# Sensitivity of freshwater mussels at two life stages to acute or chronic effects of NaCl or KCl

Chris D Ivey[1], Rebecca A Consbrock[1], James L Kunz[1], Chris G Ingersoll [1], Ning Wang[1], William Brumbaugh[1], Ed Hammer[2*], Candice R Bauer[2*], Chris M Barnhart[3]

[1]USGS, Columbia, MO USA; [2*] USEPA, Chicago, IL USA; [3]Missouri State University Springfield, MO USA

## Abstract

Native freshwater mussels are in serious global decline and urgently need protection and conservation. Nearly 70% of the 300 species in North America are endangered, threatened, of special concern, or already extinct. The declines in the abundance and diversity of North American mussels have been attributed to a wide array of human activities that cause pollution, water-quality degradation, and habitat destruction and alteration. Limited studies indicate that freshwater mussels may be more sensitive to major ions such as chloride and potassium than commonly tested organisms used to derive US Environmental Protection Agency (USEPA) ambient water quality criteria (AWQC). The objectives of these studies were to 1) Evaluate the acute sensitivity of mussel species to Cl (tested as NaCl) or K (tested as KCl), 2) Evaluate the influence of hardness on toxicity of Cl to glochidia and juveniles of fatmucket in acute exposures, 3) Evaluate the chronic effects of Cl or K on juvenile fatmucket survival, weight and biomass in 28-day exposures. Acute exposures were conducted with the glochidia and juveniles of fatmucket in waters at different hardnesses ranging from 50 to 300 mg/L as $CaCO_3$. Newly transformed or about 2 month-old juveniles of up to 10 mussel species were tested in 96-hour acute Cl exposures and newly transformed juveniles of six mussel species in 96-hour K exposures at hardness 100 or 170 mg/L as $CaCO_3$. In chronic 28-day exposures the effects of Cl or K were evaluated on juvenile fatmucket survival, weight, and biomass. Acute median effect concentrations (EC50s) from the glochidia tests increased from 409 mg Cl/L at hardness 50 mg/L to 1633 mg Cl/L at hardness 300 mg/L, and from 798 mg Cl/L at hardness 50 to 2976 at hardness 300 for juvenile fatmucket. Acute EC50s for newly transformed juveniles ranged from 1281 to 2379 mg Cl/L and the EC50s for the 2-month-old juveniles ranged from 1842 to 3172 mg Cl/L at a hardness of 100 to 170 mg/L $CaCO_3$. Most of these EC50s for Cl were below the final USEPA acute value (FAV) of 1720 mg Cl/L. The 28-d Cl EC20 for fatmucket based on biomass was 245 mg Cl/L. Acute EC50s for K for newly transformed juveniles of the six mussels ranged from 31 to 61 mg K/L, which makes these mussels the most sensitive species in a toxicity database for K. Results indicate that mussels are generally more sensitive to Cl and K compared to other species in national toxicity databases, and USEPA acute AWQC for Cl may not be protective of freshwater mussels.

## Introduction

Limited studies indicate that freshwater mussels may be more sensitive to major ions such as chloride (Cl; tested as NaCl) or potassium (K; tested as KCl) than commonly tested organisms used to derive the USEPA AWQC. The objective of these studies was to evaluate the sensitivity of various species of freshwater mussels at various life stages (glochidia, newly transformed juveniles, or about 2-month-old juveniles) in acute or chronic exposures to Cl or K.

## Objectives

- Evaluate the acute sensitivity of up to ten mussel species to Cl (tested as NaCl) or K (tested as KCl)
- Evaluate the influence of hardness on toxicity of Cl to glochidia and juveniles of fatmucket in acute exposures
- Evaluate the chronic effects of Cl or K on juvenile fatmucket survival, weight and biomass in 28-day exposures

* The views expressed herein do not necessarily represent the views of the U.S. EPA or the United States.

## Methods

**Table 1. Summary of test conditions for conducting toxicity tests with juvenile mussels in basic accordance with ASTM (2012)**

