NATIONAL ENVIRONMENTAL LABORATORY ACCREDITATION CONFERENCE (NELAC)

ON-SITE LABORATORY ASSESSMENT

TOXICITY TESTING CHECKLIST (22 PAGES TOTAL)

LABORATORY: _____

Physical Address: _____

_____

Mailing Address: _____
(if different from above)

_____

Telephone Number: _____ Facsimile Number: _____

E-mail address: _____

INSPECTED BY: (Name) (Affiliation)

_____ _____

_____ _____

_____ _____

INSPECTION DATES: _____

LABORATORY TECHNICAL DIRECTORS AND MANAGEMENT:

(Name) (Title)

_____ _____

_____ _____

_____ _____

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GENERAL INSTRUCTIONS: Before each item is a blank line and a NELAC Standard citation in **Bold Numerals**.

Place a check mark (__----) in the blank if the laboratory meets the NELAC Standard referenced.

Place an X-mark (X) in the blank if the Standard is not met and the laboratory must devise an acceptable Plan of Correction and estimated completion date. **The NELAC Standard reference must be cited in in the on-site assessment report.**

Mark **"N/A"** in the blank if the NELAC Standard is not applicable to this laboratory, either because of the nature of its business mission, because of the analytical tests it performs, or because of the situation never ever happening.

Notes:

The checklist for environmental laboratory **compliance with NELAC Standards** is found on **pages 3-17**

Photocopy pages 10-16 as necessary to assess **each Toxicity test method** for compliance with the NELAC Standards

Photocopy pages 7-8 as necessary to assess **each Chemical or Physical Test support method** for compliance with the instrument calibration and/or standardization requirements of the NELAC Standards

The requirements for adherence to EPA **test method protocols and for test acceptability criteria** for each Toxicity test method are found on **pages 18-25**

40 CFR Parts 122.21(g)(7), 122.21(h)(4) 122.41(j)(4), 403.7(b)(2)(v), 403.12(b)(5)(vi), 403.12(g)(4), & 501.15(b)(10)(iv) mandate the use of test methods approved in **40 CFR Part 136**

If the laboratory appears to meet a particular NELAC Standard but does not have the documentation to back up its claim, use the following:

_____ **5.0** Does the laboratory have **all items** identified in NELAC Chapter 5 Quality Systems **available** for on-site inspection or data audit

Comment: (list all applicable Standards where the accompanying data was not available for review)

____ **5.5.6.4(d)** Do **all** containers of prepared standards & reference materials bear a **unique identifier, expiration date, & link** to its specific **preparation record**

____ **5.5.6.4(e)** Are procedures in place to ensure that prepared reagents **meet the requirements of the test method**

Note: Reagents of appropriate quality must be selected and used. In methods where the purity of reagents is not specified, analytical reagent grade shall be used. Reagents of lesser purity than specified in the test method shall not be used. Checks of the container label to verify that the purity of the reagents complies with the test method must be documented.

____ **5.5.6.4(f)** Do containers of **prepared reagents** bear a **preparation date**

____ **5.5.6.4(f)** Is the expiration date for each prepared reagent **defined on the container or documented elsewhere** as indicated in the laboratory's quality manual or SOP

____ **D.2.6(a)** Is the **grade of all reagents** used in Toxicity tests as **specified in the test method**

____ **D.2.6(a)** Does the laboratory prepare all **reference standards** from chemicals that are **analytical reagent grade** or better

____ **D.2.6(a)** Is the **preparation** of all standards & reference toxicants **documented**

____ **D.2.6(c)** Is only **reagent-grade water** collected from **distillation or deionization units** used to prepare reagents

Alkalinity – EPA 310.1; SM2320B; ASTM D1067-92; USGS I-1030-85; AOAC 973.43
Sulfuric Acid or Hydrochloric Acid titrant
Bromcresol Green (green to yellow) or Methyl Orange (yellow to red) indicator, or pH meter to detect endpoint
Sodium Carbonate or Calcium Carbonate standard

Alkalinity – EPA 310.2; USGS I-2030-85
Methyl Orange color reagent & KHP buffer (pH 3.1) for autoanalyzer (550 nm)

Ammonia Distillation – SM4500NH3 B (required unless comparability data for representative effluents proves otherwise)
Sodium Hydroxide distillation reagent
Indicating Boric Acid receiver solution

Ammonia – EPA 350.1; SM4500NH3 H; USGS I-4523-85
Heating Bath on-line with Autoanalyzer
Sodium Phenate & Sodium Hypochlorite color reagents (630 nm)
EDTA or Sodium Potassium Tartrate to prevent precipitation of divalent metal ions
Sodium Nitroprusside catalyst

Ammonia – EPA 350.2; SM4500NH3 C; ASTM D1426-93A; USGS I-3520-85; AOAC 973.49
Nessler Reagent for colorimetry (Mercuric Iodide, Potassium Iodide, Sodium Hydroxide) (425 nm)

Ammonia – EPA 350.2; SM4500NH3 E
Sulfuric Acid titrant
Mixed Indicator (Methyl Red + Methylene Blue) (green to lavender)

Ammonia – EPA 350.2, 350.3; SM4500NH3 F, SM4500NH3 G; ASTM D1426-93B

Ammonia membrane electrode & filling solution
Sodium Hydroxide to adjust pH above 11 (immerse electrode in solution FIRST)

Ammonia – Technicon 379-75WE

Autoanalyzer with Ammonia sensitive electrode detector

Chlorine – EPA 330.1; SM4500CL D, SM4500CL E (low-level free chlorine); ASTM D1253-92

Phenylarsine Oxide titrant
Phosphate Buffer to adjust sample pH to 6.5-7.5
Platinum Electrode & Amperometric Detection System

Chlorine – EPA 330.2; SM4500CL C

Iodine or Potassium Iodate titrant
Phenylarsine Oxide or Sodium Thiosulfate
Potassium Iodide & Starch Indicator (colorless to blue), or amperometric detection

Chlorine – EPA 330.3; SM4500CL B

Phenylarsine Oxide or Sodium Thiosulfate titrant
Potassium Iodide reagent (iodine liberated upon reaction with chlorine)
Potassium Bi-iodate or Potassium Dichromate primary standard
Starch indicator (blue to colorless)

Chlorine – EPA 330.4; SM4500CL F

Ferrous Ammonium Sulfate titrant
DPD Indicator (N,N-Diethyl-p-phenylenediamine) (red to colorless)
Potassium Dichromate primary standard
Potassium Iodide, to convert monochloramine & dichloramine to chlorine
Glycine, to determine bromine+iodine & subtract from the total halogen result

Chlorine – EPA 330.5; SM4500CL G

DPD color reagent (515 nm)
Chlorine standards (KMnO4 as chlorine equivalent, or household Hypochlorite with KI added & standardized with Sodium Thiosulfate)

Hardness – EPA 130.1 (Autoanalyzer)

Ammonia Buffer, to adjust sample pH to 10
Magnesium-EDTA (releases Mg when Ca from the sample is preferentially complexed)
Calmagite Color Indicator (complexes with free Mg) (520 nm)

