# Acute sensitivity of freshwater mollusks and commonly tested invertebrates to select chemicals with different toxic modes of action

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## Abstract

Freshwater mollusks are generally under-represented in toxicity databases. A series of acute toxicity tests were conducted with five mussel and two snail species from different tribes or families, and three commonly tested invertebrates (amphipod *Hyalella azteca*, cladocerans *Ceriodaphnia dubia* and *Daphnia magna*) using 10 chemicals representing different toxic modes of action. The chemicals were chosen based on the interest of USEPA in developing or updating ambient water quality criteria (AWQC); the availability of toxicity data for non-mollusks; the sensitivity of other non-unionid mollusks to the chemicals; and toxic mode of action. Preliminary results indicate that (1) mussels representing different tribes or families generally have similar sensitivity to the tested chemicals across several toxic modes of action; (2) mussels are sensitive to 8 of the 10 tested chemicals; (3) the AWQC may not be protective of multiple mussel species from acute exposures of chloride or nickel; (4) the AWQC may need to be derived or updated for chloride, potassium, sulfate, and nickel to reflect the sensitivity of mussels to these chemicals.

## Introduction

Previous studies indicate that freshwater mollusks are more sensitive to some chemicals, such as copper and ammonia, than commonly tested organisms. However, mollusks are generally under-represented in toxicity databases. Studies are needed to compile a more comprehensive database including mollusks to compare the sensitivity of mollusks, and to evaluate the degree to which existing or proposed USEPA ambient water quality criteria (AWQC) protect mollusks.

The objectives of this ongoing study are (1) to compare the sensitivity of five juvenile mussels and two air-breathing snails from different tribes or families to that of three commonly tested invertebrates (amphipod *Hyalella azteca*, cladocerans *Ceriodaphnia dubia* and *Daphnia magna*) in acute exposures with 10 chemicals representing different toxic modes of action (Tier 1); and (2) to "screen" acute sensitivity to 10 other chemicals with a commonly tested mussel species (fatmucket *Lampsilis siliquoidea*; Tier 2). Here we present preliminary Tier 1 results.

The 10 chemicals selected for the Tier 1 study were chosen based on (1) AWQC for chemicals of interest to the USEPA, (2) availability of toxicity data for >10 non-mollusks in the USEPA Interspecies Correlation Estimation (ICE) database (Raimondo et al. 2010), (3) sensitivity of mollusks that are not unionid mussels, and (4) different toxic modes of action. The additional chemicals for the Tier 2 study with *L. siliquoidea* were selected based on (1) lack of toxicity data for *L. siliquoidea*, (2) sensitivity of mollusks that are not unionid mussels, (3) availability of toxicity data for ≥10 species in the databases used to derive the AWQC or the ICE model, and (4) different toxic modes of action.

Fatmucket (*Lampsilis siliquoidea*)

Great pond snail (*Lymnaea stagnalis*)

Cladoceran (*Ceriodaphnia dubia*)

Amphipod (*Hyalella azteca*)

## Materials and Methods

Toxicity tests were conducted following standard methods in ASTM (2013ab). Test species and test conditions are summarized in Table 1.

**Table 1.** Summary of test conditions for conducting acute toxicity tests with select mussels, snails, and three commonly tested invertebrates in accordance with ASTM (2013ab)

