13.6.16.11.1		Does the laboratory document the type of food and feeding times?				
13.10.6.1.1 13.10.6.1.2		Is temperature, DO, and pH measured at the beginning and end of each 24-h exposure period in at least one test chamber at each test concentration and in the control?				
13.10.6.1.4		Are conductivity, alkalinity and hardness measured in each new sample (3 samples collected/test for off-site testing, or daily samples for on-site) and in the control?				
13.10.6.2.3		Are the number of live young recorded each day when the live adults are transferred to fresh test solutions?				
13.10.6.2.2		Are partial broods released over a two-day period documented and counted as one brood? (ESP and USEPA Method Recommendation)				
13.10.6.2.3		Are the young discarded after counting?				

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
13.10.9.1		Is the test terminated when 60% of the control organisms have had at least 3 broods, or at the end of 8 days, whichever comes first? Is this decision made daily in 24-h increments, within a 2-h window (i.e., +/- 1 h of test initiation time)? (ESP Recommendation)				
13.10.9.2		At test termination, are the number of live young counted and recorded?				
13.10.9.1		Does the laboratory ensure offspring from fourth or higher broods are not counted and not included in the total number of neonates produced during the test? (USEPA Method Recommendation)				
13.12.1, 13.13.1.4		Are tests considered valid only if?: o At least 80% of all control organisms survive, and o 60% of surviving control females produce at least three broods, with an average of 15 or more young per surviving female, and o If more than 7 replicates in the control remain after excluding males and blocks with 50% or more of surviving organisms identified as males.				
10.2.8.2		When NPDES permits require sublethal hypothesis testing endpoints (e.g., growth or reproduction NOECs and LOECs), is within-test variability (PMSD) reviewed, and does the test PMSD exceed 47?				

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
10.2.8.2.4.1		If the test PMSD exceeds 47 and if toxicity is found at the permitted receiving water concentration (RWC) based upon the value of the NOEC or LOEC, are the results deemed acceptable?				
10.2.8.2.4.2		If the test PMSD exceeds 47 and toxicity is not found at the permitted RWC based upon the value of the effect concentration estimate (NOEC or LOEC), are the results deemed not acceptable, and a new test conducted promptly on a newly collected sample?				
		REFERENCE TOXICANT TESTS and CONTROL CHARTS				
4.7.2		Does the laboratory perform at least one acceptable reference toxicant test per month when tests are performed monthly or more frequently?				
4.7.2		Does the laboratory perform at least one acceptable reference toxicant test concurrently with each effluent toxicity test when tests are conducted only monthly, or less frequently?				
10.2.7.1		Are the following reviewed to verify that the reference toxicant test was valid? ○ Test acceptability criteria ○ Test conditions ○ Concentration-response relationship ○ Test sensitivity (PMSD)				
10.2.7.1, § 64802.15 (*l*)(3)		Does the laboratory plot reference toxicant results on control charts for both survival and reproduction?				

Reference	SOP/QM§	Does the laboratory comply with this section?	Yes	No	N/A	Observations
4.16.2		For endpoints that are point estimates (LC50s and IC25s), are the cumulative mean ($\bar{X}$) and upper and lower control limits (± 2S and ± 3S) re-calculated with each successive test result?				
4.16.4		If two or more consecutive tests do not fall within the ± 2S control limits, are the results explained and is the reference toxicant test immediately repeated? Are actions taken to correct the problem reported?				
4.16.5		When a result from a reference toxicant test is outside the 99% confidence intervals (± 3S), does the laboratory conduct an immediate investigation to assess the possible causes for the outlier?				