Hardness – EPA 130.2; SM2340C; ASTM D1126-86(92); USGS I-1338-85; AOAC 973.52B

EDTA titrant (Disodium salt of Ethylenediaminetetraacetic Acid)
Calcium Carbonate standard
Eriochrome Black T or Calmagite indicators (red to blue)
Sodium Cyanide, Sodium Sulfide, or CDTA inhibitors (to sharpen titration endpoints if necessary)
Ammonia Buffer to adjust sample pH to 10.0-10.1

Hardness – calculation from Calcium & Magnesium

pH – EPA 150.1, 9040, 9045; SM4500H+ B; ASTM D1293-84(90)A, D1293-84(90)B; USGS I-1586-85; AOAC 973.41
pH – EPA 150.2; Technicon 378-75WA

pH Glass Electrode
pH Standard Buffers
SW-846: Use EPA 9040 if Aqueous Phase > 20% of sample; otherwise, must use EPA 9045
Autoanalyzer or continuous readout flow cell (EPA 150.2, Technicon 378-75WA)

Dissolved Oxygen – EPA 360.2; SM4500O C; ASTM D888-92A; USGS I-1575-78; AOAC 973.45B

Manganous Sulfate
Alkali-Iodide-Azide reagent (Sodium or Potassium Hydroxide, Sodium Iodide, Sodium Azide)
Sodium Thiosulfate titrant (Winkler titration)
Starch indicator (blue to colorless)
Potassium Bi-iodate primary standard

Dissolved Oxygen – EPA 360.1; SM4500O G; ASTM D888-92B; USGS I-1576-78

Oxygen Membrane Electrode

Salinity – SM2520B

Synthetic Seawater samples of known Salinity, to calibrate Conductivity Meter

Salinity – SM2520C

Hydrometers, checked for accuracy annually, or Densitometer

Specific Conductance – EPA 120.1, 9050; SM2510B; ASTM D1125-91A; USGS I-1780-85; AOAC 973.40

Sodium Chloride or Potassium Chloride standards (Wheatstone Bridge with platinum electrodes)

Temperature – EPA 170.1; SM2550B

NIST-traceable thermometer, with scale graduations of 0.1 degrees Celsius

Synthetic Moderately Hard Freshwater

20% (v/v) Mineral Water ----> pH 7.9-8.3, Hardness 80-100 mg/L as CaCO3, Alkalinity 60-70 mg/L as CaCO3
96 mg/L NaHCO3, 60 mg/L CaSO4.2H2O, 60 mg/L MgSO4, & 4.0 mg/L KCl
----> pH 7.4-7.8, Hardness 80-100 mg/L as CaCO3, Alkalinity 60-70 mg/L as CaCO3

Synthetic Seawater (Salinity 30.89 g/L)

21.03 g/L NaCl, 3.52 g/L Na2SO4, 0.61 g/L KCl, 0.088 g/L KBr, 0.034 g/L Na2B2O7.10H2O,
9.50 g/L MgCl2.6H2O, 1.32 g/L CaCl2.2H2O, 0.02 g/L SrCl2.6H2O, & 0.17 g/L NaHCO3

____ **D.2.8(a)** If the laboratory uses **closed, refrigerator-size incubators**, are the **culturing & testing of organisms separated** to avoid cross-contamination

____ **D.2.8(b)** Is the **laboratory space adequate** for the **types & numbers** of Toxicity tests performed

____ **D.2.8(b)** Does the laboratory building provide **adequate cooling, heating, & illumination** for conducting testing & culturing

____ **D.2.8(b)** Is **hot & cold water available** for cleaning equipment

____ **D.2.8(c)** Is the **air used** for aeration of test solutions, dilution waters, & cultures **free of oil & fumes**

____ **D.2.8(d)** Does the laboratory or contracted outside expert **positively identify test organisms** to species on an **annual basis**

____ **D.2.8(d)** Is the taxonomic reference (citation & page(s), plus the name(s) of the taxonomic expert(s)) **kept on file** at the laboratory
Note: Outside-source suppliers of test organisms must provide the same taxonomic ID information to the laboratory

COMMENTS:

SUPPORT EQUIPMENT EVALUATED (TOXICITY): _____

____ **D.2.8(e)** Are the **instruments** used for routine support measurements of **chemical & physical parameters** (e.g., pH, Dissolved Oxygen, Conductivity, Salinity, Alkalinity, Hardness, Chlorine, Ammonia, & Weight) **calibrated and/or standardized** according to the manufacturer's instructions & the NELAC Standards

Note: As these are support measurements, the following calibration & verification requirements refer to NELAC 5.5.5.2.1

Note: Copy **page 6** as necessary to assess the initial & continuing instrument calibration associated with **each test method** for chemical & physical parameters

Note: All chemical & physical measurements and calibrations must be **documented**

____ **5.5.5.2.1(a)** Is the support equipment maintained in **proper working order**

____ **5.5.5.2.1(a)** Does the laboratory keep records of all **repair & maintenance activities** including service calls

____ **5.5.5.2.1(b)** Is the support equipment calibrated or verified **at least annually**, using NIST-traceable references when available, over the **entire range of use**

____ **5.5.5.2.1(b)** Are the **results of calibration or verification** for all support equipment **within the specifications required** for the application for which the equipment is used

____ **5.5.5.2.1(b)** Does the laboratory **remove** support equipment **from service** or **establish & maintain correction factors** to correct all measurements for the deviation when the results of the annual calibration are not within the specifications required for the support equipment

____ **5.5.5.2.1(c)** Does the laboratory **retain raw data records** to document support equipment performance

____ **5.5.5.2.1(d)** Does the laboratory check balances, ovens, refrigerators, freezers, and water baths with NIST-traceable references (where commercially available) **prior to use** on **each working day** in the **expected use range**

____ **5.5.5.2.1(e)** Are mechanical volumetric dispensing devices & burettes (except Class A glassware) **checked for accuracy** on a **quarterly** use basis

Note: Glass microliter syringes must come with a certificate attesting to established accuracy, or else the accuracy must be initially demonstrated & documented by the laboratory

____ **5.5.5.2.1(f)** Does the laboratory document the temperature, cycle time, & pressure of each use of the **autoclave** for **chemical tests** with the appropriate chemical indicators or temperature recorders & pressure gauges

____ **D.2.8(g)** Is **reagent-grade water** (prepared by any combination of distillation, reverse osmosis, ion exchange, and activated carbon & particle filtration) **meet the method-specified requirements**

____ **D.2.8(h)** Is the standard **dilution water quality** used for testing or culturing sufficient to allow **satisfactory survival, growth, & reproduction of test species** as demonstrated by negative control performance & routine reference toxicant tests

____ **D.2.8(h)** Is the water analyzed for **toxic metals & organics** whenever the **minimum acceptability criteria** for control survival, growth, & reproduction **are not met** & no other cause (e.g., contaminated glassware, poor stock of test organisms) can be identified

____ **D.2.8(h)** Does the laboratory demonstrate that the **measured concentrations or limits of detection** for toxic metals and organic analytes **meet test method requirements** or are **less than one-tenth the expected chronic value** for the most sensitive species tested and/or cultured

Note: The "US EPA Ambient Water Quality Criteria Documents" & the EPA ACQUIRE database provide guidance & data on acceptability & toxicity of individual metals & organic compounds

