Comparative Response of *Ceriodaphnia dubia* and Juvenile *Anodonta imbecillis* to Selected Complex Industrial Whole Effluents

Donald C. Wade, Robert G. Hudson, and Aubrey D. McKinney
Tennessee Valley Authority, Muscle Shoals, Alabama
Presbyterian College, Clinton, South Carolina
Tennessee Wildlife Resources Agency, Nashville

Abstract. The responses of *Ceriodaphnia dubia* as test organisms in 7-day chronic daily-renewal bioassay and 6-day-old *Anodonta imbecillis* in 9-day acute daily-renewal bioassay are compared. A value for relative sensitivity to selected complex industrial whole effluent samples is generated following determination of an observed and calculated no observable effect concentration. Results from tests using effluents from two pulp and paper facilities and a coal-fired steam electric facility ash pond indicate that juvenile mussels are more sensitive than *Ceriodaphnia* to selected effluents and should be used to assess the toxicity of effluent releases to waters with freshwater mussels.

Introduction

Treated industrial effluents have a major impact on the nation's surface waters. The U.S. Environmental Protection Agency (EPA), in the 1990 technical support document for water quality–based toxics control, suggests a comprehensive approach to establishing effluent guidelines under the National Pollutant Discharge Elimination System (NPDES). This approach includes evaluation of chemical-specific standards, whole effluent toxicity limitations based on bioassay results using standardized test methodology, and instream biosurvey information to evaluate the impact of effluent releases on the various components of the aquatic community in the receiving stream.

At present, both acute and chronic standardized whole effluent test procedures have been adopted by EPA using surrogate organisms such as *Ceriodaphnia dubia* to evaluate benthic community response to industrial releases. The nationwide decline in freshwater mussels may indicate that currently accepted bioassay methodologies are inadequate to evaluate the response of mussels to complex whole effluents or selected contaminants.

Isom and Hudson (1984) published a methodology for the transformation of freshwater mussel glochidia into juvenile mussels using complex liquid growth media in a modified in vitro cell culture technique. Wade et al. (1989) reported the successful development and use of a 9-day acute toxicity test utilizing artificially transformed juvenile (6–8 day) *Anodonta imbecillis*. The advantages of utilizing *Anodonta imbecillis* as a test species include relatively wide distribution and availability, tolerance of transport and relocation to a laboratory setting, extended annual period when adults are gravid, and successful transformation of glochidia to juveniles in culture media.

Previous bioassays using juvenile *Anodonta* exposed to selected contaminants suggest a minimum 5- to 9-day exposure is required to realize an acute endpoint effect. The availability of a 9-day acute bioassay methodology using juvenile *Anodonta imbecillis* affords the opportunity to evaluate the comparative response of juvenile mussels and *Ceriodaphnia dubia* exposured to selected industrial whole effluents.

Methods

Effluents were collected by grab sample from three industrial discharges believed to exhibit negligible waste stream variability. Approximately 10 liters are required for the 7-day chronic *Ceriodaphnia* test and 30 liters for the 9-day acute juvenile mussel test. Effluent samples are iced for transport and maintained in the laboratory at 4°C.

Seven-day daily-renewal chronic tests using *Ceriodaphnia dubia* follow the recommendations of EPA (1989), using neonates less than 24 hours old. Test concentrations generally included exposure to 100%, 50%, 25%, and 12.5% effluent. Results were statistically analyzed using a computer program

Table 1. Protocol for conducting toxicity tests using juvenile freshwater mussels.

Test species	*Anodonta imbecillis* (freshwater mussel, Unionidae)
Test type	Solid phase, static, daily renewal
Temperature	24 ± 1°C
Photoperiod	Dark
Test chamber volume	250 ml
Renewal of test solutions	Daily
Age of test organisms	6–10 days*
Number mussels per replicate	15
Replicates per concentration	3
Feeding regime	Daily – Concentrated indigenous algae bloomed in dilution water. Also clean silt (filtered through 100-µm Nitex® to facilitate visual observations)
Aeration	None
Dilution water	5 µm bag filtered receiving water – No dilution required in screening study
Test duration	9 days
Effect measured	Stress – Impaired movement Mortality – Absence of ciliary action or empty shells

* Younger mussels can be used; however, by 6 days the activity of young mussels has increased to a level that ensures inclusion of viable individuals into the test.

provided by EPA. Toxicity endpoints include reproduction and mortality. Data are interpreted to establish values for 96-hour LC_{50}, 7-day LC_{50}, and 7-day no observable effect concentration (NOEC).