| Parameter | Conditions |
|---|---|
| Test species | Mussels:<br>Family Unionidae<br>Amblemini tribe, *Amblema plicata*<br>Anodontini tribe, *Utterbackia imbecillis*<br>Lampsilini tribe, *Lampsilis siliquoidea*<br>Quadrulini tribe, *Megalonaisas nervosa*<br>Family Margaritiferidae, *Margaritifera falcata*<br>Snails:<br>Family Physidae, *Physa gyrina*<br>Family Lymnaeidae, *Lymnaea stagnalis*<br>Commonly tested species:<br>Cladoceran, *Ceriodaphnia dubia*<br>Cladoceran, *Daphnia magna*<br>Amphipod, *Hyalella azteca* |
| Test type | Static-renewal (flow-through for ammonia test) |
| Duration | 96 hour (48 hour for cladocerans and amphipod) |
| Temperature | 23°C (20°C for *M. falcata*) |
| Light quality | Ambient laboratory light |
| Light intensity | About 500 lux |
| Photoperiod | 16L:8D |
| Test chamber size | Static renewal: 50-ml glass beaker<br>Flow through:160-ml glass chamber |
| Test solution volume | Static renewal: 30 ml<br>Flow through: 80 ml |
| Renewal of solution | Static renewal: After 48 h<br>Flow through: 9 volume additions per day |
| Age of organism | Mussel, snail, amphipod: about 1-week-old juveniles<br>Cladocerans: <24-h-old neonates |
| Organisms/chamber | Static renewal: 5<br>Flow through: 10 |
| Replicates | 4 |
| Feeding | None |
| Aeration | None |
| Dilution water | Diluted well water: hardness about 100 mg/L and alkalinity 90 mg/L as $CaCO_3$, pH 8.3, Ca 24 mg/L, Mg 8.4 mg/L, K 0.9 mg/L, Na 9.0 mg/L, Cl 10 mg/L, $SO_4$ 19 mg/L, and dissolved organic carbon 0.5 mg/L |
| Dilution factor | 0.5 |
| Test concentrations | 5 concentrations plus a control (and a solvent control for organic chemicals) |
| Chemical residues | Water samples for chemical analysis were collected at the beginning and/or the end of test |
| Water quality | Dissolved oxygen, pH, conductivity, hardness, alkalinity, dissolved organic carbon, Ca, K, Mg, Na, $SO_4$, and Cl measured periodically during the exposures |
| Endpoint | Survival |
| Test acceptability | ≥ 90% control survival |
| Data analysis | EC50s were determined using Toxicity Relationship Analysis Program (Erickson 2012) |

## Results and Discussion

**Figure 1.** EC50s for ten chemicals in toxicity tests with five mussel and two snail species from different tribes or families, and three commonly tested invertebrates, compared to final acute value (FAV) in the USEPA ambient water quality criteria (AWQC, USEPA 2013a; normalized to test water). EC50s were estimated based on measured concentrations except nominal concentrations for two organic chemicals (measured concentrations changed substantially in the exposures of the organic chemicals). Error bar indicate 95% confidence limit (CL). No CL for some EC50s due to only one or no partial kill.

- EC50s for each of 10 tested chemicals generally differed by less than a factor of 2 among the five mussel tribes of the family Unionidae or between the two mussel families Unionidae and Margaritiferidae (Figure 1), indicating that different mussel species may have similar sensitivity to the tested chemicals.
- Snails had greater EC50s for ammonia, potassium, or sulfate compared to mussels whereas the EC50s for each of the other seven tested chemicals were similar for mussels and snails (Figure 1), indicating mussels may be more acutely sensitive than snails to some chemicals.
- EC50s for *C. dubia* were about equal to or less than the EC50s from two other commonly tested species (*D. magna* and *H. azteca*), except that *H. azteca* had a lower EC50 for metolachlor (Figure 1). EC50s for *C. dubia* were also about equal to or less than the EC50s from mussels and snails, except that mussels had consistently lower EC50 for ammonia or potassium (Figure 1), indicating *C. dubia* may represent sensitivity of snails, but not mussels, for some chemicals.

**Figure 2.** Ranked freshwater species mean acute values (SMAVs) in the USEPA Interspecies Correlation Estimation database (Raimondo et al. 2010) for alachlor, metolachlor, potassium, chromium, and nickel; in a chloride database used to derive the Iowa State chloride criteria in 2009; in a sulfate database used to derive the Illinois sulfate criteria in 2008; in the copper database used to derive USEPA ambient water quality criteria (AWQC) for copper (USEPA 2007); in the ammonia database used to derive AWQC for ammonia (USEPA 2013b); and in a zinc database (DeForest and Van Gendere 2012); with addition of SMAVs for freshwater mussels of different tribes or families tested in this study and other studies. Dashed line indicates the final acute value (FAV) in the AWQC.

- Species mean acute values (geometric mean of EC50s for a test species) for mussels based on the current study and previous studies were generally in the lower 30th percentile of the species sensitivity distributions for the tested chemicals, except for two organic chemicals (Figure 2). Importantly, mussels were among the five most sensitive species in the databases for ammonia, potassium, sulfate, chloride, copper, and nickel (Figure 2).
- Of the six tested chemicals with USEPA AWQC, the EC50s for chloride or nickel were about equal to or less than the final acute value (FAV) used to derive USEPA AWQC (Figures 1 and 2). Notably, the EC50s for ammonia from two mussel species tested in this study were below the FAV in the recently updated AWQC for ammonia at pH 8.2 and 23°C (Figure 1).