____ **D.2.8(i)** Is the **quality of the food** used for testing or culturing sufficient to **allow satisfactory survival, growth, & reproduction of the test species** as demonstrated by routine reference toxicant tests & negative control performance

____ **D.2.8(i)** Does the laboratory have **written procedures** for the **statistical evaluation of food acceptance**

____ **D.2.8(j)** Is a **subset of organisms used in bioaccumulation tests** analyzed at the start of the test (baseline) **for the target compounds to be measured** in the bioaccumulation tests

____ **D.2.8(n)** Do all organisms used in tests, or used as breedstock to produce neonate test organisms (e.g., cladocerans & larval fish), **appear healthy, show no signs of stress or disease**, & exhibit acceptable survival (90% or greater) during the **24-hour period immediately preceding use** in tests

____ **D.2.8(o)** Are **materials used in test chambers**, culture tanks, tubing, & **other items coming in contact** with test samples, solutions, control water, sediment, soil, or food must be **non-toxic & cleaned** as described in the test methods

____ **D.2.8(o)** Do these materials **not reduce or add to sample toxicity**

Note: Appropriate materials for use in toxicity testing are described in the EPA Toxicity manuals

COMMENTS: where the above NELAC Standards are not being met

____ **D.2.8(p)** Does the laboratory make & **record light intensity** measurements on a **yearly basis**

____ **D.2.8(p)** Does the laboratory verify & **document photoperiod** (e.g., 16 hr light, 8 hr dark) at least **quarterly**

____ **D.2.8(q)** Does the laboratory document the **health & culturing conditions** of all organisms used for testing

____ **D.2.8(q)** Does this documentation include **culture conditions** (e.g., salinity, hardness, temperature, pH) & **observations** of any stress, disease, or mortality

____ **D.2.8(q)** Does the laboratory **obtain written documentation** of these water quality parameters & biological observations for **each lot** of organisms received from an **outside source**

____ **D.2.8(q)** Does the laboratory **record these observations & water quality parameters** upon the arrival of the organisms at the laboratory

____ **D.2.8(x)** Is the **culturing of Ceriodaphnia dubia** adequate such that **blocking by parentage** can be established

____ **D.2.8(s)** Does the **maximum holding time** for samples (lapsed time between sample collection to first use in a test) **not exceed 36 hours**

Note: Samples may be used for renewal up to 72 hours after first use, except as prescribed by the method & approved by the regulatory agency having authority for program oversight

____ **D.2.8(t)** Are all samples **chilled to 0-6 degrees Celsius** during or immediately after collection & maintained at that temperature range (except as prescribed by the method & approved by the regulatory agency having authority for program oversight)

COMMENTS:

TOXICITY TEST METHODS

TOXICITY TEST METHOD & TEST SPECIES EVALUATED: _____

Note: Copy **pages 11-16** as necessary to assess each Whole Effluent Toxicity test method & test species

____ **5.5.4.1.2(a)** Does the laboratory have an **in-house methods manual** for each accredited **analyte** or **method**
Note: This manual may consist of copies of published or referenced test methods

____ **5.5.4.1.2(b)** Does the laboratory **clearly indicate** in its methods manual **any modifications** made to the referenced test method and **describe any changes or clarifications** where the referenced test method is ambiguous or provides insufficient detail

Does each test method in the in-house methods manual include or reference, where applicable:

____ **5.5.4.1.2(b)(1)** **Identification** of the test method
____ **5.5.4.1.2(b)(2)** Applicable **matrix or matrices**
____ **5.5.4.1.2(b)(3)** **Method Detection Limit**
____ **5.5.4.1.2(b)(4)** **Scope & application**, including components to be analyzed
____ **5.5.4.1.2(b)(5)** **Summary** of the test method
____ **5.5.4.1.2(b)(6)** **Definitions**
____ **5.5.4.1.2(b)(7)** **Interferences**
____ **5.5.4.1.2(b)(8)** **Safety**
____ **5.5.4.1.2(b)(9)** **Equipment & supplies**
____ **5.5.4.1.2(b)(10)** **Reagents & standards**
____ **5.5.4.1.2(b)(11)** Sample **collection, preservation, shipment, & storage**
____ **5.5.4.1.2(b)(12)** **Quality control**
____ **5.5.4.1.2(b)(13)** **Calibration & standardization**
____ **5.5.4.1.2(b)(14)** **Procedure**
____ **5.5.4.1.2(b)(15)** **Data Analysis & Calculations**
____ **5.5.4.1.2(b)(16)** Method **performance**
____ **5.5.4.1.2(b)(17)** Pollution **prevention**
____ **5.5.4.1.2(b)(18)** **Data assessment & acceptance criteria** for quality control measures
____ **5.5.4.1.2(b)(19)** **Corrective actions** for out-of-control data
____ **5.5.4.1.2(b)(20)** Contingencies for **handling out-of-control or unacceptable data**
____ **5.5.4.1.2(b)(21)** **Waste management**
____ **5.5.4.1.2(b)(22)** **References**
____ **5.5.4.1.2(b)(23)** **Tables, diagrams, flowcharts, validation data**

____ **D** Does the laboratory have **procedures** for developing **acceptance/rejection criteria** for this Toxicity test method

____ **D** Does the laboratory ensure that the **essential standards** outlined in Appendix D are incorporated into the method manuals and/or Quality Manual

COMMENTS:

TOXICITY TEST METHOD EVALUATED: _____

____ **5.5.4.2.2(a) C.1** Has the laboratory performed a **satisfactory demonstration of method capability** prior to the acceptance & institution of this test method

Note: See Appendix D.2.1(a)(1) for the **specific procedural requirements** for Toxicity testing

____ **C.1** Does the laboratory **document** in its Quality Manual **other adequate approaches** to **Demonstration of Capability** if this procedure is **not required** by the mandated test method or regulation and if the laboratory **elects not to perform** this procedure

____ **5.5.4.2.2(d) C.2** Does the laboratory use the **NELAC-specified certification statement** to document the **completion of each Demonstration of Capability** (initial & continuing)

____ **C.2** Are copies of these certification statements retained in the **personnel records** of each **employee performing the test method**

____ **5.5.2.6(c)(3) D.2.1(a)(3)(iv)** Does each Analyst have **documentation** of **continued proficiency** by at least **one of the following once per year**:

- Acceptable performance of a **blind sample** (single blind to the analyst)
- Another **demonstration of capability or initial measurement system evaluation**
- Successful performance of a blind performance sample on a **similar test method** using the **same technology** (the acceptable limits must be determined prior to analysis)
- At least **4 consecutive** laboratory **control samples** with **acceptable levels** of precision & accuracy (acceptable ranges for precision & accuracy must be determined prior to analysis)
- Analysis of **authentic samples** that have been analyzed by **another trained analyst** with **statistically identical results**

Note: See App. D.2.1(a)(2) for the minimum requirements for the laboratory, but the frequency for an analyst need not exceed method-specified requirements & App. D.2.1(a)(3)

____ **5.5.4.2.2(d)** Does the laboratory **retain all associated supporting data** necessary to **reproduce the analytical results** summarized in the appropriate certification statement

____ **5.5.4.2.2(e) C.1** Does the laboratory **complete a demonstration of capability each time** there is a **change** in **instrument type, personnel, or test method**

