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Developing Standardized Guidance for Conducting Toxicity Tests With Glochidia of Freshwater Mussels

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Abstract

A joint three-year research project between USGS, USFWS, and USEPA has been started to evaluate the methods for conducting toxicity tests with various life stages of freshwater mussels. One of the objectives of this research is to develop standardized guidance for conducting toxicity tests with glochidia of freshwater mussels. Multiple tests were conducted to assess the survival times of glochidia isolated from different mussel species or different individuals of the same species. Copper and ammonia toxicity tests with glochidia were also conducted to examine the influences of exposure periods, different test chambers, and different individuals or ages of glochidia to test results. Glochidia were held in 24-well polystyrene tissue-culture plates, in crystallizing dishes, or in beakers under flow-through conditions. There were no obvious differences in the survival times of glochidia held in various containers. Over 90% survival of glochidia was observed for > 4 d with *Potamilus ohiensis* and *Lampsilis siliquoidea*. In contrast, the survival of glochidia of other species (*Quadrula quadrula*, *Q. pustulosa, Leptodea fragili, Venustaconcha ellipsiformis*) was >90% for less than 1 or 2 d. EC50s of both chemicals decreased over the exposure periods of 6 to 96 h. Copper concentrations reduced substantially in polystyrene plates but not in glass dishes after 48 h exposure period, whereas ammonia concentrations reduced in both plates and dishes. Different EC50s were observed when using glochidia isolated from different females of *Lampsilis siliquoidea* but not found when using 2 or 24-h old glochidia from a female. These results suggest that the toxicity tests with glochidia of many freshwater mussels could be conducted for 24 or 48 h with a control survival of over 90% although shorter or longer tests might be needed for a particular species depending on glochidia survival time and the life history characteristics of this species.

Background

Glochidia of freshwater mussels have been found to be sensitive to many chemicals compared to other invertebrates, fish, or amphibians that are commonly tested (e.g., Keller and Ruessler 1997; Augspurger et al. 2003). However, USEPA has expressed concerns regarding the use of toxicity data generated with glochidia in the derivation of water quality criteria (Charles Stephan, USEPA, Duluth, MN; personal communication). A joint three-year research project between USGS, USFWS, and USEPA has been started to evaluate the methods for conducting toxicity tests with various life stages of freshwater mussels.

Study Questions

- How long should a toxicity test with glochidia be conducted?
- Is there any different result when using various test chambers?
- What criteria should be used to judge acceptability of toxicity tests with glochidia?
- Is there any different result when using glochidia from different females of the same species or using glochidia of different ages?

Materials and Methods

Survival tests:

- Adult mussels: Collected from Missouri; held in flow-through baths.
- Glochidia collection: Flushed from the gills of a female (Photo 1).
- Test water: Reconstituted water, hardness 170 mg/L as $CaCO_3$, pH 8.3, 20°C.
- Glochidia viability: Confirmed by about 300 glochidia using saturated NaCl solution; tests started if viability >90%.
- Test chambers: 24-well polystyrene tissue-culture plates, 200-ml crystallizing dishes, or 250-ml beakers in an intermittent flow diluter system (Photo 2 and 3).
- No. glochidia per replicate: 20 to 30 in a well, 1000 to >5000 in a dish or beaker.
- No. replicates: 3 wells or 3 subsamples of about 100 individuals from 3 dishes or beakers.
- Water addition: every other day for wells and dishes; 7 times/d for beakers in the diluter system.
- Survival duration: determination of percentage survival of glochidia each day by their response to the addition of a saturated solution of NaCl.

