i. Satisfies the requirements of N.J.A.C. 7:18–3.3(a)2;

ii. Has a range of at least 0 to 40 grams;

iii. Is readable within 0.1 grams;

iv. Provides reproducibility of at least ±0.05 grams.

6. Laboratories performing acute toxicity testing shall have and use one or more pH meters that satisfy the requirements of N.J.A.C. 7:18–3.3(a)3.

7. Laboratories performing acute toxicity testing shall have and use one or more conductivity instruments that satisfy the requirements of N.J.A.C. 7:18–3.3(a)6.

8. Laboratories performing acute toxicity testing shall have and use one or more dissolved oxygen meters that satisfy the requirements of N.J.A.C. 7:18–5.2(a)17.

9. Laboratories performing tests with Cladoceran, shall have the following equipment:

i. A light meter capable of measuring in Lux or footcandles in the range of at least 0 to 200 footcandles;

ii. Medicine droppers or pipettes with 1.0 to 3.0 mm bores;

iii. Borosilicate glass beakers with covers, or test chambers made of another approved material under (a)1 above; and

iv. All testing equipment to be constructed with materials as approved for invertebrates in (a)1 above.

10. A laboratory shall have a refrigerator that is capable of storing the required sample volumes and that satisfies the requirements of N.J.A.C. 7:18–3.3(a)7.

11. Laboratories performing zooplankton or macrocrustacean toxicity tests shall have and use a low-power magnification device, for working with invertebrate species.

12. A laboratory shall use only glassware, plasticware and metal utensils that satisfy the requirements of N.J.A.C. 7:18–3.3(a)8. The laboratory shall use plasticware only if it is made of inert, nontoxic materials approved under (a)1 above. When manually establishing test solutions, the laboratory shall use Class "A" volumetric flasks or graduated cylinders, calibrated "to deliver."

13. Dilution water sample containers used by the laboratory for discrete samples shall meet the following requirements:

i. The laboratory shall use only wide-mouthed containers equipped either with stoppers, screw caps or an equivalent closure;

ii. The laboratory shall use only containers and cap liners constructed of materials approved under (a)1 above; and

iii. The laboratory shall clean each container after each use, in accordance with N.J.A.C. 7:18–7.4(c).

14. A laboratory performing discrete effluent sampling shall use containers meeting the following requirements:

i. The laboratory shall use either wide-mouthed glass containers, disposable unplasticized plastic containers, or disposable unplasticized plastic liners for containers that are leakproof and constructed of materials meeting the requirements of (a)1;

ii. The laboratory shall not reuse containers made of materials listed in (a)1ii above unless they have been cleaned in accordance with N.J.A.C. 7:18–7.4(c);

iii. The laboratory shall discard after one use any containers made of materials specified in (a)1iii above, and not cleaned and reused unless the laboratory has demonstrated pursuant to (a)1iii above that the container can be decontaminated without significant degradation;

iv. Container closures shall be leakproof and constructed of materials meeting the requirements of (a)1 above; and

v. The laboratory shall store containers in a manner that prevents contamination.

Administrative change.
See: 28 N.J.R. 4098(a).
Amended by R.1997 d.192, effective May 19, 1997.
See: 28 N.J.R. 4149(a), 29 N.J.R. 2275(a).
Rewrote (a)1vii(1); and in (a)9, deleted reference to labs performing tests with *Daphnia sp.*

7:18–7.4 General laboratory procedures

(a) A laboratory performing acute toxicity tests shall have available and use glass-distilled or deionized water, referred to in this chapter as laboratory pure water, that satisfies the following requirements:

1. The laboratory pure water shall have conductivity of less than 1.0 micromho/cm at 25 degrees Celsius, and shall not contain any of the constituents listed in Table 7.4(a) in a concentration greater than or equal to the limit specified in Table 7.4(a).

