

USEPA Research on Mussels and Magna: Developing Toxicity Testing Methods And Guidance for New Species for Effluents and Ambient Waters

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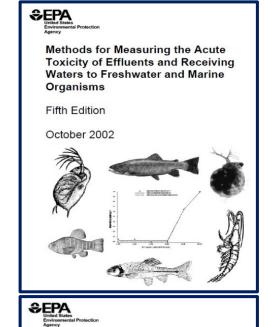
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EPA'S TOXICITY TEST METHODS

- > EPA has established aquatic toxicity test methods for conducting water quality assessments on both effluents and receiving waters that are used all over the U.S. for determining possible impacts to aquatic life, and some are also used by other countries for the same purpose.
- > EPA has numerous toxicity methods for acute and short-term chronic test species* used for states across the U.S. (freshwater tests) and for states along the Atlantic Ocean and Gulf of Mexico (estuarine/marine tests) (Photos). All of EPA's effluent toxicity test methods (<u>www.epa.gov/cwa-methods/whole-effluent-toxicity-</u> <u>methods</u>) provide guidance so that the toxicity data generated are representative of the sample(s) assessed, and produce valid and scientifically defensible data. This is accomplished by having both mandatory and recommended provisions in the toxicity methods. EPA's toxicity manuals provide standardized test methods which include quality assurance/quality control procedures that support scientific



Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater

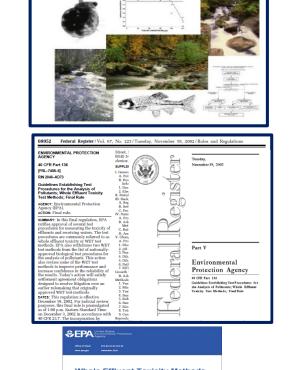
Fourth Edition

October 2002

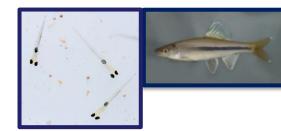
OBJECTIVE AND APPROACHES

- > The objective of this project is to develop invertebrate assays test methods for short-term chronic toxicity test methods, specifically
 - Mussel, fatmucket (*Lampsilis siliquoidea*): acute (96 h) and 7-d Short-term Test to Estimate **Chronic Toxicity**
 - Cladoceran (*Daphnia magna*): 4, 5 d Short Term Chronic Test to Estimate Chronic Toxicity
- > Method development will include testing of several single/reference chemicals and effluents for both species along with the standard WET species at two of EPA's research labs (i.e., Duluth and Cincinnati). Interlaboratory testing with both labs for both species is planned for both short term growth methods to estimate chronic toxicity for *D. magna* and the fatmucket.
 - Both labs will test the same 5 reference materials (KCl, ZnSO₄, ammonia chloride, phenol, bifenthrin) three times each, and conduct a minimum of 5 effluents from wastewater treatment plants and industrial discharges. This will be done to demonstrate that the test can be reliably performed in different labs using new test method endpoints.

- defensibility.
- > In this poster, we provide details for methods that are under development within the Office of Research and Development Laboratories in Duluth, MN and Cincinnati, OH. The current focus is the development of an effluent test protocol for a mussel for acute and short-term chronic tests and the addition of a short-term chronic method for the cladoceran, *Daphnia magna*. The current species for both the freshwater acute and short-term chronic test methods for effluents & ambient waters are shown below (CFR Table IA 40 CFR 136.3)



Toxicity, **acute**, fresh water organisms, LC₅₀, percent effluent (CFR Table IA 40 CFR 136.3)



Pimephales promelas &

Acute Method **2000.0**

Fathead minnow,

bannerfish shiner,

Cyprinella leedsi

48 or 96 h

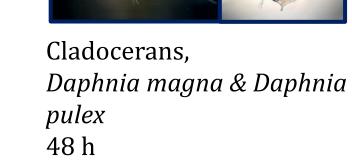






Photo credit: G. Novak

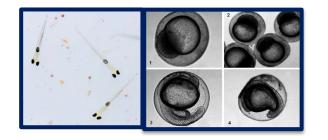
Cladoceran, Rainbow trout, Oncorhynchus Ceriodaphnia dubia *mykiss,* & and brown trout 48 h Salvelinus fontinalis Acute Method **2002.0** 48 or 96 h Acute Method **2019.0**



Acute Method **2021.0**

1-2 mm length)