____ **5.5.4.2.2(f)** Does the laboratory **fully document** the achievement of **demonstration of capability requirements** for each **specialized work cell**

Note: A work cell is defined as a group of analysts with specifically defined tasks that together perform the test method

____ **5.5.4.2.2(g)** Does the laboratory demonstrate & document acceptable performance through **acceptable continuing performance checks** (e.g, laboratory control samples) **each time** that **membership** in a work cell **changes**

____ **5.5.4.2.2(g)** Do the **new members** of the work cell **work with experienced analysts** in the specialty area

____ **5.5.4.2.2(g)** Does the laboratory **repeat a Demonstration of Capability** with the new work cell if the **first 4 continuing performance checks** following the change in personnel **produce a failure** in any sample batch acceptance criteria

____ **5.5.4.2.2(g)** Is the **Demonstration of Capability repeated** if the entire **work cell is changed or replaced**

____ **5.5.4.2.2(h)** Is the **performance of the work cell** as a group **linked to the training records** of the **individual members** of the work cell

TOXICITY TEST METHOD & TEST SPECIES ASSESSED: _____

Note: Refer to **pages 15-22** for specific requirements in EPA Whole Effluent Toxicity methods

____ **D.2.8(f)** Does the laboratory maintain the **test temperature as specified** in the test method

____ **D.2.8(f)** Is the temperature control equipment **adequate to maintain** the required test temperature

____ **D.2.8(f)** Is the **average daily temperature** of the test solutions maintained **within the method-specified range**

____ **D.2.8(f)** Is the temperature measured **at least once per 24-hour period**

____ **D.2.8(f)** Is the test temperature for **continuous flow toxicity tests** recorded & **monitored continuously**

____ **D.2.8(f)** Where **electronic data loggers are used**, is temperature monitored at a frequency **sufficient to capture temporal variations** of the environmental control system

____ **D.2.8(k)** Are the **test chamber sizes & test solution volumes** as specified in the test method

____ **D.2.8(k)** Are **all test chambers** used in a test **identical** (of the same size)

____ **D.2.8(l)** Are the test organisms **fed the quantity & type of food** specified in the test method

____ **D.2.8(l)** Are the test organisms **fed at the time intervals** specified in the test method

____ **D.2.8(m)** Are **all test organisms** used in the test **from the same source**
Note: Where available, certified seeds are used for soil tests

____ **D.2.8(p)** Is the **light intensity maintained** as specified in the test method

____ **D.2.8(p)** Does the laboratory **maintain the photoperiod** as specified in the test method during the test

____ **D.2.8(p)** For **algal & plant tests**, is the light intensity measured & recorded **at the start of each test**

____ **D.2.8(r)** Do the **age & age range of the test organisms** comply with the specifications in the test method

____ **D.2.8(r)** Does the laboratory document supporting information such as **hatch dates & times, time of brood releases, & metrics** (e.g., chironomid head capsule width)

____ **D.2.8(u)** Are **organisms used in a given test** from the **same batch**

____ **D.2.8(v)** Does this test have the **minimum number of replicates per treatment** as prescribed by the method

____ **D.2.8(w)** Does the control population of **Ceriodaphnia** in chronic effluent or receiving water tests contain **no more than 20% males**

____ **D.2.8(y)** Are the **dissolved oxygen & pH** in aquatic tests within the **acceptable range at test initiation**
Note: Minimal aeration is provided to tests if and only if acceptable Dissolved Oxygen concentrations cannot otherwise be obtained or if specified in the test method

____ **D.2.8(z)** Are **test soils or sediments** must be within the **geochemical tolerance range** of the test organism

TOXICITY TEST METHOD & TEST SPECIES ASSESSED: _____

____ **D** Does the laboratory **assess & evaluate** all **quality control measures** on an **on-going basis**

____ **D** Does the laboratory **use** quality control **acceptance criteria** to determine the **validity of the data**

____ **5.1.1** Does the laboratory **demonstrate compliance** with requirements in **mandated test methods** or **environmental regulations** that are more stringent than the NELAC Standards
Note: Refer to pages 17-24 in this checklist & examine any wastewater discharge permits that the laboratory is supporting through its testing

____ **5.5.9.2(d)** **D** Does the laboratory's **Toxicity data** indicate that the **quality control protocols** in the test methods manual **are being followed**

____ **D.2.1(a)(1)** Has the laboratory demonstrated its ability to **obtain consistent results with standard reference toxicants** (SRT's) & **complete an Initial Demonstration of Capability** (DOC) in order to attain accreditation for this Toxicity testing method

____ **D.2.1(a)(1)(i)** Has the initial DOC been accomplished with **at least 5 acceptable standard reference toxicant tests** for each method, species, & endpoint, with different batches of organisms

____ **D.2.1(a)(1)(i)** In this initial DOC are **appropriate negative controls** (water, sediment, or soil) tested at the frequency & duration specified in the test method
Note: Initial DOC's must be prepared in accordance with the requirements in Appendix C

____ **D.2.1(a)(1)(ii)** Has the laboratory recorded **control performance** & **statistical endpoints** (e.g., NOEC or ECp) for each method, species, & endpoint on control charts
Note: Initial DOC is established by maintenance of SRT test results on control charts, where 95% of these results fall within the control limits established in accordance with Appendix D.2.1(a)(1)(iii) below & meet Test Acceptability Criteria

____ **D.2.1(a)(1)(ii)** Has the laboratory **evaluated precision** (i.e., coefficient of variation (CV)) or **sensitivity** (i.e., statistical minimum percent difference (SMSD)) measures for these tests against method-specific or (lacking the former) laboratory-developed criteria, to determine the validity of the initial DOC

____ **D.2.1(a)(1)(iii)** For endpoints that are **point estimates** (e.g., ICp & ECp), are control charts plotted as **cumulative mean & the control limits**, which consist of the upper & lower 95% confidence limits (**+/- 2 std. dev**.)
Note: For highly variable point estimates that exceed method-specific criteria, the control chart limits are adjusted accordingly

____ **D.2.1(a)(1)(iii)** For endpoints from **hypothesis tests** (e.g., NOEC & NOAEC), are the values **plotted directly**, with the control limits consisting of **one concentration interval above & below** the **central tendency** (i.e., the mode)

____ **D.2.1(a)(1)(iv)** Does the laboratory **calculate** the cumulative mean CV (for endpoints that are point estimates) & SMSD (for endpoints that are hypothesis tests) & **maintain these values on control charts**

TOXICITY TEST METHOD & TEST SPECIES ASSESSED: _____

____ **D.2.1(a)(2)** Does the laboratory **demonstrate on-going performance** through **routine SRT testing** for this test method, species, & each endpoint
Note: See **D.2.1(a)(3)** for minimum frequency requirements

____ **D.2.1(a)(2)(i)** **D.2.2** Does the laboratory **determined interlaboratory precision** on an on-going basis through the use of **control charts** (as established in Appendix D.2.1(a)(1)(ii))

____ **D.2.1(a)(2)(i)** Are the control charts plotted as **point estimate values** (e.g., EC25 for chronic tests & LC50 for acute tests) or as **hypothesis test values** (e.g., NOEC or NOAEC) over time

____ **D.2.1(a)(2)(ii)** After the initial DOC is determined, does the laboratory **adjust the control limits & CV** for each method, species, & endpoint as **additional test results are obtained**