Table 7.4(a)
Constituents in Laboratory Pure Water

Constituent	Limit
Arsenic, Chromium(VI) and Nickel	10.0 µg/L each
Total Organic Carbon (TOC)	2.0mg/L
Fluoride	100 µg/L
Un-ionized Ammonia	12.5 µg/L
Lead and Copper	5.0 µg/L each
Silver	2.0 µg/L
Mercury	0.30 µg/L
Total Residual Chlorine	0.5mg/L
Cadmium	1.0 µg/L
Aldrin	0.03 µg/L

Constituent	Limit
Chlordane	0.5 μg/L
DDT and DDE pesticides	0.13 μg/L each
Dieldrin	0.05 μg/L
Endosulfan I & II	0.06 μg/L each
Endrin	0.10 μg/L
Heptachlor	0.09 μg/L
Lindane	0.08 μg/L
PCBs (as PCB 1242)	0.07 μg/L
Toxaphene	1.00 μg/L
Standard (Heterotrophic) Plate Count	100 colony forming units (CFU)/100 mL
Bacteriological Water Suitability Test	0.8-3.0 Ratio
Total Solids	10 mg/L

2. The laboratory shall have the laboratory pure water analyzed at least monthly for conductivity or resistivity, and for total residual chlorine. The laboratory shall document the results.

3. The laboratory shall have the laboratory pure water analyzed at least semi-annually for standard plate count, and at least annually for TOC, total solids, fluoride, un-ionized ammonia, arsenic, hexavalent chromium, copper, lead, nickel, cadmium, mercury, silver, bacteriological water suitability test, all listed pesticides, and PCBs. The laboratory shall document the results.

(b) A laboratory performing acute toxicity tests shall have available and use a supply of water of constant quality for the holding, spawning, and rearing of aquatic organisms, referred to in this subchapter as laboratory grade water. The laboratory may reconstitute the laboratory grade water from laboratory pure water or obtain it from a natural source. The laboratory shall use only laboratory grade water that satisfies the following requirements:

1. The laboratory grade freshwater supplies shall be constant in quality and shall not contain any of the constituents listed in Table 7.4(b)1 in a concentration greater than the limit specified in Table 7.4(b)1.

Table 7.4(b)1
Constituents in Laboratory Grade Freshwater

Constituent	Limit
Salinity	3.5 parts per thousand (ppt)
Suspended solids	80 mg/L
TOC	10 mg/L
Un-ionized ammonia	12.5 μg/L
Total residual chlorine	0.5 μg/L
Aldrin	3.0 μg/L
Chlordane	0.5 μg/L
DDT & DDE	0.13 μg/L each
Dieldrin	0.05 μg/L
Endosulfan I & II	0.06 μg/L each
Endrin	0.10 μg/L
Heptachlor	0.09 μg/L
Lindane	0.08 μg/L
PCBs (as PCB 1242)	0.5 μg/L
Toxaphene	1.00 μg/L
Fluoride	100 μg/L
Antimony	146 μg/L
Arsenic	40.0 μg/L
Cadmium	e(0.7852 [In (Hardness)]-3.49) μg/L
Hexavalent chromium	11 μg/L
Copper	e(0.8545 [In (Hardness)]-1.465) μg/L
Lead	e(1.273 [In (Hardness)]-1.460) μg/L
Mercury	0.30 μg/L
Nickel	e(0.84 [In (Hardness)]-1.1645) μg/L
Selenium (recoverable inorganic selenite)	35 μg/L
Silver	e(1.72 [In (Hardness)]-6.52) μg/L
Zinc	e(0.8473 [In (Hardness)] + 0.7614) μg/L

2. The laboratory grade saltwater supplies shall be constant in quality, have a salinity greater than 3.5 ppt with a range favorable to the test organisms, and shall not contain any of the constituents listed in Table 7.4(b)2 in a concentration greater than the limit specified in Table 7.4(b)2.

Table 7.4(b)2
Constituents in Laboratory Grade Saltwater

Constituent	Limit
Suspended solids	80 mg/L
TOC	10 mg/L
Un-ionized ammonia	12.5 μg/L
Aldrin	1.3 μg/L
Chlordane	0.5 μg/L
DDT & DDE	0.13 μg/L each
Dieldrin	0.05 μg/L
Endosulfan I & II	0.05 μg/L each
Endrin	0.10 μg/L
Heptachlor	0.09 μg/L
Lindane	0.08 μg/L
PCBs (as PCB 1242)	0.5 μg/L
Toxaphene	1.0 μg/L
Fluoride	1400 μg/L
Antimony	146 μg/L
Arsenic	136 μg/L
Cadmium	2.0 μg/L
Hexavalent chromium	50 μg/L
Copper (dissolved)	2.9 μg/L
Lead	5.6 μg/L
Mercury	0.2 μg/L
Nickel	8.3 μg/L
Selenium (recoverable inorganic selenite)	54 μg/L
Silver	5.0 μg/L
Zinc	86 μg/L

3. The laboratory shall have the laboratory grade freshwater and saltwater analyzed at least monthly for pH, salinity, alkalinity, and un-ionized ammonia. Suspended solids should be analyzed monthly.