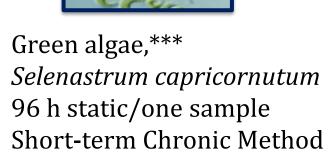
Toxicity, **chronic**, fresh water organisms, NOEC or IC25, percent effluent (CFR Table IA 40 CFR 136.3)



Fathead minnow, *Pimephales promelas* 7-d, daily renewal or 8-d teratogenicity test Short-term Chronic Methods 1000.0 & 1001.0



Cladoceran, Ceriodaphnia dubia 7-d renewal, daily Short-term Chronic Method 1002.0



- Various test parameters will be assessed including food, water, number of replicates, culture procedures and proficiency guidance for users.
- For the *D. magna* growth method, the test duration to be tested will include a 4-day and 5-d test period. An evaluation of both the (4 day and 5 day) methods will be assessed based on test sensitivity, test method precision and test power.
- For the fatmucket, comparisons of the age of initial organisms will be compared with the various effluents and reference toxicants. With the age range of 1, 2 or 3 week old, measurements of the starting age will be needed to determine the growth on a consistent basis (increase from initial weights). In addition the test development will consider test sensitivity, test method precision, and test power.
- > Test method development will include an assessment of the relevance and reliability of the test method protocol as well as the refinement/optimization of the method and development of test protocols.

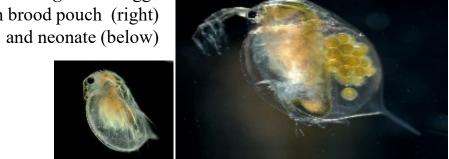
Test Parameter	Mussel, <i>L. siliquoidea,</i> Method	Cladoceran, <i>D. magna</i> Method
Test Type	Static renewal	Static renewal
Test Duration	7-d	4-d, 5-d
Temperature	25 ± 1 °C	25 ± 1 °C
Test chamber size	4 oz cup, with layer of silica sand, sieved to 0.2 mm particles	60 ml (suggested)
Test solution volume	100 ml	50 ml (suggested)
Test water	Reconstituted water	Reconstituted water
Renewal of solutions	Daily renewal, ~80% replacement	Daily (required)
Age of test organism	1, 2, or 3 week post-metamorphosis	< 24 h old (12 h age window) (required)
Organisms /replicate	10 juvenile mussels /replicate	20 neonates (suggested)/replicate
Replicates	4 replicates /concentration	4 replicates / concentration
Test Concentrations	Minimum of 5 with the control	Minimum of 5 with the control
Feeding	 Algal mixture prepared daily, ramped diet (see photo below) 2 mL / 2X day for 1- to 2-wk-olds 3 mL / 2X daily for 3-wk-olds 	Algae (<i>Raphidocelis subcapitata</i>) & alfalfa extract (required)
Endpoint	Mortality (gaped shell with swollen tissue and empty shell), growth (maximum shell length)	Survival and growth (mean dry weight) (required)
Test acceptability	≥80% survival in the controls and	\geq 90% or greater control survival
criterion	growth criteria (percent increase) TBD	and growth 10X initial dry weight (required)

1003.0

- * The EPA method manuals (available at <u>www.epa.gov/cwa-methods/whole-effluent-toxicity-methods</u>) are incorporated by reference into the US Code of Federal Regulations (CFR) Rulemaking (https://www.govinfo.gov/content/pkg/FR-2002-11-<u>19/html/02-29072.htm</u>]
- ** The micro alga has been renamed, Raphidocelis subcapitata, formerly Selenastrum capricornutum (as shown above and in the published manuals and CFR rule publication).
- *** For an interim it was also named *Pseudokirchneriella subcapitata*. All names are synonymized.

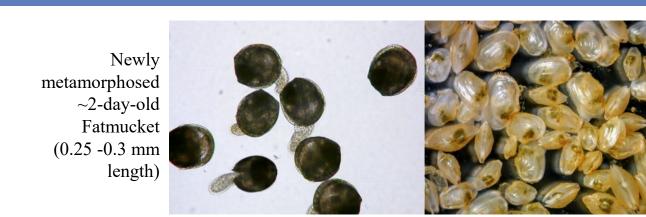
TWO TEST METHODS UNDER DEVELOPMENT

Adult *D. magna* with eggs in brood pouch (right)



Cladoceran, Daphnia magna sublethal test

- Daphnids are sensitive species and have been demonstrated to have reproducible results in a laboratory setting.
- Species is frequently used for toxicity testing for chemicals and for acute tests with effluents.
- As the *D. magna* chronic test, a three-brood test, is a 21-d test, this method focuses on developing growth test for *D. magna* at 25°C (rather than 20°C). Tests will be initiated with <24 h old young and the development of the method will include assessing growth at 4-d and 5-d. The growth endpoint will be a growth measurement rather than the three brood young production.