| | |
|---|---|
| Test species: | Up to 10 freshwater mussel species |
| Test chemicals: | Sodium chloride (NaCl) or Potassium chloride (KCl) |
| Test type: | Static renewal (acute); Flow through (chronic) |
| Test Duration: | 96 h (acute); 28 d (chronic) |
| Temperature: | 20 or 23±1°C |
| Light quality: | Ambient laboratory light |
| Light intensity: | About 500 lux |
| Photoperiod: | 16L:8D |
| Test chamber size: | 50 ml (acute); 300 ml (chronic) |
| Test solution volume: | 30 ml (acute); 2 volume additons/day (chonic) |
| Renewal of solution: | After 48 h (acute) |
| Age of test organism: | <5-d to 4-month old (acute); about 2-month old (chronic) |
| No. organisms per test chamber: | 5 (acute); 10 (chronic) |
| Replicates/concentration: | 4 |
| Feeding: | No feeding (acute); 2ml algal solution 2x day (chronic) |
| Chamber cleaning: | None (acute); bi-weekly renewal of sand (chronic) |
| Dilution water: | (1) Reconstituted ASTM moderately hard water (hardness 100 mg/L as $CaCO_3$ (2) CERC well water (hardness about 300 mg/L), (3) diluted well water (50, 100, and 200 mg/L hardness) |
| Dilution factor: | 0.5 |
| Chemical residues: | Cl, and K measured throughout the study |
| Water quality: | DO, pH, conductivity, hardness, and alkalinity determined at the control, medium, and high concentrations of chemicals at beginning and end of test. |
| Endpoint: | Survival (foot movement), dry weight and biomass (chronic only) |
| Test acceptability criterion: | >90% control survival (acute); >80% control survival (chronic) |

**Table 2. Summary of test conditions for conducting toxicity tests with glochidia in basic accordance with ASTM (2012)**

| | |
|---|---|
| Test species: | Fatmucket (*Lampsilis siliquoidea*) |
| Glochidia collection: | Flush female mussel gills with water from syringe |
| Test chemical: | Sodium chloride (NaCl); Potassium chloride (KCl) |
| Test type: | Static |
| Test duration: | 24 h |
| Temperature: | 20±1°C |
| Light quality: | Ambient laboratory light |
| Light intensity: | About 500 lux |
| Photoperiod: | 16:8 h light:dark |
| Test chamber: | 300 ml glass beaker |
| Test solution volume: | 100 ml |
| Renewal of solution: | None |
| Age of test organism: | <2 h after releasing from marsupium |
| Organisms/chamber: | About 500 |
| Replicates/concentration: | 3 |
| Feeding: | None |
| Dilution water: | (1) Reconstituted ASTM moderately hard water; hardness 100 mg/L as $CaCO_3$ (2) CERC well water (hardness about 300 mg/L), (3) diluted well water (50, 100, and 200 mg/L hardness) |
| Dilution factor: | 0.5 |
| Chemical residues: | Cl, and K measured throughout the study |
| Water quality: | Dissolved oxygen, pH, hardness, and alkalinity measured in the control, medium, and high exposure concentrations, and salinity and conductivity measured in all concentrations at 0 and 24 h |
| Endpoint: | Survival (valve closure with the addition of NaCl) |
| Test acceptability: | ≥90% survival in controls |

Photo 1. CERC Biologist Ning Wang checking juvenile mussel survival

Photo 2. Fatmucket mussel juvenile

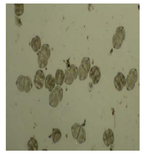


Photo 3. Dwarf mussel glochidia

## Results and Discussion

### Chloride (Cl; tested as NaCl)

Figure 1A. Acute EC50s for Cl in 96-hour exposures with juvenile mussels; hardness 100-170 mg/L as $CaCO_3$
*Error bars indicate 95% confidence limits (CL) or standard deviation; no CL due to no or only one partial kill*

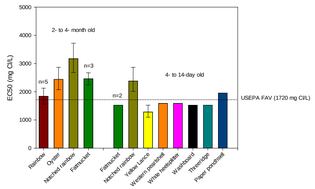
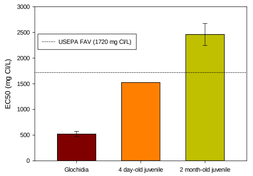



Figure 2A. Acute EC50s for Cl in 96-hour exposures with early life stages of fatmucket; hardness 100-170 mg/L as $CaCO_3$
*Error bars indicate 95% confidence limits (CL) or standard deviation; no CL due to no or only one partial kill*




### Potassium (K; tested as KCl)

Figure 1B. Acute EC50s for K in 96-hour exposures of newly transformed mussels; hardness 100 mg/L as $CaCO_3$
*Error bars indicate 95% confidence limits (CL); no CL due to no or only one partial kill*

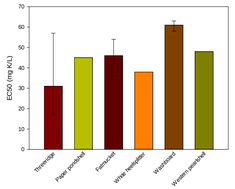



Figure 2B. Acute EC50s for K in 96-hour exposures for early life stages of fatmucket; hardness 100 mg/L as $CaCO_3$
*Error bar indicates 95% CL; no CL for piecewise regression*

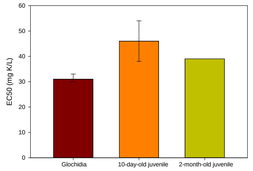



Figure 3. Acute EC50s for Cl at different hardnesses for glochidia or juveniles of fatmucket
*Error bars indicate 95% confidence limits (CL); no CL due to no or only one partial kill*