Toxicity tests:

- Test chemicals: Copper or ammonia
- Test type: Static or static-renewal
- Test Duration: 6, 24, 48, 72, or 96 h
- Temperature: 20±1°C
- Light quality: Ambient laboratory light
- Light intensity: 10-20 uE/m^2/s
- Photoperiod: 16L:8D

- Test chamber size: 24-well polystyrene plate or 200-mL crystallizing dish
- Test solution volume: 4 mL in well, 100 ml in dish
- Renewal of solution: After 48 h
- Age of test organism: <2-h old (or depending on the study objective)
- Organisms/chamber: 20 to 30/well, 1000 to 2000/dish
- Replicates/concentration: 3 wells or 3 subsamples of about 100 individuals from 3 dishes
- Feeding: None
- Aeration: None
- Dilution water: Reconstituted water (160-180 mg/L as $CaCO_3$, pH 8.3)
- Dilution factor: 0.5
- Test concentration: 6 or 7 concentrations including a control
- Water quality: DO, pH, conductivity, hardness, and alkalinity at least at the start and end of tests
- Endpoint: Survival if valve closure with NaCl (EC50 calculated based on nominal concentration; TOXSTAT 3.5, West, Inc. 1996)
- Test acceptability: >90% survival in control

Results and Discussion

Survival tests

- Survival times of glochidia of six species ranged from <2 to >8 d (Figure 1); over 90% survival of glochidia was observed for ≥4 d with *Potamilus ohiensis* and *Lampsilis siliquoidea* but for ≤1 d with *Quadrula quadrula, Q. pustulosa, Leptodea fragilis*, and *Venustaconcha ellipsiformis*.
- There was no substantial difference in the survival of glochidia of *Lampsilis siliquoidea* held in various test chambers under static-renewal or flow-through conditions during the first three days (Figure 2).
- These results suggest that the toxicity tests with glochidia of *P. ohiensis* and *L. siliquoidea* could be conducted for 48 h with a control survival of over 90% in multi-well plates, in crystallizing dish, or in beakers under flow-through conditions, whereas toxicity tests with the other four species might need to be conducted for shorter periods to maintain a control survival of >90%.
- Previous studies have indicated that glochidia of most species tested can maintain >80% survival for more than 1 or 2 d (Table 1).

Copper toxicity tests

- Some difference in copper EC50s was observed between glochidia from two individual females of *L. siliquoidea* (Figure 3), suggesting the potential variation of toxicity sensitivity between individual females.
- Copper EC50s decreased over the exposure periods (Figure 3), suggesting that a 48-h toxicity test should be considered if glochidia are able to survive for >2 d.
- Copper EC50s for glochidia held in glass dishes were consistently lower than in 24-well polystyrene plates (Figure 3). This was consistent with the measured copper concentrations, which decreased during 48-h exposures in the plates but did not

change in the dishes (Figure 4). The decrease of copper concentration in the plates presumably resulted from adsorption losses of copper on the polystyrene surface.

- Copper EC50s were similar when using 2- or 24-h old glochidia of *L. siliquoidea* (Figure 5), indicating that sensitivity of glochidia held for 24 h after removal from a female was similar to newly released glochidia.

Ammonia toxicity tests

- Ammonia EC50s decreased over the exposure periods (Figure 6), as observed in copper tests (Figure 3).
- Ammonia EC50s for glochidia held in dishes were similar to those in plates (Figure 6).
- Ammonia concentrations decreased during 48-h exposures in both plates and dishes (Figure 7), except one in dish after 48-h exposure (perhaps due to the mass of dead glochidia), indicating the necessity of renewing ammonia solution within 24 h.

Conclusions

- 24 or 48 h was a reasonable time period to conduct toxicity tests with glochidia of most species evaluated although shorter or longer tests might be needed for a particular species depending on glochidia survival time and the life history characteristics of this species.
- >80% control survival could be considered as the acceptability of a 24- or 48-h toxicity test with glochidia.
- Using large glass dishes for toxicity tests had more volume of water available for conducting water quality and chemical analyses, and might maintain more stable chemical concentration during the 48-h exposure period than using polystyrene multi-well plates.

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Table 1. Survival time of glochidia after removal from female mussels.