4. The laboratory shall have the laboratory grade freshwater analyzed at least monthly for total residual chlorine and total hardness.

5. The laboratory shall have the laboratory grade waters analyzed at least semi-annually for TOC, all listed pesticides, PCBs, fluoride, and all trace elements and metals specified in N.J.A.C. 7:18–7.4(b)1 for freshwater and 2 for saltwater.

6. The laboratory shall document the analyses performed under (b)3, 4 and 5 above.

7. A source of laboratory grade fresh water shall be considered to be of constant quality if the monthly ranges of total hardness, alkalinity, conductivity, and salinity are less than 10 percent of the average values, and the pH range is less than 0.4 standard units.

8. No adjustment to the salinity of a natural saltwater shall be made except, when necessary, as follows:

i. To reduce the salinity of the water, the laboratory may add either laboratory pure water or laboratory grade freshwater; or

ii. To increase the salinity, the laboratory may add hypersaline brine prepared in accordance with the procedure specified in the NJDEPE, "Standardized Culturing Method for the Sheepshead Minnow, Cyprinodon variegatus," #CM004, commercial dry sea salts, or a strong solution of artificial laboratory grade saltwater.

9. Before using laboratory grade saltwater obtained from natural sources to culture invertebrate species, the laboratory shall filter the water through a filter no larger than 20 microns.

(c) A laboratory performing acute toxicity tests shall clean the equipment and containers used in the tests, pursuant to the procedures listed in (c)1 through 3 below.

1. The laboratory shall clean all new materials and containers, except for approved materials marked and sold as "Medical Grade" or "Food Grade," using the procedures in N.J.A.C. 7:18–7.3(a)1vii.

2. The laboratory shall clean all reusable test vessels, sample containers, toxicant delivery systems, and any other equipment used in testing that has come in contact with a toxicant or effluent. To clean the equipment, the laboratory shall:

i. Scrub in a one percent solution, preferably 50 degrees Celsius or warmer, of a non-toxic, phosphate free, synthetic laboratory detergent, such as Linbro 7X® tissue cleaning agent, and tap water;

ii. Rinse three times in hot tap water;

iii. For organic contamination or stains that are not removed after using the procedures in (c)2i and ii above, rinse or soak in a 200 mg/L solution of sodium hypochlorite. Do not use acid and hypochlorite together;

iv. Rinse the equipment three times with laboratory pure water;

v. To remove heavy metal contamination, soak smaller equipment or containers in freshly prepared five percent by volume or stronger HCl for at least one hour. Rinse equipment or containers too large to soak twice with fresh five percent by volume or stronger HCl;

vi. Rinse at least three times in laboratory pure water;

vii. Rinse twice with fresh 100 percent acetone followed by two rinses with 100 percent methanol;

viii. Rinse three times with laboratory pure water; and

ix. Either air or oven dry.

3. After each use, the laboratory shall clean all reusable glassware, tanks, containers, and equipment used for culturing and for dilution water sampling and delivery for testing. To clean the equipment, the laboratory shall:

i. Scrub in a one percent solution, preferably 50 degrees Celsius or warmer, of non-toxic, phosphate free, laboratory detergent, such as Linbro 7X® tissue culture cleaning agent, and either laboratory grade freshwater or tap water;

ii. If contamination with disease or parasites is suspected, disinfect the tanks, equipment and containers by either of the following:

(1) Soak for at least one hour with either a 200 mg/L solution of sodium hypochlorite or a 0.5 percent solution of commercial chlorine bleach; or

(2) Rinse with either a 200 mg/L solution of sodium hypochlorite or a 0.5 percent solution of commercial chlorine bleach; or;