Figure 1

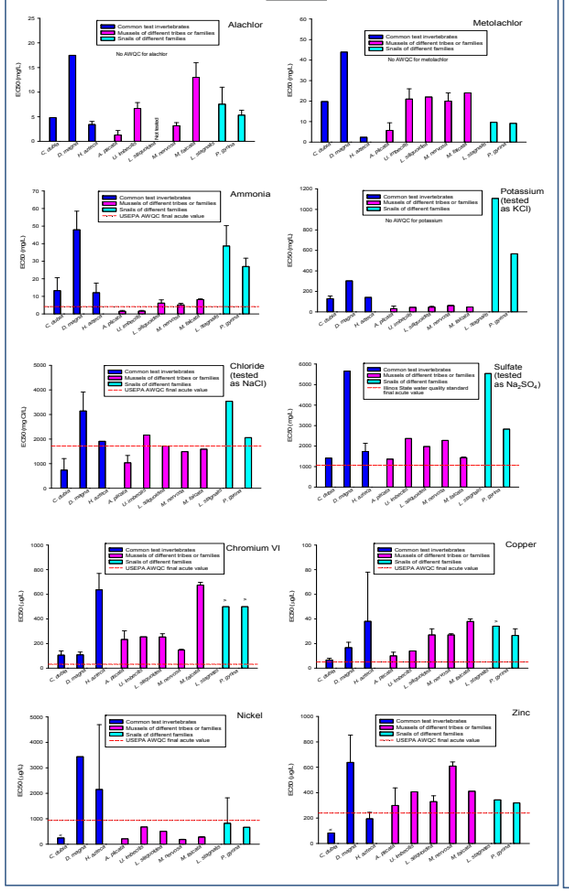
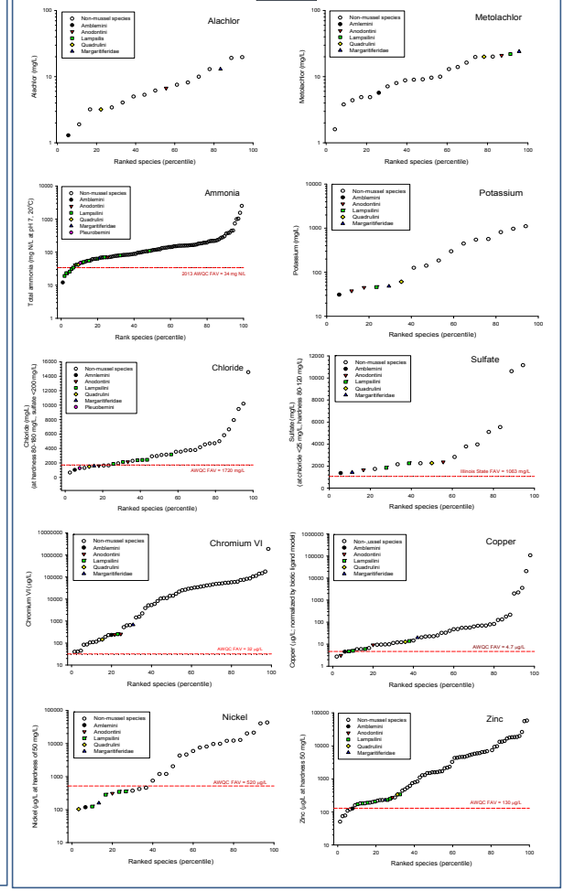



Figure 2




## Conclusions

- Mussels representing different tribes or families generally have similar sensitivity to toxicants across several toxic modes of action.
- Mussels are generally among the more sensitive species to 8 of the 10 chemicals tested.
- The AWQC may need to be derived or updated for chloride, potassium, sulfate, and nickel to reflect the sensitivity of mussels to these chemicals, and the AWQC for ammonia may need to be adjusted to protect mussels in some locations.
- We will continue this study to conduct more toxicity tests with additional mussel species and other chemicals (Tier 2 study). We also plan to repeat a few tests in which measured exposure concentrations of organic chemicals were not stable or EC50s could not be calculated (such as less than or greater than values) or have a large range of confidence limits (Figure 1), and to develop a more complete toxicity database for each of the chemicals.

## Acknowledgement

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Contact Ning Wang (nwang@usgs.gov) with any questions.