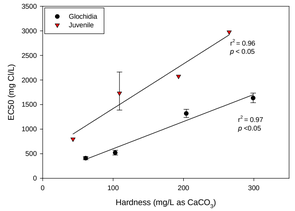



Figure 4A. Chronic 28-d EC20s for Cl based on survival, dry weight, or biomass in water-only exposures; hardness 100 mg/L.
*Error bar indicates 95% confidence limit (CL); no CL for no or only one partial kill*

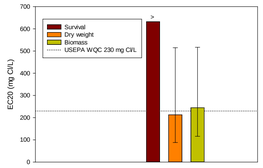
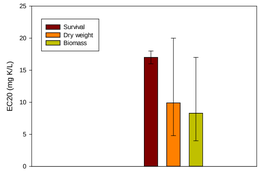



Figure 4B. Chronic 28-d EC20s for K based on survival, dry weight, or biomass of fatmucket; hardness 100 mg/L
*Error bar indicates 95% confidence limit*




## Results and Discussion (Continued)

- Control survival in all exposures met the test acceptability requirement of >90% in acute exposures and >80% in chronic exposures.
- Acute 96-h EC50s for Cl exposures for six mussel species were below the USEPA water quality criteria final acute value (FAV) of 1720 mg Cl/L (Fig. 1A). Acute 96-hour Cl EC50s for 4- to 14-day-old juveniles were slightly lower than 96-h EC50s for 2- to 4-month-old juveniles (Fig. 1A). Acute 96-hour EC50s for K were generally similar for each of the mussel species tested (Fig. 1B). Mussels were the most sensitive species to K among all tested species (Wang 2013 poster).
- Acute EC50s for Cl exposures with early life stages of fatmucket increased from glochidia to 2-month-old juveniles (Fig. 2A); however acute EC50s for K exposures with early life stages of fatmucket were not significantly different (Fig. 2B). 96-h EC50s for Cl for glochidia and 4-day-old juveniles of fatmucket were below the FAV of 1720 mg Cl/L (Fig. 2A). No FAV for K has been established.
- Acute EC50s for Cl increased for juveniles and glochidia of fatmucket as hardness increased from about 50 to 300 mg/L as $CaCO_3$ (Fig. 3A).
- Chronic EC20s for Cl or K in 28-d exposures based on the sublethal endpoints of dry weight or biomass were lower than the lethal survival endpoint. The EC20s for Cl based on dry weight and biomass were at or below the USEPA chronic water quality criterion of 230 mg Cl/L. No AWQC for K has been established, but the chronic EC20s were the lowest of species tested (USEPA ECOTOX database 2013).

## Conclusions

- Most of the mussel species tested had EC50s and EC20s for Cl below the chronic and acute water quality criteria, and therefore may not be protective of all mussel species.
- The need for K water quality criteria should be furthered evaluated based upon these data, as well as data in Wang et al 2013 poster and USEPA ECOTOX database 2013 (consisting of multiple species from seven different families) to ensure that K toxicity does not negatively affect aquatic life.
- Fatmucket glochidia are more acutely sensitive to Cl than fatmucket juveniles and their EC50s were significantly lower at a hardness of 100-170 mg/L as $CaCO_3$; however, K had similar acute effects on all life stages of fatmucket.
- Acute Cl toxicity decreased with increasing water hardness for fatmucket glochidia and juvenile mussels

## References

- American Society for Testing and Materials (ASTM). 2012. ASTM International standard guide for conducting early life stage toxicity tests with fishes (E12421-05). Annual Book of ASTM Standards Volume 11.06, West Conshohocken, PA.
- USEPA ECOTOX database. 2013. http://cfpub.epa.gov/ecotox/.
- Ning Wang, Chris Ingersoll, Chris Ivey, John Besser, Bill Brumbaugh, David Alvarez (USGS, Columbia, MO); Ed Hammer, Candice Bauer (USEPA, Chicago, IL); Tom Augspurger (USFWS, Raleigh, NC); Sandy Raimondo (USEPA, Gulf Breeze, FL); Burt Shephard (USEPA, Seattle, WA); Joe Bartoszek (USFWS, Burbank, WA); Chris Barnhart (Missouri State University, Springfield, MO); Nathan Eckert (USFWS, Genoa, WI). Acute sensitivity of freshwater mollusks and commonly tested invertebrates to select chemicals with different toxic modes of action . 2013. SETAC poster MP030.

## Acknowledgments

Jamie Hughes, John Besser, Doug Hardesty, Ning Wang, Dave Whites, Brittany King, Eric Brunson, Dave Mount, and Dave Soucek



Presented at 34th annual meeting of SETAC NA, Nashville, TN November 18, 2013
civey@usgs.gov