(3) Autoclave at a temperature of 121 degrees Celsius and a pressure of 1.1 lb. per cm^2 (15 psi) for 15 minutes;

iii. If not autoclaving, rinse at least three times with either hot laboratory grade fresh water or tap water; and

iv. Rinse at least three times with laboratory pure water;

(d) A laboratory performing acute toxicity tests shall use only organisms approved by the Department and identified to species using systematic keys appropriate for the test organism. The approved test organisms for acute toxicity testing are as follows:

1. If the receiving water immediately downstream of the discharge being tested has a natural salinity of less than or equal to 3.5 parts per thousand (ppt) at mean high tide, the laboratory shall use the following freshwater organisms as specified in the applicable NJPDES permit:

i. The following species of cold-water fishes:

(1) Rainbow trout—*Oncorhynchus mykiss*

(2) Brown trout—Salmo trutta;

(3) Brook trout—Salvelinus fontinalis.

ii. The following species of warmwater fishes:

(1) Fathead minnow—*Pimephales promelas*

(2) Bluegill—*Lepomis macrochirus*

iii. The following invertebrate species of freshwater zooplankton:

(1) Cladoceran:

(A) *Daphnia magna;*

(B) *Daphnia pulex*;

(C) *Ceriodaphnia dubia.*

2. If the receiving water immediately downstream of the discharge being tested has a natural salinity, at mean high tide, of greater than 3.5 ppt, or if the receiving water is a marine water (that is, a tidal saltwater), the laboratory shall use the following saltwater organisms as specified in the applicable NJPDES permit:

i. The following estuarine and marine species of saltwater fishes:

(1) Sheepshead minnow—*Cyprinodon variegatus*;

(2) Tidewater silverside—Menidia peninsulae;

(3) Atlantic silverside—Menidia menidia;

(4) Inland silverside—Menidia beryllina.

ii. The following marine and estuarine invertebrate species of saltwater macrocrustaceans:

(1) Grass shrimp—*Palaemonetes pugio*

(2) Mysid—*Mysidopsis bahia*

(e) A laboratory performing acute toxicity tests shall prepare test organisms in accordance with the following requirements:

1. All organisms used in a test shall be from the same source, the same age group or life stage, and the same species.

i. All fish shall be from the same year class and the total length of the longest fish shall not be more than twice that of the shortest fish. The laboratory shall make the total length measurements either upon a 10 percent sample of each group of organisms used for a test, or upon all of the surviving control test organisms after a test.

ii. The laboratory shall use test organisms collected from the sources listed in (e)1ii(1) through (4) below.

(1) Cladoceran used for toxicity tests shall be reared in the testing facility from laboratory cultures;

(2) Warm-water, estuarine and marine fishes and macrocrustaceans shall be obtained from commercial suppliers, hatcheries, or laboratory cultures. If such fishes or macrocrustaceans are not available from any such sources, they may be obtained from the wild;

(3) Cold-water fishes shall be obtained from commercial suppliers or hatcheries, certified disease-free (free of infections, pancreatic necrosis, furunculosis, kidney disease, and whirling disease);

(4) The laboratory shall not use organisms captured by the use of electroshocking, chemical treatment, and gill nets for either toxicity testing or culture brood.

iii. The laboratory shall determine the age of test organisms at the beginning of a toxicity test. The age of the test organisms shall satisfy the following requirements:

(1) Daphnia magna or D. pulex shall be neonates between one and 24 hours old;

(2) *Ceriodaphnia dubia* shall be less than 24 hours old;

(3) Mysidopsis sp shall be between one and five days old, and no more than a 24 hour range in age;

(4) Pimephales promelas and Lepomis macrochirus shall be one to 14 days old, and no more than a 24 hour range in age;

(5) The coldwater fishes shall be:

(A) Oncorhynchus mykiss—15 to 30 days (after yolk sac absorption to 30 days)

(B) Salvelinus fontinalis—30 to 60 days

(C) Salmo trutta—30 to 60 days

(6) Cyprinodon variegatus shall be one to 14 days old, and no more than a 24 hour range in age;

(7) Menidia menidia, M. peninsulae, and M. beryllina shall be nine to 14 days old, and no more than a 24 hour range in age; and

(8) Palaemonetes pugio shall be one to 60 days old.

