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Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels[1]

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1. Scope

1.1 This standard guide describes methods for conducting laboratory toxicity tests with early life stages of freshwater mussels including glochidia and juvenile mussels in water-only and effluent exposures (Annex A1). Future revisions to this standard may describe methods for conducting toxicity tests with endpoints of reproduction, behaviors, and biomarkers.

1.2 Freshwater mussels (order Unionida) are one of the most imperiled groups of animals in the world, and environmental contamination has been linked as a contributing factor to the decline of mussel populations (Lydeard et al. 2004 (**1**); Strayer et al. 2004 (**2**); Haag 2012 (**3**); Lopes-Lima et al. 2017 (**4**)).[2] Three critical life stages (glochidia, juvenile mussels, and adults) have been used in toxicity assessments and the toxicity studies are separated according to the medium of exposure (water, sediment, and host fish (Ingersoll et al. 2007 (**5**)). Recent studies on early life stages of mussels have demonstrated that the mussels are among the most sensitive freshwater species to a variety of contaminants, including ammonia, some metals (for example, aluminum, copper, nickel, and zinc), and major ions (for example, chloride, nitrate, potassium, and sulfate) (Bringolf et al. 2007 (**6**); Newton et al. 2007 (**7**); Wang et al. 2007ab, 2010, 2011ab, 2016, 2017ab, 2018abc, 2020ab (**8-20**); Cope et al. 2008 (**21**); Gillis et al. 2008, 2010, 2011, 2021 (**22-25**); Miao et al. 2010 (**26**); Salerno et al. 2020 (**27**)). These studies indicate that environmental guideline values for individual chemicals established for the protection of aquatic organisms may not be adequately protective of sensitive stages of freshwater mussels. For example, when freshwater mussel toxicity data were included in an update to the United States Environmental Protection Agency (USEPA) ambient water quality criteria (WQC) for ammonia, the acute criterion decreased by about a 1.4 fold and the chronic criterion decreased by 2.4 fold (USEPA 2013) (**28**).

1.3 *Summary of Life History of Freshwater Mussels:*

1.3.1 Freshwater mussels are bivalve mollusks belonging to the taxonomic Order Unionida (section 10.1). Like most bivalves, mussels are totally aquatic, relatively sedentary, filter-feeding animals, and spend most of their lives partially or completely burrowed in the substrate of streams, rivers, or lakes. Freshwater mussels have an unusual and complex life cycle that includes a larval stage, the glochidium, that is briefly parasitic on fish (Fig. 1).

1.3.2 The successful transfer of mature glochidia to a suitable host constitutes a critical event in the life cycle of most freshwater mussels (Haag 2012) (**3**). Once the glochidia are released from the female, the glochidia need to attach to the gills or the fins of an appropriate fish host and encyst to complete development. Although glochidia may survive for months during brooding in the female mussel, glochidia typically survive for only a few days after release unless the glochidia reach a compatible host. Host fish specificity varies among mussels. While some mussel taxa appear to require a single host species, others can utilize several species of host fish. Encapsulation on the host occurs by overgrowth of host tissue. Within the capsule, glochidia obtain nutrition from the host, continue their development, and metamorphose within days to weeks. Metamorphosis is followed by excapsulation (drop-off) and transition to self-sustained existence as a benthic organism.

1.3.3 Both juvenile and adult mussels live embedded in sediment and feed using ciliary mechanisms to capture fine particles (Haag 2012) (**3**). However, young juvenile mussels (~0.2 mm –10 mm) may bury several centimeters in sediment where they feed and respire from interstitial water, while larger adult mussels can access the water column. Water column and substrate conditions suitable for adult life stages may not be protective of juvenile life stages of freshwater mussels.

1.4 *Summary of Toxicity Testing Conditions:*

1.4.1 Section 4 provides a summary of conditions for conducting toxicity tests with glochidia and juvenile mussels. Annex A1 provides guidance for conducting water-only laboratory toxicity tests with glochidia and juvenile mussels. Recommended test conditions for conducting these laboratory toxicity tests are based on various published methods and are

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[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