Nine-day daily renewal acute tests using juvenile (6 days old) *Anodonta imbecillis* follow the methodology reported by Wade et al. (1989). Tests used daily static renewal where 15 juvenile mussels in 250-ml test chambers were exposed to three replicates of each effluent concentration. Each daily replicate was provided with 800 mg of prescreened (100 µm) silt and 6 ml of algal concentrate per liter. Mussels were confined in 50-mm-diameter glass cylinders fitted with a bottom of 100 mm Nitex^R screen to facilitate handling, observation, and daily renewal. Wade (1990) summarized both the test protocol (Table 1) and toxicity endpoints (Table 2). During the test, physical/chemical monitoring includes dissolved oxygen, temperature, and pH measurements initially and at the conclusion of each 24-hour period prior to renewal. Data are interpreted to establish acute LC_1, LC_{50}, and LC_{99} concentrations.

Table 2. Toxicity endpoints: criteria used to evaluate stress and differentiate between living and dead juveniles.

Alive/active	Foot protrudes from shell within a minute of settling to the bottom of the dish; foot locates dish bottom and "flips" shell into an upright position; mussel glides along dish bottom extended foot.
Alive/stressed	Shell remains closed or gapes open with foot immobile and protruding; close observation reveals only slight foot motion; ciliary action obvious.
Dead	Shell gaped open with detrital material adhered to soft tissues just within the shell (indicating absence of ciliary movement which sweeps this area clean in live, active and stressed, mussels); and/or decomposition of soft parts (indistinguishable foot, organs, etc., usually accompanied by protozoans within the shell); or empty shells.

Results and Discussion

There are several possibilities for developing a chronic NOEC value from data generated by an acute test. EPA (1990) recommends the use of an acute to chronic ratio (ACR) for establishing a NOEC for the release of complex whole effluents. An ACR of 10 (where the acute $LC_{50}/10$ = chronic NOEC) is recommended where good acute data are available but chronic data are either inadequate or unavailable. Another approach is recommended by Schweinforth and Wade (1990), in which an acute 9-day test and a sub-chronic 90-day test are conducted utilizing a reference toxicant. The ACR resulting from the use of manganese as a reference is 6 (where the acute $LC_{50}/6$ = chronic NOEC).

The relative sensitivities of *Ceriodaphnia dubia* in a 7-day chronic test and *Anodonta imbecillis* in a 9-day acute test are presented for two comparative methodologies as follows and in Tables 3 and 4. Comparative results using both EPA and manganese source reference methodologies are summarized in Table 5.

Establishing a chronic NOEC from an acute 9-day test utilizing *Anodonta imbecillis* should take into consideration that this mussel is likely to be more tolerant of selected pollution-related stresses than are many other mussel species. As a group, *Anodonta* are considered to be quite tolerant of elevated temperature, low dissolved oxygen, siltation, and extended periods of no flow. *Anodonta*'s successful exploitation of ponds in agricultural settings may indicate a tolerance for selected organic contaminants.

EPA advocates the use of standardized bioassay methodology for whole effluent tests and has

Table 3. Sensitivity of juvenile mussels (*Anodonta imbecillis*) and *Ceriodaphnia* to industrial complex effluents from three sources using the EPA-recommended ACR.

	*Ceriodaphnia**	*Anodonta**
Pulp and paper effluent		
LC_{50}	>100%	38.6%
NOEC : Survival	>100%	
NOEC : Growth	25%	3.86%**
Relative sensitivity	25.00/3.86 = 6.50	
Paper processing effluent		
LC_{50}	9.6%	2.2%
NOEC : Survival	6.3%	
NOEC : Growth	3.13%	0.22%**
Relative sensitivity	3.13/0.22 = 14.2	
Steam plant ash pond effluent		
LC_{50}	>100%	100%
NOEC	>100%	
Relative sensitivity: No toxicity endpoint reached		

* *Ceriodaphnia*: 7-day chronic test. *Anodonta*: 9-day acute test.

** Based on EPA suggested conversion LC_{50} to NOEC (ACR = 10) for nonpersistent waste.

Table 4. Sensitivity of juvenile mussels (*Anodonta imbecillis*) and *Ceriodaphnia* to paper company complex effluents using observed ACR for manganese.

	*Ceriodaphnia**	*Anodonta**
Pulp and paper effluent		
End-of-test mortality in 100% effluent	0%	100%
Test endpoint*		
Survival	NOEC > 100%	LC_{50} = 38.63%
Growth	NOEC = 25%	NOEC = 6.4%**
Relative sensitivity	25/6.4 = 3.9	
Paper processing effluent		
End-of-test mortality in 100% effluent	100% (10% in 6.25% effluent)	100% (100% in 5% effluent)
Test endpoint*		
Survival	NOEC = 6.25%	LC_{50} = 2.20%
Growth	NOEC = 3.13%	NOEC = 0.37%**
Relative sensitivity	3.13/0.37 = 8.7	

* *Ceriodaphnia*: 7-day chronic test. *Anodonta*: 9-day acute test.