2. The laboratory shall satisfy the following requirements in collecting test organisms for use in toxicity testing:

i. If using laboratory-reared specimens, report the original source and strain;

ii. If collecting organisms from the wild, or obtaining organisms from a commercial supplier or hatchery, report the time, place and method of collection, transportation, and handling;

iii. Do not collect organisms from areas known to be polluted;

iv. Do not collect organisms in poor condition, such as organisms that are diseased, parasitized, or exhibit deformities;

v. Collect macrocrustaceans and smaller fishes (with a total length of less than 30 mm) near shore using dip nets or coarse plankton nets, or by hand. Collect larger specimens in seines. If the specimens are located offshore then trawls shall be used.

(1) To prevent organisms from being damaged during collection, short hauls with a duration of 10 minutes or less shall be made with seines or trawls. The nets shall not collect debris that will injure the organisms;

(2) The seine bag shall be left in the water at the end of a haul. Organisms shall be dipped with a container from the bag and transferred directly to prepared holding tanks. Do not collect allow overcrowding of the animals. When trawling, bring the trawl up to the boat and over the side quickly without letting the catch hit the side of the boat. Immerse the portion of the net with the catch in it in a tank of water. Open the trawl, dip out organisms with a container or a small mesh hand net, and transfer to a holding tank;

(3) The water temperature, salinity, dissolved oxygen, and pH shall be determined at the collection site and recorded in a log. During transport to and acclimation in the laboratory, the organism holding tanks shall be aerated to ensure dissolved oxygen levels at or near saturation. Dissolved oxygen levels in the holding tanks shall not fall below 60 percent saturation. The holding tank water temperature shall be maintained within ± three degrees Celsius of the temperature of the water at the collection site at the time of collection for at least 24 hours;

(4) When collecting freshwater fish, between 0.1 and 0.3 percent table salt (NaCl) should be added to the holding tank water prior to the introduction of the collected specimens;

(5) Prophylactic treatments with antibiotics shall not be used; and

(6) Collected organisms shall be observed for injury. Injured organisms shall be discarded.

3. The laboratory shall use only test organisms that have been held, handled, and conditioned in accordance with the following requirements:

i. All field collected organisms shall be quarantined for at least fourteen days to observe for parasites and diseases, and to recover from the stress of collection and transport. Test organisms obtained from a culture source with demonstrated ability to supply healthy, disease-free stock shall be quarantined for at least two days. Organisms in culture in the testing facility do not need to be quarantined before use in a toxicity test. A log shall be kept documenting the test organism quarantine procedures used, recording the observations (physical measurements and biological) made, and recording any mortality;

(1) If during quarantine more than 10 percent of the organisms either die within two days of their arrival in the laboratory or if they suffer from parasites or diseases that cannot be controlled, the entire batch of organisms shall be destroyed. All containers and equipment that came in contact with the organisms shall be cleaned and sterilized before reuse by the procedures specified in (e)3i(2) below.

(2) To sterilize tanks, containers or equipment, the laboratory shall use at least a one-hour soaking in either a 200 mg/L sodium hypochlorite solution or a 0.5 percent solution of commercial chlorine bleach. The residual chlorine shall be removed by rinsing at least three times with either laboratory grade or laboratory pure water. Disinfection by autoclaving shall also be acceptable as specified in (c)3ii(3) above.

ii. After the quarantine period, disease-free organsims shall be acclimated to laboratory grade water and temperature, or to test dilution water and test temperature.

(1) Acclimation of fish and grass shrimp to either laboratory grade water or test dilution water shall be done by gradually and incrementally making no more than a 50 percent tank volume exchange of water in each holding tank per 12 hours over a 24 hour period;

(2) Mysids are collected from gravid females held in culture water at a salinity within ± two ppt of the dilution water to be used in the test and Cladoceran are transferred from stock cultures held in laboratory grade water to the test dilution water. No other acclimation would be necessary for these organisms;

(3) Changes in water temperature shall not exceed three degrees Celsius within a 24–hour period.