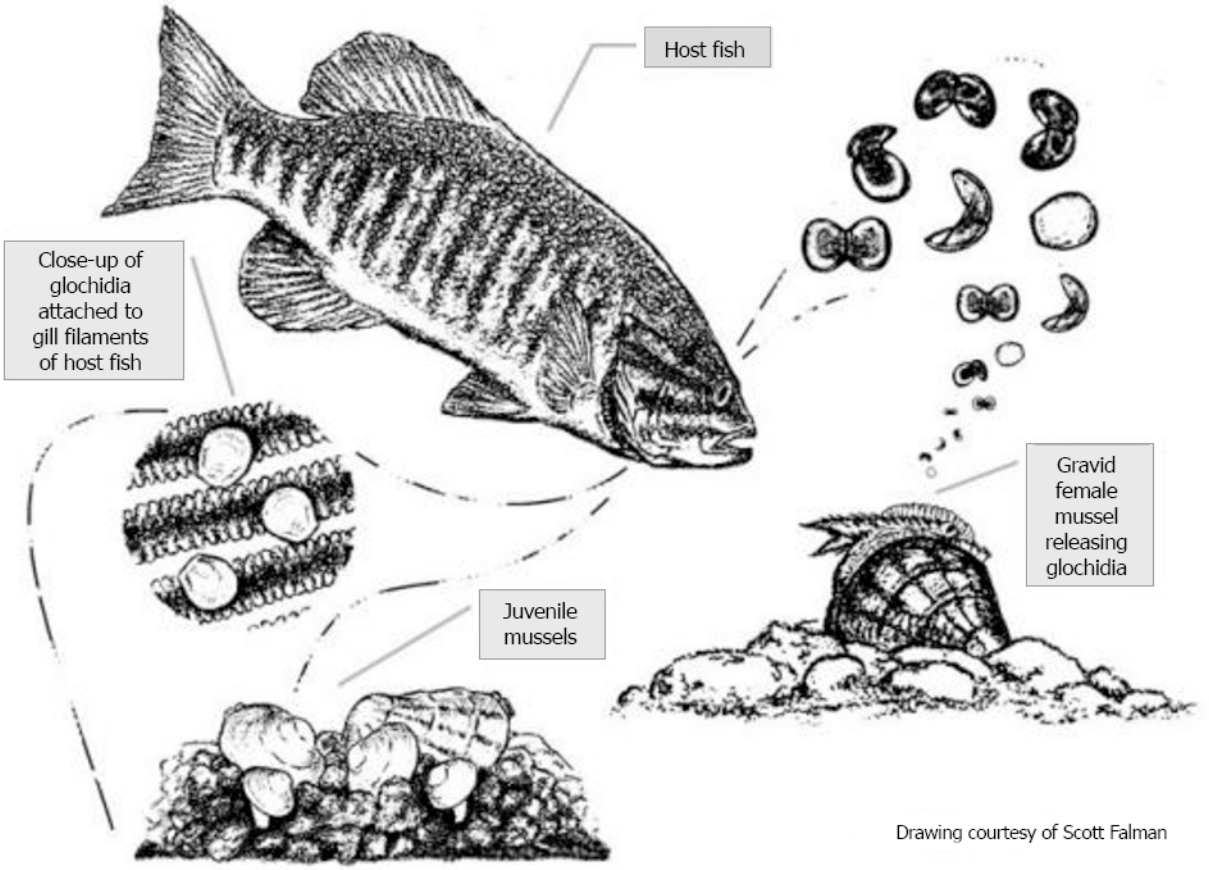

FIG. 1 Life Cycle of a Freshwater Mussel (Scott Faiman, Missouri Department of Conservation, Columbia, MO, USA)

based on the conditions used to conduct an intra- or inter-laboratory toxicity test with glochidia and juvenile mussels (section 16.5). Female mussels brooding matured glochidia are only available on a seasonal basis. Section 10 describes procedures for collecting brooding female mussels from the field and holding them in the laboratory to obtain glochidia for conducting toxicity tests or for obtaining glochidia to propagate juvenile mussels using a host fish. Juveniles propagated using host-free (*in vitro*) methods may or may not produce comparable results (section 10.5.4.2).

1.4.2 In the field, mussels may be exposed to contaminants in water, sediment, or food. This standard only addresses effects associated with exposure of mussels to contaminants in water.

1.4.3 Guide E1706 provides guidance for conducting sediment toxicity tests with juvenile mussels. Guide E2122 provides guidance for conducting *in situ* field exposures with caged mussels.

1.4.4 Results of tests, even those with the same species, using procedures different from those described in Annex A1 may not be comparable. Comparison of results obtained using modified versions of these procedures might provide useful information concerning new concepts and procedures for conducting toxicity tests with aquatic organisms. If tests are conducted with procedures different from those described in this standard, additional tests are required to determine comparability of results. General procedures described in this standard might be useful for conducting tests with other aquatic organisms; however, modifications may be necessary.

1.5 This standard is arranged as follows: