



Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels¹

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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 exposures (**Annex A1**). Future revisions to this standard may describe methods for conducting toxicity tests with (1) adult freshwater mussels and (2) contaminated sediments using various life stages of freshwater mussels.

1.2 Many factors are cited as potentially contributing to the decline of freshwater mussel populations in North America. Of the nearly 300 taxa of freshwater mussels in North America, 70 species (23 %) are listed as endangered or threatened and another 40 species (14 %) are candidates for possible listing (Williams et al 1993 (**1**); Neves 1997, 2004 (**2**, **3**)).² Habitat alteration, introduction of exotic species, over-utilization, disease, predation and pollution are considered causal or contributing factors in many areas of the United States (Neves et al 1997) (**4**). Over the past decade, there have been over 75 published studies conducted that have evaluated the role of contaminants in the decline of populations of freshwater mussels (Kernaghan et al 2005) (**5**). In these studies, early life stages of mussels of several species are highly sensitive to some metals and ammonia in water exposures when compared to many of the most sensitive species of other invertebrates, fish, or amphibians that are commonly used to establish U.S. Environmental Protection Agency Water Quality Criteria (WQC; Augspurger et al 2003 (**6**), Keller et al 2005 (**7**), Kernaghan et al 2005 (**5**); USGS (2005a,b) (**8**, **9**) section 1.5). Importantly, results of these previous studies indicate WQC for individual chemicals established for the protection of aquatic organisms may not be adequately protective of sensitive stages of freshwater mussels.

¹ This guide is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

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

1.3 Summary of Life History of Freshwater Mussels:

1.3.1 Freshwater mussels are bivalve mollusks belonging to the family Unionidae or Margaritiferidae (section 10.1). Adults are sedentary animals, spending their entire lives partially or completely burrowed in the bottoms of streams, rivers, or lakes. Adult mussels are filter feeders, using their gills to remove suspended particles from the water column. The microscopic, juvenile stage uses foot (pedal) feeding to some degree for the first several months of their lives, feeding on depositional materials in pore water of sediment, including bacteria, algae, and detritus. Freshwater mussels have an unusual and complex mode of reproduction, which includes a brief, obligatory parasitic stage on fish or other host organisms called glochidia (**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. 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. Encystment on the host occurs by overgrowth of host tissue. Metamorphosis of juvenile mussels on the fish host occurs within days or weeks, depending on species and temperature. Host fish specificity varies among mussels. While some mussel species appear to require a single host organism, other species can transform their glochidia into juvenile mussels on several species of host fish. Following proper host infestation, glochidia transform into microscopic juveniles and excyst (drop off) and settle into suitable habitat to survive. The transformation of glochidia to juveniles results in the development of internal organs necessary for self-sustained existence as a benthic organism.

1.3.3 Newly-transformed juvenile mussels have a life style different from adult mussels. Transformed juvenile mussels may be at the sediment-water interface or may burrow several centimeters into sediment and rely on water percolating between substrate particles of sediment for food and oxygen. Newly-transformed juvenile mussels feed using ciliary currents