____ **D.2.1(a)(2)(ii)** Are control charts maintained & calculated with **only the most recent 20 data points**

____ **D.2.1(a)(2)(iv)** Has the laboratory developed **acceptance/rejection policies**, consistent with the test method, **for reference toxicant data** that considers test dilution factor, test sensitivity (for hypothesis test values), testing frequency, out-of-control test frequency, relative width of acceptance limits, control chart CV, & degree of difference between test results & acceptance limits

____ **D.2.1(a)(2)(v)** If reference toxicant data **fails to meet control chart acceptance criteria**, are the test data **examined for defects, corrective action taken,** & the **test repeated** if necessary with different batch of organisms, or else is the **data qualified**

____ **D.2.1(a)(3)(i)** For test methods conducted at a frequency **greater than monthly**, are the SRT tests conducted at an **on-going frequency of monthly**
Note: The frequency of on-going laboratory SRT testing can be less frequent if the method **specifically requires less frequent SRT tests** (e.g., sediment tests)

____ **D.2.1(a)(3)(ii)** For test methods & species commonly used in the laboratory but are tested at a frequency of **monthly or less**, are the SRT tests **conducted concurrently** with the environmental test

____ **D.2.1(a)(3)(iii)** If the test organisms **are obtained from an outside source**, does the laboratory **determine the sensitivity of each batch of organisms** received from a supplier via a **concurrent SRT test**
Note: The laboratory is exempted from this requirement if the supplier provides control chart data for the last 5 SRT tests using the same SRT & test conditions, but **supplied SRT data may not be older than 6 months**

____ **D.2.1(a)(4)** Does the laboratory use the **same** reference toxicant, test concentrations, dilution water, & data analysis methods for **all reference toxicant tests** conducted for this test method & species

____ **D.2.1(a)(4)** Does the laboratory use **dilution factors of 0.5x or greater** for both acute & chronic tests

____ **D.2.1(a)(4)** Does the laboratory follow any **state or permitting authority requirements** for a reference toxicant or dilution series

____ **D.2.1(a)(5)** Does the laboratory conduct reference toxicant tests with the **same procedures** as environmental toxicity tests for which the precision is being evaluated unless specified in the test method
Note: As an example, 10-day sediment toxicity tests employ 96-hour water-only reference toxicant tests

TOXICITY TEST METHOD & TEST SPECIES ASSESSED: _____

____ **D.2.1(a)(5)** Does the laboratory **employ the same** test duration, laboratory dilution water, feeding, organism age, range & density, test volumes, renewal frequency, water quality measurements, and the number of test concentrations, replicates, & organisms per replicate **as specified in the environmental toxicity tests** for reference toxicant tests

____ **D.2.1(b)(1)** Does the laboratory follow the standards for use, type, & frequency of testing for **negative controls** (e.g., Control, Brine Control, or Dilution Water) as **specified by the test method & by the permit**

____ **D.2.1(b)(1)** Is a **negative control included with each test** to evaluate test performance and the health & sensitivity of the specific batch of organisms

____ **D.2.1(b)(2)** Does the laboratory include **additional negative controls** when sample adjustments (e.g., thiosulfate for dechlorination) or solvent carriers are used in the test

____ **D.2.1(b)(3)** Does the laboratory **achieve test acceptability criteria** as specified in the test method for **both the reference toxicant & effluent or environmental sample toxicity test**

____ **D.2.1(b)(3)** **D.2.8(aa)** Does the laboratory **calculate** the test acceptability criteria **& meet the method specified requirements** for performing toxicity

Note: An individual test may be conditionally acceptable if temperature, dissolved oxygen, pH, & other specified conditions fall outside specifications, depending upon the degree of departure & the objectives of the test; in this circumstance the acceptability of the test shall depend upon the experience & professional judgment of the technical director & the permitting authority

____ **D.2.4(a)** Does the laboratory **calculate the statistical minimum significant difference** (SMSD) according to the formula in the test method & **report it with the test results**

Note: The statistical minimum significant difference only needs to be calculated & reported for hypothesis test values, such as NOEC or NOAEC

____ **D.2.4(b)** For point estimates (e.g., LCp, ICp, or ECp), does the laboratory **report confidence intervals** as a measure of the **precision around the point estimate value** (when the calculation is possible)

____ **D.2.4(c)** Does the laboratory calculate and report statistical minimum significant differences **only for hypothesis test values** (e.g., NOEC & NOAEC)

____ **D.2.5(a)** Does the laboratory **perform data analysis & calculate endpoints** as specified in regulations, permits, or the test method

____ **D.2.5(b)** Does the laboratory **plot Toxicity data** in the form of a **dose response curve** that relates the dose of the chemical to cumulative percentage of test organisms demonstrating a response such as death

____ **D.2.5(b)** Have evaluation criteria been established for **interpretation of concentration or dose response curves**

COMMENTS: List test species & NELAC Standards where the above requirements are not being fulfilled

EPA-REQUIRED TEST CONDITIONS & TEST ACCEPTABILITY CRITERIA

ACUTE TOXICITY – All Test Species

Test Type:	Static non-renewal, static renewal, or flow-through
Duration:	24 hours, 48 hours, or 96 hours
Light Intensity:	10-20 uE/m2/sec (50-100 foot-candles) (ambient laboratory levels)
Photoperiod:	16 hours light, 8 hours darkness
Test Solution Renewal:	After 48 hours (minimum)
Test Concentrations:	Effluents: Minimum of 5 concentrations plus a control Receiving Waters: 100% receiving water & a control
Dilution Series:	>= 0.5 dilution series
Endpoint:	Effluents: Mortality (LC50 or NOEC) Receiving Waters: Mortality (significant difference from control)
Test Acceptability Criteria:	90% or greater survival in controls

EPA 2002, 2021 - Freshwater Acute Toxicity – Ceriodaphnia dubia; Daphnia pulex, Daphnia magna

Temperature:	20 +/- 1 C, or 25 +/- 1 C
Test Chamber Size:	30 mL (minimum)
Test Solution Volume:	15 mL (minimum for Ceriodaphnia dubia) 25 mL (minimum for Daphnia pulex & Daphnia magna)
Age of Test Organisms:	Less than 24 hours old
# Organisms per Test Chamber:	5 (minimum for effluent & receiving water tests)
# Replicate Chambers per Concentration:	4 (minimum for effluent & receiving water tests)
# Organisms per Concentration:	20 (minimum for effluent & receiving water tests)
Feeding Regime:	Feed YCT & Selenastrum while holding prior to the test; Newly-released young should have food available 2 hr prior to use; Add 0.1 mL each YCT & Selenastrum 2 hr prior to renewal at 48 hr
Dilution Water:	Moderately hard synthetic freshwater
Sampling Requirements:	Grab or Composite, 36-hour holding time, 1 L sample required

EPA 2000 - Freshwater Acute Toxicity – Pimephales promelas, Cyprinella leedsi

Temperature:	20 +/- 1 C, or 25 +/- 1 C
Test Chamber Size:	250 mL (minimum)
Test Solution Volume:	200 mL (minimum)
Age of Test Organisms:	1-14 days; 24-hour range in age
# Organisms per Test Chamber:	10 (minimum for effluent & receiving water tests)
# Replicate Chambers per Concentration:	2 (minimum for effluent tests) 4 (minimum for receiving water tests)
# Organisms per Concentration:	20 (minimum for effluent tests) 40 (minimum for receiving water tests)
Feeding Regime:	Artemia nauplii made available while holding prior to the test; Add 0.2 mL Artemia concentrate 2 hr prior to renewal at 48 hr
Test Solution Aeration:	None unless dissolved oxygen falls below 4.0 mg/L; Rate should not exceed 100 bubbles/min
Dilution Water:	Moderately hard synthetic freshwater
Sampling Requirements:	Grab or Composite, 36-hour holding time, 2 L sample required

EPA 2019 - Freshwater Acute Toxicity – Oncorhynchus mykiss, Salvelinus fontinalis

Temperature:	12 +/- 1 C
Test Chamber Size:	5 L (minimum)
Test Solution Volume:	4 L (minimum)
Age of Test Organisms:	Rainbow trout: 15-30 days (after yolk-sac absorption to 30 days) Brook trout: 30-60 days
# Organisms per Test Chamber:	10 (minimum for effluent & receiving water tests)
# Replicate Chambers per Concentration:	2 (minimum for effluent tests) 4 (minimum for receiving water tests)
# Organisms per Concentration:	20 (minimum for effluent tests) 40 (minimum for receiving water tests)
Feeding Regime:	Nor required for this test
Test Solution Aeration:	None unless dissolved oxygen falls below 6.0 mg/L; Rate should not exceed 100 bubbles/min
Dilution Water:	Moderately hard synthetic freshwater
Sampling Requirements:	Grab or Composite, 36-hour holding time; 20 L effluent sample required, 40 L receiving water required

EPA 2007 - Saltwater Acute Toxicity – Mysidopsis bahia, Homesimysis costata

Temperature:	20 +/- 1 C, or 25 +/- 1 C (12 or 25 C for Homesimysis)
Test Chamber Size:	250 mL (minimum)
Test Solution Volume:	200 mL (minimum)
Age of Test Organisms:	1-5 days; 24-hour range in age
# Organisms per Test Chamber:	10 (minimum for effluent & receiving water tests)
# Replicate Chambers per Concentration:	2 (minimum for effluent tests) 4 (minimum for receiving water tests)
# Organisms per Concentration:	20 (minimum for effluent tests) 40 (minimum for receiving water tests)
Feeding Regime:	Artemia nauplii made available while holding prior to the test; Add 0.2 mL Artemia concentrate < 24 hr old daily
Test Solution Aeration:	None unless dissolved oxygen falls below 4.0 mg/L; Rate should not exceed 100 bubbles/min
Dilution Water:	Uncontaminated seawater, hypersaline brine or sea salts in H2O; 0.5-3.0% for Mysidopsis, 3.2-3.4% for Homesimysis
Sampling Requirements:	Grab or Composite, 36-hour holding time, 2 L sample required

EPA 2004, 2006 - Saltwater Acute Toxicity – Cyprinodon variegatus; Menidia beryllina, M. menidia, M. peninsulae

Temperature:	20 +/- 1 C, or 25 +/- 1 C
Test Chamber Size:	250 mL (minimum)
Test Solution Volume:	200 mL (minimum)
Age of Test Organisms:	1-14 days; 24-hour range in age
# Organisms per Test Chamber:	10 (minimum for effluent & receiving water tests)
# Replicate Chambers per Concentration:	2 (minimum for effluent tests) 4 (minimum for receiving water tests)
# Organisms per Concentration:	20 (minimum for effluent tests) 40 (minimum for receiving water tests)
Feeding Regime:	Artemia nauplii made available while holding prior to the test; Add 0.2 mL Artemia concentrate 2 hr prior to renewal at 48 hr
Test Solution Aeration:	None unless dissolved oxygen falls below 4.0 mg/L; Rate should not exceed 100 bubbles/min
Dilution Water:	Uncontaminated seawater, hypersaline brine or sea salts in H2O 0.5-3.2% for Cyprinodon, 0.1-3.2% for M. beryllina, 1.5-3.2% for other Menidia sp. (tolerances for salinity +/- 10% RSD)
Sampling Requirements:	Grab or Composite, 36-hour holding time; 1 L effluent sample sample required, 2 L receiving water required

CHRONIC TOXICITY – All Test Species

Test Concentrations:	Effluents: Minimum of 5 concentrations & a control Receiving Waters: 100% receiving water or minimum of 5 concentrations & a control
Dilution Factor:	Effluents: Greater than or equal to 0.5 Receiving Waters: None, or greater than or equal to 0.5

EPA 1000 – Freshwater Chronic Toxicity (Pimephales promelas)

Test Type:	Static Renewal
Test Duration:	7 days
Temperature:	25 +/- 1 degrees Celsius
Light Quality:	Ambient laboratory illumination
Light Intensity:	10-20 uE/m2/sec (50-100 foot-candles) (ambient laboratory levels)
Photoperiod:	16 hours light, 8 hours dark
Test Chamber Size:	500 mL (minimum)
Test Solution Volume:	250 mL (minimum)
Renewal of Test Solution:	Daily
Age of Test Organisms:	Newly-hatched larvae less than 24 hours old; If shipped, less than 48 hours old & 24-hour range in age
# Larvae per Test Chamber:	15 (minimum of 10)
# Replicate Chambers per Concentration:	4 (minimum of 3)
# Larvae per Concentration:	60 (minimum of 30)
Feeding Regime:	0.1 mL newly-hatched (<24 hr old) brine shrimp nauplii 3 times daily at 4-hour intervals; OR at a minimum, 0.15 g twice daily, 6 hours between feedings (at the beginning of the work day prior to renewal, & at the end of the work day after renewal); Sufficient nauplii added to provide an excess Larvae fish not fed during final 12 hours of test
Cleaning:	Siphon daily, immediately before test solution renewal
Aeration:	None unless Dissolved Oxygen concentration falls below 4.0 mg/L; Rate should not exceed 100 bubbles/min
Dilution Water:	Moderately hard synthetic water prepared using Millipore Milli-Q, or DI water with reagent-grade chemicals or 20% DMW
Endpoints:	Survival & Growth (weight)
Sampling Requirements:	On-Site Tests: Samples collected daily, used within 24 hours of time they are removed from the sampling device Off-Site Tests: Minimum of 3 samples collected on days one, three, & five; maximum 36-hour holding time before first use
Sample Volume Required:	2.5 L per day
Test Acceptability:	80% or greater survival in controls; average dry weight of surviving organism in control chambers equals or exceeds 0.25 mg

EPA 1001 – Freshwater Chronic Toxicity (Pimephales promelas)