(4) Changes in salinity during acclimation shall not exceed three ppt in a 12–hour period.

iii. Organisms used in range-finding toxicity tests do not have to be acclimated to the test dilution water and test temperature prior to use in a test; the organisms shall have been acclimated to laboratory grade water and laboratory temperature for at least two days, in accordance with the procedures in (e)3ii(1) through (4) above.

iv. Organisms to be used in N.M.A.T. or N.O.A.E.C.* definitive and definitive acute toxicity tests shall be acclimated to the test dilution water and the test temperature prior to their use in the toxicity test. Acclimation shall be performed in accordance with the criterion stated in (e)3ii(1) through (4) above. If the

organisms were held in laboratory grade water, and the laboratory grade water is to be used as test diluent water, and the holding temperature is identical to the test temperature, then acclimation is not necessary.

v. After the test organisms are acclimated to laboratory grade water and laboratory temperature, or to the test temperature and dilution water, the laboratory shall hold the test organisms under conditions of salinity and temperature that do not change more than specified in (e)3ii(3) and (4) above, for the following periods:

(1) Fish and grass shrimp shall be held for at least 24 hours prior to use in a test; and

(2) Cladoceran and Mysids do not have to be held any additional time prior to use in a test.

vi. If more than five percent of a group of test organisms dies during the acclimation and holding period, the laboratory shall take the following steps:

(1) For Cladoceran or Mysids, discard the group, and acclimate and hold a new group; and

(2) For fish or grass shrimp, either discard the group or hold it for an additional ten days in the test dilution water and at test temperature. If mortality for the group of organisms is more than three percent during the final 48 hours of the additional 10 days of holding, discard the entire group, and acclimate and hold a new group.

vii. The laboratory shall satisfy the following requirements in handling organisms:

(1) Follow culturing activities and procedures designed to minimize handling;

(2) Discard organisms that touch dry surfaces, are dropped, or are injured during handling;

(3) Do not use dip nets made of small mesh netting or cloth for organisms smaller than 0.01 grams each. Handle organisms smaller than 0.01 grams by a large-bore pipette;

(4) Use fire-polished smooth glass tubes or large-bore pipettes for transferring Cladoceran and Mysid;

(5) Clean and sanitize nets and other equipment used for handling organisms between uses;

(6) Analysts shall wash their hands with detergent leaving no toxic residue before handling or feeding organisms;

(7) Maintain dissolved oxygen concentrations in containers for holding fishes, mysids or grass shrimp between 60 percent and 100 percent of saturation. If there is danger of supersaturation with gases, keep the water in an open system, passed over baffles or otherwise aerated to bring it into equilibrium with the air;

(8) Thoroughly clean tanks and equipment regularly, removing or flushing out excessive growths and wastes;

(9) Remove all uneaten food from tanks and containers within 24 hours of feeding;

(10) Cover tanks and containers to prevent organisms from jumping out, unless the nature of the organism and the distance between the top of the water and the top of the container make it unlikely that the organisms can jump out;

(11) Shield tanks and containers to protect organisms from nearby movements and noise;

(12) In flow-through holding tanks without any form of biofiltration, maintain an exchange rate of at least two tank-volumes per 24 hours;

(13) In holding tanks with recirculation systems, maintain a flow of water through the biofiltration systems sufficient to ensure removal of excreted nitrogen compounds and excess suspended solids;

(14) Shrimp and bottom-dwelling fish may be provided with either a silica sand substrate or an oyster shell/crushed coral substrate in the holding tanks;

(15) Feed Cladoceran and coldwater freshwater fish until the beginning of a test but not during the test. Feed mysids and grass shrimp before and during a test. Feed all warmwater freshwater and all saltwater fish before the beginning of the test and at two hours prior to the 48 hour renewal;

(16) Each day during holding and acclimation, observe organisms carefully for signs of disease, stress, damage, and mortality. Record observations in a log. Discard injured, dead and abnormal individuals; and

(17) Do not use organisms used in a test (including those used in a control treatment) in a subsequent test, or as culture stock.

4. The laboratory shall comply with the following procedures when culturing test organisms:

i. Maintain a daily log of organism feeding, behavioral observations, treatments, and mortalities;

ii. Feed all organisms, except for Cladoceran, at least once per day;

iii. Destroy zooplankton and saltwater macrocrustaceans that become diseased or infested. If fishes are treated to either prevent or cure diseases, fungal infections or parasitic infections, with any material other than table salt (NaCl), the laboratory shall:

(1) If contamination with disease or parasites is suspected, disinfect the tanks, equipment and containers by one of the following:

(A) Soak for at least one hour with either a 200 mg/L solution of sodium hypochlorite or a 0.5 percent solution of commercial chlorine bleach, and then rinse at least three times with laboratory grade or pure water; or

(B) Rinse with either a 200 mg/L solution of sodium hypochlorite or a 0.5 percent solution of commercial chlorine bleach and then rinse at least three times with laboratory grade or pure water; or

(C) Autoclave using the procedures specified in (c)3ii(3) above;

(2) The laboratory shall not use in toxicity tests fish from tanks contaminated with parasites or disease, until:

(A) Seven days since the contamination have elapsed, and there is no evidence of disease; and

(B) Ten days have elapsed after all treatments are stopped.

iv. The Department recommends that a laboratory culturing test organisms use the applicable method listed in (e)4iv(1) through (7) below.

(1) The Department recommends that a laboratory culturing Oncorhynchus mykiss, Salmo trutta, or Salvelinus fontinalis do so in accordance with "Standardized Culturing Methods for Cold-water Fishes," NJDEPE—#CM001.

(2) The Department recommends that a laboratory culturing Pimephales promelas do so in accordance with "Standardized Culturing Methods for the Fathead Minnow, Pimephales promelas" NJDEPE—#CM002.

(3) The Department recommends that a laboratory culturing Daphnia magna or D. pulex do so in accordance with "Standardized Culturing Methods for Daphnia magna and Daphnia pulex and Ceriodaphnia dubia," NJDEPE—#CM003.

(4) The Department recommends that a laboratory culturing Cyprinodon variegatus do so in accordance with "Standardized Culturing Methods for the Sheepshead Minnow," NJDEPE—#CM004.

(5) The Department recommends that a laboratory culturing Palaemonetes pugio shall do so in accordance with "Standardized Culturing Methods for Grass Shrimp," NJDEPE—#CM005.

(6) The Department recommends that a laboratory culturing Menidia menidia, M. beryllina, or M. peninsulae do so in accordance with "Standardized Culturing Methods for the Atlantic, Tidewater, and Inland Silversides," NJDEPE—#CM006.

(7) The Department recommends that a laboratory culturing Mysidopsis bahia do so in accordance with "Standardized Culturing Methods for Mysid Shrimp," NJDEPE—#CM007.

Administrative change.
See: 28 N.J.R. 4098(a).
Amended by R.1997 d.192, effective May 19, 1997.
See: 28 N.J.R. 4149(a), 29 N.J.R. 2275(a).
In (d)1iii, amended species list; in (e)1ii(1), (e)3ii(2), (e)3v(2), (e)3vi(1), (e)3vii(4) and (15), and (e)4ii, deleted reference to Daphnids preceding reference to Cladoceran; and in (e)3iv, inserted "N.O.A.E.C.".

7:18–7.5 Acute toxicity testing methodology

(a) A laboratory shall not use an acute toxicity test experimental design unless it satisfies all applicable requirements of this section.

(b) When the purpose of a definitive acute toxicity test is to determine compliance with an LC_{50} or EC_{50} permit limitation, the test shall satisfy all of the following requirements:

1. The test shall include at least one control treatment, and a series of at least five effluent concentrations;

2. The laboratory shall perform each control treatment and each effluent concentration at least in duplicate, and shall conduct additional replicate series when necessary to achieve required test precision. The laboratory shall use only true replicates, with no water connections between test chambers;

3. If the toxicity of the effluent to the test organism is not known, the laboratory shall select concentrations that are evenly spaced on either a logarithmic scale or a geometric scale. The concentration of effluent in each treatment (except for the highest concentration and each control) shall be at least 50 percent of the next highest one;

4. If the toxicity of the effluent to the test organism is known approximately, the laboratory shall select concentrations of effluent that are evenly spaced (on either a logarithmic scale or geometric scale) around the expected LC_{50} or EC_{50}. Except for the highest concentration and each control(s), the test concentration shall be at least 60 percent of the next higher one. The use of a 100 percent effluent concentration is not required where the inclusion of such concentration is not within the expected range of the LC_{50};

5. Every toxicity test shall include a dilution water control treatment consisting of the same dilution water, conditions, procedures, type and number of organisms as used in the effluent treatments, except that the laboratory shall add none of the effluent being tested to the dilution water. Whenever the laboratory uses artificial sea salts to adjust the salinity of either the dilution water sample or effluent sample, an additional control treatment shall be included. This additional control treatment shall consist

of replicate chambers containing only artificial saltwater, made with the same artificial sea salts used to adjust the samples. The artificial saltwater shall be made to the same standardized salinity and pH as the other test treatments; and

6. The laboratory shall expose at least 20 test organisms to each effluent concentration and each control treatment. For example, when the laboratory is conducting the test in duplicate, it shall expose at least 10 organisms per test chamber. The number of organisms used in each effluent concentration shall be equal to the number used in other effluent concentrations and to the number used in the control.

(c) When the effluent is known to generally have an LC of greater than or equal to 100 percent and the laboratory is conducting an N.M.A.T. definitive acute toxicity test for determining compliance with a "no measurable acute toxicity" permit limitation, the toxicity test design shall meet the following requirements:

1. The test series shall consist of at least one control treatment, and a series of at least five effluent concentrations;

2. The laboratory shall perform each control treatment and each effluent concentration at least in duplicate, and shall conduct additional replicate series when necessary to achieve required test precision. The laboratory shall use only true replicates, with no water connections between test chambers;

3. The laboratory shall expose at least 20 test organisms to each effluent concentration and each control treatment. For example, when the laboratory is conducting the test in duplicate, it shall expose at least ten organisms per test chamber. The number of organisms used in each effluent concentration shall be equal to the number used in other effluent concentrations and to the number used in the control; and

4. Every toxicity test shall include a dilution water control treatment consisting of the same dilution water, conditions, procedures, type and number of organisms as used in the effluent treatments, except that the laboratory shall add none of the effluent being tested to the control treatment. Whenever the laboratory uses artificial sea salts to adjust the salinity of either the dilution water sample or the effluent sample, an additional control treatment shall be included. This additional control treatment shall consist of replicate chambers containing only artificial saltwater, made with the same artificial sea salts used to adjust the samples. The artificial saltwater shall be made to the same standardized salinity as the other test treatments.

(d) When there is no historical aquatic toxicological data available on an effluent, the laboratory shall conduct a range-finding toxicity test to ascertain the range of effluent concentrations for subsequent definitive tests. The range-finding toxicity test shall satisfy the following requirements:

1. The range-finding toxicity test shall consist of one or more control treatments, and at least four treatments which shall include a 100 percent effluent-by-volume, 50 percent effluent-by-volume, and 12.5 percent effluent-by-volume. The laboratory shall use either a single test series or replicates;

2. Every range-finding test shall include a dilution water control treatment. This treatment shall consist of the same dilution water, conditions, procedures, type and number of organisms as used in the effluent treatment, except that none of the effluent being tested shall be added to the dilution water; and

3. Five or more test organisms shall be exposed to each control treatment and each effluent treatment.

(e) The laboratory shall conduct tests as either static, renewal, or flow-through tests in accordance with the following:

1. The laboratory shall conduct the following as either a renewal test or a flow-through test:

i. Any definitive toxicity test with cold-water fishes, warm-water fishes, saltwater fishes or saltwater macrocrustaceans; and

ii. Any N.M.A.T. or N.O.A.E.C. definitive toxicity test with cold-water fishes, warm-water fishes, saltwater fishes or saltwater macrocrustaceans;

2. The laboratory shall conduct as either a static test or a flow-through test any range-finding toxicity test with coldwater fishes, warmwater fishes, saltwater fishes or saltwater macrocrustaceans; and

3. The laboratory shall conduct the following as a static test:

i. Any definitive toxicity test with freshwater zooplankton;

ii. Any N.M.A.T. or N.O.A.E.C. definitive toxicity test with freshwater zooplankton; and

iii. Any range-finding toxicity test with freshwater zooplankton.

(f) The laboratory shall conduct toxicity tests for the durations described below:

1. Cladoceran range-finding toxicity test duration shall be at least 24 hours;

2. Cladoceran definitive toxicity test, N.O.A.E.C. and N.M.A.T. definitive toxicity test durations shall be 48 hours;

3. Mysid range-finding toxicity test duration shall be at least 24 hours;

4. Mysid definite toxicity test, N.O.A.E.C. and N.M.A.T. definitive toxicity test durations shall be at least 96 hours;