Species	Temp. C	Survival time Day (% survival)	References
Actinonaias pectorosa	10	13 (75)	Zimmerman and Neves (2002)
	25	5 (75)	Zimmerman and Neves (2002)
	20	>2 (90)*	Jacobson et al. (1997)
Anodonta anatina	13	>3 (90)	Huebner and Pynnonen 1992
Anodonta cygnea	13	>3 (90)	Huebner and Pynnonen 1992
Anodonta cataracta	10	>14 (90)	Jacobson (1990)
Anodonta grandis	10	>14 (90)	Jacobson (1990)
Elliptio complanata	5	7 (NR)	Matterson (1948)
Lampsilis cardium	21	>2 (90)*	Lasee (1991)
Lampsilis fasciola	20	>2 (90)*	Jacobson et al. (1997)
Lampsilis r. siliquoidea	10	9 (NR)	Tedla and Fernado (1969)
Lampsilis siliquoidea	20	4 (90)	This study
	25	>2 (80)*	Keller and Ruessler (1997)
Lampsilis teres	25	0.2 (80)*	Keller and Ruessler (1997)
Leptodea fragilis	20	1 (90)	This study
Margaritifera falcata	11	11 (NR)	Murphy (1942)
Medionidus conradicus	20	>2 (90)*	Jacobson et al. (1997)
Megalonaias nervosa	25	>1 (80)*	Keller and Ruessler (1997)
Potamilus ohiensis	20	5 (90)	This study
Pyganodon grandis	20	>1 (90)*	Jacobson et al. (1997)
Quadrula quadrula	20	1 (90)	This study
Quadrula pustulosa	20	<1 (90)	This study
Utterbackia imbecillis	21	10 (80)	Fisher and Dimock (2000)
	25	>2 (80)*	Keller and Ruessler (1997)
	25	>2 (80)*	Klaine et al. (1997)
	20	>1 (95)*	Johnson et al. (1990, 1993)
Venustaconcha ellipsiformis	20	1 (90)	This study
Villosa iris	10	8 (75)	Zimmerman and Neves (2002)
	25	2 (75)	Zimmerman and Neves (2002)
	22	>1 (80)*	Goudreau et al. 1993
	20	>1 (80)*	Scheller (1997)
	20	>2 (90)*	Jacobson et al. (1997)
Villosa lienosa	25	>2 (80)*	Keller and Ruessler (1997)
Villosa villosa	25	>2 (80)*	Keller and Ruessler (1997)
Villosa nebulosa	20	>2 (90)*	Jacobson (1990)

* The value is based on control survivals of 24- or 48-h toxicity tests.

NR: not reported.

Photo 1. Glochidia were gently flushed from a female Mussel using a syringe with water.

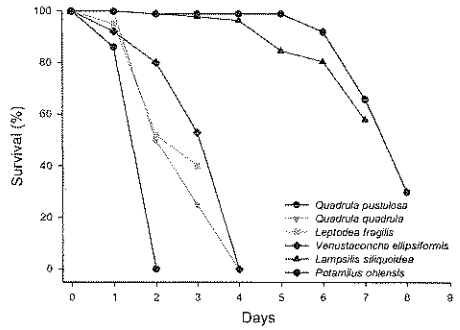

Figure 1. Survival of glochidia after removal from female mussels of six species.

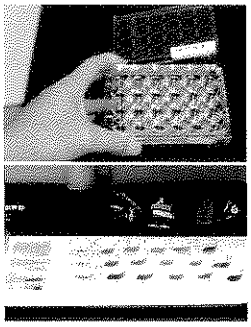

Photo 2. 24-well polystyrene tissue-culture plates and 200-ml crystallizing dishes.

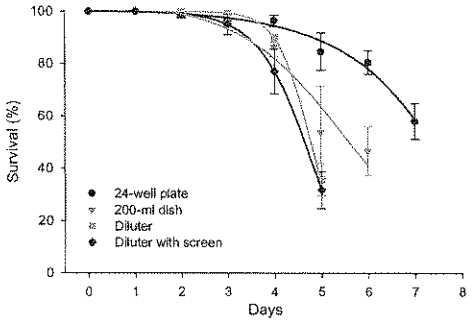

Figure 2. Mean survival (±SE, n=5) of glochidia isolated from *L. siliquoidea* female and held in different test chambers. Regression lines (r^2>0.95) are best fits showing a general trend of the data.