The Fatmucket - Lampsilis siliquoidea

- Widely distributed in North America.
- Long-term brooder, available through the year for culture/testing. The fatmucket appears to be among the most sensitive of all aquatic species to some contaminants.
- It is relatively easy to culture in the laboratory
- Sensitive and reproducible results are obtained in a laboratory setting.
- ASTM Standard Methods include
 - 24 h acute test with glochidia (viability)
 - 96 h acute with juvenile mussels (survival)
 - 28-d exposure with juvenile mussels for the growth and survival effects

LOOKING FOWARD

As the method development continues, the results will be presented at scientific meetings and collaborating with scientists that are members of the new Aquatic Toxicity Testing Interest Group (ATTIG) North America Society of Environmental Toxicology and Chemistry (SETAC) which has a charter for scientific exchange on toxicity methods for effluent. The ATTIG was formed to communicate the methods and learn from the scientific community and the ATTIG is composed of volunteers members that conduct toxicity testing (academia, government, and the business sector). This is a peer to peer discussion and has been effective for moving method development for a variety of species forward in a collaborative fashion.

Longer-term additional effluent toxicity test method may be needed for other invertebrate taxonomic groups so that we can enhance our understanding of the range of species sensitivities, aid



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DEVELOPMENT AND VALIDATION OF A DAPHNIA MAGNAFOUR-DAY SURVIVAL AND GROWTH TEST METHOD

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Abstract—Zooplankton are an important part of the aquatic ecology of all lakes and streams. As a result, numerous methods have been developed to assess the quality of waterbodies using various zooplankton species. Included in these is the freshwater specie Daphnia magna. Current test methods using D. magna involve acute lethality test methods ranging from 24 to 96 h in duration and chronic test methods with durations of 21 to 28 d. Whereas the current acute and chronic test methods are useful, a need exist for a shorter-duration test method that will provide a chronic or subchronic endpoint with this species. In the present study, a 4-d, static-renewal survival and growth test was developed for use with D. magna. The test results were compared to per for mance criteria and results from 7-d survival and reproduction tests with Ceriodaphnia dubia to determine the level of comparability between the two methods. Results from the 4-d D. magna survival and growth test method indicated that this method will produce consistent results with various reference toxicant materials and provide data that are both reproducible and useful for detecting potential toxicity in aquatic environments.

Keywords—Daphnia magna Test method Short-chronic Growth Ceriodaphnia dubia

1.3 Summary of Life History of Freshwater Mussels: 1.1 This standard guide describes methods for conducting laboratory toxicity tests with early life stages of freshwater in the family Unionidae or Margaritiferidae (section 10.1). Adults mussels including glochidia and juvenile mussels in water-only exposures (Annex A1). Future revisions to this standard may describe methods for conducting toxicity tests with (1) adult freshwater mussels and (2) conducting toxicity tests with (1) adult freshwater mussels and (2) contaminated sediments using various life stages of freshwater mussels. arious life stages of freshwater mussels.

U.S. Environmental Protection Agency Office of Research and Development

 Method development tests will be initiated with juveniles (1- to 3-wk-old) and after 7-

d, growth rates are determined.

This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee. Designation: E2455 – 06 (Reapproved 2013)

Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels

This standard is issued under the fixed designation E2455; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (e) indicates an editorial change since the last revision or reapproval.

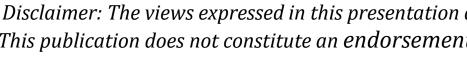
Algal mixture prepared daily: adding 1-mL Nano 3600[™] and 2-mL Shellfish Diet 1800[™] into 1.8-L water (~500 nl cell volume/mL; ASTM

2018)

in the detection of both known and unknown chemical and biological contaminants in wastewater and ambient waters and provide procedures to aid in prioritizing problem contaminants and mixtures.



Amphipod, *Hyalella azteca* Midge, Chironomus dilutus Mayfly (*Neocloeon triangulifer*)



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