** Based on ACR = 6; 9-day acute vs. 90-day subchronic exposure of juvenile mussels to Mn.

Table 5. Comparison of sensitivity* as calculated using the EPA method and the TVA 9-day acute/90-day subchronic exposure to manganese reference.

	EPA	Mn reference
Pulp and paper effluent	6.5	3.9
Paper processing effluent	14.2	8.7
Steam plant ash pond effluent	1	1
	No toxicity endpoint reached	

* Listed factors by which *Anodonta* are more sensitive than *Ceriodaphnia* in whole-effluent bioassay.

published procedures utilizing surrogate organisms to represent critical lifestages of the benthic community organisms. Results to date indicate that *Ceriodaphnia dubia* are less sensitive than *Anodonta imbecillis* to selected complex whole effluents and may be inadequate as a surrogate for the freshwater mussel component of the benthic community.

The need for a standardized bioassay test procedure utilizing juvenile mussels in an acute test is, at present, weighed against both the considerable expense of gathering sufficient information to support the adoption of a standard methodology, including the response of juvenile mussels to reference toxicants, and the fact that so few states have significant mussel resources that it is difficult to raise mussel bioassay to a priority status.

At the federal level, two independent programs may find an increasing need for juvenile mussel testing. First, a rapidly increasing number of native freshwater mussels are either listed or are proposed for listing as federally protected endangered species by the U.S. Fish and Wildlife Service. Juvenile mussel testing could be a valuable component of required recovery plans and identification of sources of toxicity. Second, EPA has recently undertaken to establish criteria for contaminants in sediments. Because available bioassay test procedures for juvenile mussels are readily adaptable to evaluate both sediment and sediment interstitial (pore) water, there is an opportunity to promote adoption of a standardized methodology. Additionally, McCann and Neves (1992) have clearly demonstrated the utility of early life stage mussel bioassay for evaluation of the toxicity of selected contaminants. A cooperative effort by states with significant mussel resources could result in whole effluent bioassay evaluations more protective of mussels than currently recognized methodology using surrogate species. Research is needed to evaluate the response of juvenile mussels to a wide variety of reference toxicants and the various categories of complex whole industrial effluents. Municipal discharges with significant industrial pretreatment sources are also candidates for evaluation.

Acknowledgments

This project is a cooperative effort which includes the Tennessee Department of Environment and

Conservation, Division of Water Pollution Control. *Ceriodaphnia* testing was conducted by EMPE, Inc. of Nashville, Tennessee. Janet Posey, TVA Aquatic Research Laboratory, played a major role in this effort.

Literature Cited

Isom, B.G., and R.G. Hudson. 1982. In vitro culture of parasitic freshwater mussel glochidia. Nautilus 96(4): 147–151.

Isom, B.G., and R.G. Hudson. 1984. Culture of freshwater mussel glochidia in an artificial habitat utilizing complex liquid growth media. U.S. Patent 4,449,480.

McCann, M.T., and R.J. Neves. 1992. Toxicity of coal related contaminants to early lifestages of freshwater mussels in the Powell River, Virginia. Virginia Cooperative Fish and Wildlife Research Unit, Department of Fisheries and Wildlife Services. Virginia Polytechnic Institute and State University, Blacksburg. 92 pp.

Schweinforth, R.L., and D.C. Wade. 1990. Effects from subchronic 90-day exposure of invitro transformed juvenile freshwater mussels (*Anodonta imbecillis*) to manganese. Abstract 11th annual meeting, Society of Environmental Toxicology and Chemistry, Arlington, Virginia, 11–15 November.

U.S. Environmental Protection Agency. 1989. Cladocern survival and reproduction test. EPA/600/4-89/001, Method 1002.00.

U.S. Environmental Protection Agency. 1990. Technical support document for water quality–based toxics control.

Wade, D.C. 1990. Screening toxicity evaluation of Wheeler Reservoir sediments using juvenile freshwater mussels (*Anodonta imbecillis* Say) exposed to sediment interstitial water. Tennessee Valley Authority. WR/AB-90/13. 28 pp.

Wade, D. C., R. G. Hudson, and A. D. McKinney. 1989. The use of juvenile freshwater mussels as a laboratory test species for evaluating environmental toxicity. Poster presented at 10th annual meeting, Society of Environmental Toxicology and Chemistry, Toronto, Ontario, 28 October–2 November.