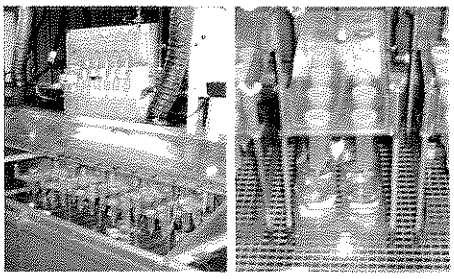

Photo 3. 250-ml beakers in an intermittent flow diluter system; 7 additional water every day.

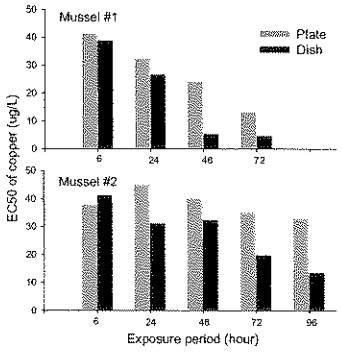

Figure 3. Copper EC50s for glochidia isolated from two female *L. siliquoidea* and held in 24-well plates and 200-ml dishes over various exposure periods.

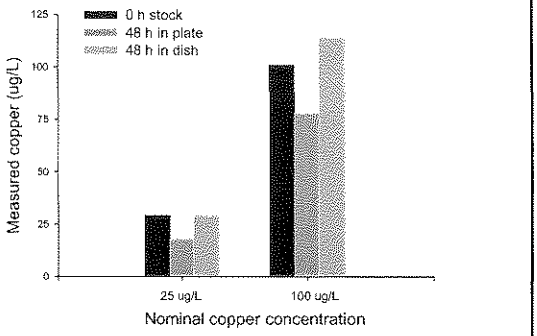

Figure 4. Measured concentrations of copper in 24-well polystyrene plates and in 200-ml glass dishes at the beginning and end of a 48-h exposure.

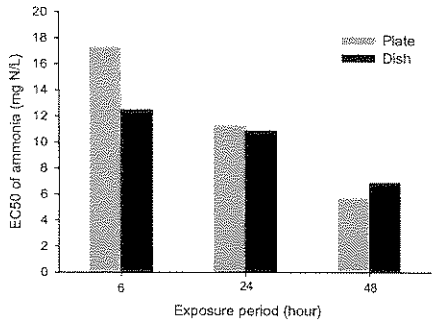

Figure 6. EC50s of total ammonia for glochidia of *P. ohiensis* held in 24-well polystyrene plates and 200-ml glass dishes.

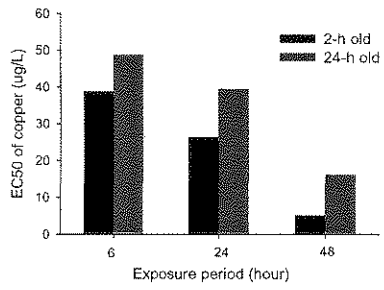

Figure 5. Copper EC50s for 2- or 24-h-old glochidia of *L. siliquoidea* over three exposure periods.

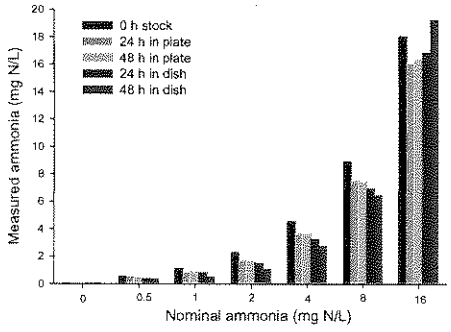

Figure 7. Measured concentrations of total ammonia in 24-well plates or in 200-ml glass dishes during the 48-h exposure.