Test Type:	Static Renewal
Test Duration:	7 days
Temperature:	25 +/- 1 degrees Celsius
Light Quality:	Ambient laboratory illumination
Light Intensity:	10-20 uE/m2/sec (50-100 foot-candles) (ambient laboratory levels)
Photoperiod:	16 hours light, 8 hours dark
Test Chamber Size:	150 mL (minimum)
Test Solution Volume:	70 mL (minimum)
Renewal of Test Solution:	Daily
Age of Test Organisms:	Embryos < 36 hours old; maximum of 48 hours if shipped
# Embryos per Test Chamber:	15 (minimum of 10)
# Replicate Chambers per Concentration:	4 (minimum of 3)
# Embryos per Concentration:	60 (minimum of 30)
Feeding Regime:	Feeding not required
Aeration:	None unless Dissolved Oxygen concentration falls below 4.0 mg/L; Rate should not exceed 100 bubbles/min
Dilution Water:	Moderately hard synthetic water prepared using Millipore Milli-Q, or DI water with reagent-grade chemicals or 20% DMW; Hardness should exceed 25 mg/L (CaCO3) to ensure hatching success
Endpoints:	Combined Mortality (dead & deformed organisms)
Sampling Requirements:	On-Site Tests: Samples collected daily, used within 24 hours of time they are removed from the sampling device Off-Site Tests: Minimum of 3 samples collected on days one, three, & five; maximum 36-hour holding time before first use
Sample Volume Required:	1.5-2.5 L per day (depending on volume of test solutions used)
Test Acceptability:	80% or greater survival in controls

EPA 1002 – Freshwater Chronic Toxicity (Ceriodaphnia dubia)

Test Type:	Static Renewal
Test Duration:	Until 60% of surviving control organisms have 3 broods (max. 8 days)
Temperature:	25 +/- 1 degrees Celsius
Light Quality:	Ambient laboratory illumination
Light Intensity:	10-20 uE/m2/sec (50-100 foot-candles) (ambient laboratory levels)
Photoperiod:	16 hours light, 8 hours dark
Test Chamber Size:	30 mL (minimum)
Test Solution Volume:	15 mL (minimum)
Renewal of Test Solution:	Daily
Age of Test Organisms:	Less than 24 hours old; all released within 8-hour period
# Neonates per Test Chamber:	1
# Replicate Chambers per Concentration:	10
# Neonates per Concentration:	10
Feeding Regime:	Feed 0.1 mL YCT & 0.1 mL algal suspension (30-35 million cells/mL) in each test chamber daily
Cleaning:	Use freshly-cleaned glass beakers or new plastic cups daily
Aeration:	None
Dilution Water:	Moderately hard synthetic water prepared using Millipore Milli-Q, or DI water with reagent-grade chemicals or 20% DMW
Endpoints:	Survival & Reproduction
Sampling Requirements:	On-Site Tests: Samples collected daily, used within 24 hours of time they are removed from the sampling device Off-Site Tests: Minimum of 3 samples collected on days one, three, & five; maximum 36-hour holding time before first use
Sample Volume Required:	1 L per day
Test Acceptability:	80% or greater survival in controls; average of 15 or more young per surviving females in control solutions (60% of surviving control organisms must produce 3 broods)

EPA 1003 – Freshwater Chronic Toxicity (Selenastrum capricornutum)

Test Type:	Static Non-renewal
Test Duration:	96 hours
Temperature:	25 +/- 1 degrees Celsius
Light Quality:	"Cool white" fluorescent lighting
Light Intensity:	86 +/- 8.6 uE/m2/sec (400 +/- 40 foot-candles) (or 4306 lux)
Photoperiod:	Continuous illumination
Test Chamber Size:	125 or 250 mL
Test Solution Volume:	50 or 100 mL
Renewal of Test Solution:	None
Age of Test Organisms:	4-7 days
Initial Cell Density in Test Chambers:	10000 cells/mL
# Replicate Chambers per Concentration:	4 (minimum of 3)
Shaking Rate:	100 cpm continuous, or twice daily by hand
Dilution Water:	Algal stock culture medium; enriched surface water; synthetic water prepared using Millipore Milli-Q, or DI water with reagent-grade chemicals or 20% DMW
Endpoints:	Growth (cell counts, chlorophyll fluorescence, absorbance, biomass)
Sampling Requirements:	On-Site Tests: 1 sample collected at test initiation & used within 24 hours of time it is removed from the sampling device Off-Site Tests: Maximum 36-hour holding time
Sample Volume Required:	1-2 L (depending on test volume)
Test Acceptability:	1000000 cells/mL with EDTA; OR 200000 cells/mL without EDTA in the controls (variability of controls should not exceed 20%)

EPA 1004 – Saltwater Chronic Toxicity (Cyprinodon variegatus)

Test Type:	Static Renewal
Test Duration:	7 days
Salinity:	2.0-3.2% (+/- 0.2% of selected test Salinity)
Temperature:	25 +/- 1 degrees Celsius
Light Quality:	Ambient laboratory illumination
Light Intensity:	10-20 uE/m2/sec (50-100 foot-candles) (ambient laboratory levels)
Photoperiod:	16 hours light, 8 hours dark
Test Chamber Size:	600-1000 mL (beakers or equivalent)
Test Solution Volume:	500-750 mL per replicate (loading & DO restrictions must be met)
Renewal of Test Solution:	Daily
Age of Test Organisms:	Newly-hatched larvae less than 24 hours old; 24-hour range in age
# Larvae per Test Chamber:	15 (minimum of 10)
# Replicate Chambers per Concentration:	4 (minimum of 3)
# Larvae per Concentration:	60 (minimum of 30)
Food Source:	Newly-hatched Artemia nauplii (< 24 hr old)
Feeding Regime:	Feed once per day 0.10 g wet weight per replicate on Days 0-2 of test; 0.15 g wet weight per replicate on Days 3-6
Cleaning:	Siphon daily, immediately before test solution renewal & feeding
Aeration:	None unless Dissolved Oxygen concentration falls below 4.0 mg/L, then aerate all chambers; Rate should not exceed 100 bubbles/min
Dilution Water:	Uncontaminated source of natural seawater; OR hypersaline brine or artificial sea salts mixed with DI water
Endpoints:	Survival & Growth (weight)
Sampling Requirements:	On-Site Tests: Samples collected daily, used within 24 hours of time they are removed from the sampling device Off-Site Tests: Minimum of 3 samples collected on days one, three, & five; maximum 36-hour holding time before first use
Sample Volume Required:	6 L per day
Test Acceptability:	80% or greater survival in controls; average dry weight of surviving organism in control chambers > 0.60 mg if unpreserved (or > 0.50 mg after < 7 days in 4% formalin or 70% ethanol)

EPA 1005 – Saltwater Chronic Toxicity (Cyprinodon variegatus)

Test Type:	Static Renewal
Test Duration:	9 days
Salinity:	0.5-3.2% (+/- 0.2% of selected test Salinity)
Temperature:	25 +/- 1 degrees Celsius
Light Quality:	Ambient laboratory illumination
Light Intensity:	10-20 uE/m2/sec (50-100 foot-candles) (ambient laboratory levels)
Photoperiod:	16 hours light, 8 hours dark
Test Chamber Size:	400-500 mL
Test Solution Volume:	250-400 mL per replicate (loading & DO restrictions must be met)
Renewal of Test Solution:	Daily
Age of Test Organisms:	Less than 24 hours old
# Embryos per Test Chamber:	15 (minimum of 10)
# Replicate Chambers per Concentration:	4 (minimum of 3)
# Embryos per Concentration:	60 (minimum of 30)
Feeding Regime:	Not required
Aeration:	None unless Dissolved Oxygen concentration falls below 4.0 mg/L, then aerate all chambers; Rate should not exceed 100 bubbles/min
Dilution Water:	Uncontaminated source of natural seawater; OR hypersaline brine or artificial sea salts mixed with DI water
Endpoints:	Percent hatch; percent larvae dead or with debilitating morphological and/or behavioral abnormalities (e.g., gross deformities, curved spine, disoriented, abnormal swimming behavior); surviving normal larvae from original embryos
Sampling Requirements:	On-Site Tests: Samples collected daily, used within 24 hours of time they are removed from the sampling device Off-Site Tests: Minimum of 3 samples collected on days one, three, & five; maximum 36-hour holding time before first use
Sample Volume Required:	5 L per day
Test Acceptability:	80% or greater survival in controls

EPA 1008 – Saltwater Chronic Toxicity (Arbacia punctulata)

Test Type:	Static
Test Duration:	80 minutes
Salinity:	3.0% (+/- 0.2% of selected test Salinity)
Temperature:	20 +/- 1 degrees Celsius
Light Quality:	Ambient laboratory light during test preparation
Light Intensity:	10-20 uE/m2/sec (50-100 foot-candles) (ambient laboratory levels)
Test Vessel Size:	Disposable glass liquid scintillation vials (20-mL capacity)
Test Solution Volume:	5 mL
# Sea Urchins:	Pooled sperm from 4 males & pooled eggs from 4 females used per test
# Egg & Sperm Cells per Test Chamber:	About 2000 eggs & 5000000 sperm cells per vial
# Replicate Chambers per Concentration:	4 (minimum of 3)
Dilution Water:	Uncontaminated source of natural seawater; OR hypersaline brine or artificial sea salts mixed with DI water
Endpoints:	Fertilization of Sea Urchin Eggs
Sampling Requirements:	One sample collected at test initiation & preferably used within 24 hours of the time it's removed from the sampling device
Sample Volume Required:	1 L per test
Test Acceptability:	70-90% fertilization of eggs in controls

EPA 1006 – Saltwater Chronic Toxicity (Menidia beryllina)

Test Type:	Static Renewal
Test Duration:	7 days
Salinity:	0.5-3.2% (+/- 0.2% of selected test Salinity)
Temperature:	25 +/- 1 degrees Celsius
Light Quality:	Ambient laboratory illumination
Light Intensity:	10-20 uE/m2/sec (50-100 foot-candles) (ambient laboratory levels)
Photoperiod:	16 hours light, 8 hours dark
Test Chamber Size:	600-1000 mL (beakers or equivalent)
Test Solution Volume:	500-750 mL per replicate (loading & DO restrictions must be met)
Renewal of Test Solution:	Daily
Age of Test Organisms:	7-11 days post-hatched larvae; 24-hour range in age
# Larvae per Test Chamber:	15 (minimum of 10)
# Replicate Chambers per Concentration:	4 (minimum of 3)
# Larvae per Concentration:	60 (minimum of 30)
Food Source:	Newly-hatched Artemia nauplii (survival of 7-9 day old test larvae improved by feeding 24-hour old Artemia)
Feeding Regime:	Feed once per day 0.10 g wet weight per replicate on Days 0-2 of test; 0.15 g wet weight per replicate on Days 3-6
Cleaning:	Siphon daily, immediately before test solution renewal & feeding
Aeration:	None unless Dissolved Oxygen concentration falls below 4.0 mg/L, then aerate all chambers; Rate should not exceed 100 bubbles/min
Dilution Water:	Uncontaminated source of natural seawater; OR hypersaline brine or artificial sea salts mixed with DI water
Endpoints:	Survival & Growth (weight)
Sampling Requirements:	On-Site Tests: Samples collected daily, used within 24 hours of time they are removed from the sampling device Off-Site Tests: Minimum of 3 samples collected on days one, three, & five; maximum 36-hour holding time before first use
Sample Volume Required:	6 L per day
Test Acceptability:	80% or greater survival in controls; OR when 7-day old larvae are used, average dry weight of control larvae (dried immediately after test termination) > 0.50 mg if unpreserved (or > 0.43 mg after no more than 7 days in 4% formalin or 70% ethanol)

EPA 1009 – Saltwater Chronic Toxicity (Champia parvula)

Test Type:	Static, Non-renewal
Test Duration:	2 days exposure to effluent, then 5-7 day recovery period in control medium for cystocarp development
Salinity:	3.0% (+/- 0.2% of selected test Salinity)
Temperature:	23 +/- 1 degrees Celsius
Light Quality:	Cool white fluorescent lights
Light Intensity:	75 uE/m2/sec (500 foot-candles)
Photoperiod:	16 hours light, 8 hours dark
Test Chamber Size:	200-mL polystyrene cups or 250 mL Erlenmeyer flasks
Test Solution Volume:	100 mL (minimum)
# Organisms per Test Chamber:	5 female branch tips & 1 male plant
# Replicate Chambers per Concentration:	4 (minimum of 3)
# Organisms per Concentration:	24 (minimum of 18)
Dilution Water:	3.0% salinity natural seawater; OR 1:1 3.0% salinity natural seawater & 3.0% salinity artificial seawater
Endpoints:	Reduction in cystocarp production relative to controls
Sampling Requirements:	One sample collected at test initiation & preferably used within 24 hours of the time it's removed from the sampling device
Sample Volume Required:	2 L per test
Test Acceptability:	80% or greater survival & average of 10 cystocarps per plant in controls

EPA 1007 – Saltwater Chronic Toxicity (Mysidopsis bahia)

Test Type:	Static Renewal
Test Duration:	7 days
Salinity:	2.0-3.0% (+/- 0.2% of selected test Salinity)
Temperature:	26 +/- 1 degrees Celsius
Light Quality:	Ambient laboratory illumination
Light Intensity:	10-20 uE/m2/sec (50-100 foot-candles) (ambient laboratory levels)
Photoperiod:	16 hours light, 8 hours dark, with phase in/out period
Test Chamber Size:	400 mL glass beakers, or 8 oz plastic disposable cups
Test Solution Volume:	150 mL per replicate
Renewal of Test Solution:	Daily
Age of Test Organisms:	7 days
# Organisms per Test Chamber:	5 (minimum)
# Replicate Chambers per Concentration:	8 (minimum)
# Organisms per Concentration:	40 (minimum)
Food Source:	Newly-hatched Artemia nauplii (< 24 hr old)
Feeding Regime:	Feed 150 nauplii per mysid daily, half after test solution renewal & half after 8-12 hours
Cleaning:	Pipette excess food from cups daily, immediately before test solution renewal & feeding
Aeration:	None unless Dissolved Oxygen concentration falls below 4.0 mg/L, then gentlely aerate in all cups
Dilution Water:	Uncontaminated source of natural seawater; OR hypersaline brine or artificial sea salts mixed with DI water
Endpoints:	Survival, Growth, & Egg Development
Sampling Requirements:	On-Site Tests: Samples collected daily, used within 24 hours of time they are removed from the sampling device Off-Site Tests: Minimum of 3 samples collected on days one, three, & five; maximum 36-hour holding time before first use
Sample Volume Required:	3 L per day
Test Acceptability:	80% or greater survival & average dry weight > 0.20 mg in controls; (Fecundity may be used if > 50% of females in controls produce eggs)