A Department of Ecology Report

Toxicity Testing of Potassium Chloride and Magnesium Chloride on New Zealand Mud Snails

Abstract

Using biological assays, the Washington State Department of Ecology (Ecology) tested the toxicity of two salts (potassium chloride and magnesium chloride) to the aquatic nuisance species, New Zealand Mud Snail (*Potamopyrgus antipodarum)*. This species is not native to Washington State and is considered highly invasive. The goal of these bioassays was to find an effective chemical treatment for decontaminating field gear, to prevent spreading snails during field work.

We exposed New Zealand Mud Snails to various concentrations of these salts for a set time period and then assessed mortality after a set recovery time. Concentrations for potassium chloride ranged from 65 to 260 grams per liter; concentrations for magnesium chloride ranged from 36 to 340 g/L. Exposure time ranged from 10 to 30 minutes, and mortality was assessed after a 48-hour recovery time.

None of the treatments we tested were reliably 100% lethal to the snails.

We do not recommend using these salts to decontaminate field gear exposed to New Zealand Mud Snails, since they were not found effective at exposures of up to 30 minutes. Alternative decontamination methods are available.

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Background

The New Zealand Mud Snail *Potamopyrgus antipodarum* (NZMS) is a highly invasive aquatic nuisance species known to inhabit a number of natural water bodies in the Pacific Northwest, including some in Washington State. It can be spread through activities such as recreational fishing, boating, firefighting, and research. Because it is a clonal reproducer, only a single NZMS specimen needs to be transported to infect a new water body (Benson et al., 2012).

Once this species is established within a water body, it is impossible to control or eradicate. Due to high potential population density (up to 500,000 per square meter) the NZMS can dominate a river system's ecology, to the detriment of native species (Kerans et al., 2005). Juvenile salmon and other fish are known to feed on the NZMS (Bersine et al., 2007) but are deprived of nutrition due to the mud snail's capacity to seal up inside its shell and survive passage through fish digestive systems (Vinson et al., 2006).

To prevent personnel from inadvertently spreading the NZMS as a result of field work, Ecology has made efforts to both increase staff awareness and improve field decontamination procedures. Potential field decontamination methods to remove the NZMS include:

Visual Inspection and Removal	Physical Treatment	Chemical Immersion
• Ineffective due to tiny size of the NZMS (often less than 2 mm)	• Drying requires longer than 48 hours • Freezing is impractical between sites on the same day • Heating requires special equipment	• More practical to implement in the field • May pose risk of sample contamination • The NZMS is resistant to chemical immersion due to closable operculum

The main concern regarding chemical immersion is the ability of the NZMS to survive treatment by closing its operculum (a shell plate which closes the shell when the snail retracts). Of the chemical immersion methods previously investigated by others, few are 100% effective at destroying the NZMS. For example, the NZMS is reported to survive exposure to some bleach solutions (Dwyer et al., 2003).

Currently, one effective chemical immersion method uses quaternary ammonia compounds (QACs) which have been demonstrated to result in 100% mortality to the NZMS using reasonable concentrations and exposure times (Schisler et al., 2008). Unfortunately, these QACs pose some risk of ammonia contamination for some samples, negating their use in certain situations.

The salts, potassium chloride (KCl) and magnesium chloride ($MgCl_2$), were chosen as possible alternatives to QACs based on a review of the literature. Potassium chloride is reported to be toxic to many shellfish, while magnesium chloride was reported to induce relaxation in some shellfish (Acosta-Salmon and Davis, 2007). Both salts are inexpensive and highly soluble. Potassium chloride is available as a fertilizer and magnesium chloride is available as a road de-icer. These salts are not likely to pose health issues for personnel. However, these salts potentially pose corrosion issues for metal equipment. This potential problem must be assessed before these salts are recommended for use as a decontamination agent.

The NZMS may become less susceptible to chemical immersion as water temperature increases. A previous study reports that chemical exposures made at low temperature (5°C) were more lethal than those performed at higher temperature (15°C), due to slower operculum closure in cold water (Hosea and Finlayson, 2005).

Study Design and Methods

Goal

The study goal was to test potassium chloride and magnesium chloride as possible treatments for decontaminating field gear exposed to the NZMS. Treatments must be found to be 100% lethal to the NZMS for consideration as potential decontamination options.

NZMS Collection and Storage

The NZMS specimens were collected by gathering aquatic plant material from the canal bottom and gently shaking the material underwater inside a catch net. Snails were transferred from the net to glass jars filled with canal water. Additional water was also collected from the canal to hold the snails prior to testing, for rinsing during the test, and for holding during recovery.

Measurements of pH, temperature, and conductivity of the canal water were made at the time of collection using an Orion 250A pH meter and an Orion 125A+ conductivity meter.

The jars were transported to an unheated storeroom at the Washington Department of Fish and Wildlife (WDFW) Nahcotta field station for holding. The air temperature in the storeroom was similar to the water temperature of the canal (approximately 1-2°C warmer in the storeroom).

Jars remained open to allow oxygen exchange. To provide algae as a food source, fluorescent lights were operated 10 hours per day above the storage jars. Jars and all testing solutions were left in the storeroom overnight to allow temperatures to stabilize, prior to exposing the NZMS to testing solutions.

Species identification was provided by Robyn Draheim of Portland State University, based on specimen photographs.

Test Organism Selection

We selected ten specimens for each test group, randomized by selecting specimens closest to a random grid node. To avoid testing compromised specimens, only active snails (feeding or moving) were selected.

Chemical Exposures

As described in the Quality Assurance Project Plan (Newell, 2009), chemical exposure was done by placing the test group of NZMS in a 600 ml beaker filled with 250 ml of test solution. The test beaker was swirled for a few seconds to ensure contact with all specimens and then allowed to rest for the allotted exposure period. After the exposure period, specimens were decanted onto a wire strainer, briefly rinsed with storage water, and then returned to 500 ml of storage water for the recovery period.

Chemical solutions were prepared at Ecology's Central Regional Office in Yakima, before snail collection. De-ionized water was used for all mixtures. Potassium chloride was obtained as Morton® potassium chloride water softener pellets. Magnesium chloride was obtained from Manchester Environmental Laboratory as magnesium chloride hexahydrate crystals. Conductivities of the final mixed solutions could not be recorded because they exceeded our instrument calibration range.

Three sets of exposures were performed for this study:

Relaxation Screen

The purpose of these exposures was to find a chemical relaxant that might help slow or prevent operculum closure. As mentioned in the Background section above, the main defense of the NZMS to chemical immersion is a closable operculum. The following compounds were tested for relaxation effects on the NZMS:

- 30 g/L $MgCl_2$
- 65 g/L KCl + 30 g/L $MgCl_2$
- 130 g/L KCl + 30 g/L $MgCl_2$
- 195 g/L KCl + 30 g/L $MgCl_2$
- 260 g/L KCl + 30 g/L $MgCl_2$
- 36 g/L $MgSO_4$
- MS-222 Fish Relaxant
- Clove oil
- Control group (source water)

For each solution listed above, 10 snails were placed into 100 mL of the solution. Snails remained in the solution for 20 minutes.

After 20 minutes the snails were observed under a microscope for signs of relaxation. Snails remained in the relaxation solution during this observation. Relaxation was defined as the snails extending out of their shell with behavior other than normal feeding/movement.

Toxicity Screen

The purpose of this test was to test a wide variety of mixtures to see if any were effective at killing the NZMS. Four types of solutions were evaluated, at varying concentrations:

- KCl
- $MgCl_2$
- KCl plus 10% Simple Green® degreaser
- a presoak exposure to $MgCl_2$ followed by a longer exposure to KCl

The first two types of solutions listed above are simple salt solutions. For the third type of solution, we added Simple Green® degreaser to the KCl solution in hopes of lowering the solution's surface tension enough to allow the solution to leak into the closed operculum. For the fourth type of solution, we presoaked the specimens in $MgCl_2$ relaxant prior to exposing them to a KCl solution, in hopes of slowing their operculum closure. The presoak solution was 36 g/L $MgCl_2$, chosen based on relaxation test results.

Details of screening test solutions and exposure times are presented in Table 1.

Table 1. Compounds, Concentrations, and Exposure Times Used for Toxicity Screen.

Exposure time and Compound	Concentrations Tested (g/L)
30 minute exposure to KCl	65, 130, 195, 260
30 minute exposure to $MgCl_2$	36, 85, 170, 255, 340
30 minute exposure to mixture of 10% Simple Green® Concentrate plus KCl	65, 130, 195, 260
10 minute exposure to 36 g/L MgCl2 followed by 20 minute exposure to KCl	65, 130, 195, 260

Each solution was tested on a group of 10 snails. Two control groups were also exposed to storage water using an identical method to the other exposed groups.

Following exposure, the snails were rinsed and held for recovery for 48 hours, as described above. Mortality was then assessed as described below.

Definitive Toxicity Test

The purpose of this test was to check the effectiveness of the two most promising solutions from the screening test, using several different exposure times.

To verify effectiveness, two chemical solutions were chosen for further testing. Based on the screening test, it appeared that 260 g/L of KCl and 340 g/L of $MgCl_2$ achieved 100% lethality. In practice, we found that it was quite difficult to dissolve the full amount of 260 g/L of KCl in water, and we anticipated that this would cause problems in preparing this solution for use by field crews. Therefore we decided to lower the dissolved concentration for this salt slightly before further testing, using 230 g/L instead of 260 g/L.

We tested the two salt solutions (230 g/L KCl and 340 g/L $MgCl_2$) on 4 replicate groups of 10 snails each, using three exposure times: 10, 20, and 30 minutes. Four control groups were exposed to storage water using an identical method to the other exposed groups.

Following exposure, the snails were rinsed and held for recovery for 48 hours, as described above. Mortality was then assessed as described below.

Mortality Assessment

Mortality was assessed after the 48-hour recovery period using the following criteria:

- Death was positively identified in specimens with rotting tissue (operculum missing, loose or easily opened and gray rotted tissue observed).
- Death was also considered likely in a number of cases, but could not be verified. These snails were classified as alive for this report. These snails were either out of the shell, or could be pulled out of the shell, and were unresponsive during a brief observation period (60-90 seconds). Despite their lack of response, some of these animals could feasibly have recovered.
- In cases where the operculum was firmly closed and not easily opened, the animal was considered alive.
- Any movement such as twitching antenna indicated live snails. It is possible that some of the live snails died later as a result of the test.

Study Results

NZMS Collection

After obtaining scientific collection permits from the WDFW, we collected the NZMS twice: on 3/9/09 and 3/22/09. Bruce Kauffman (WDFW) provided valuable assistance in obtaining the specimens. Specimens were obtained in the canal immediately downstream of the 315th St. Bridge near G Street in Ocean Park (45.5300 N, 124.05652 S).

Water properties in the canal during these collections are shown in Table 2.

Table 2. Canal Water Properties During Sample Collection.

Collection Date	Water Temp (°C)	Lab Testing Temp (°C)	pH (Standard Units)	Specific Conductivity (uS/cm)
3/9/2009	7.5	9.4	7.6	256
3/23/2009	9.0	10.4	7.4	252

An example photo of collected specimens is shown in Figure 1.

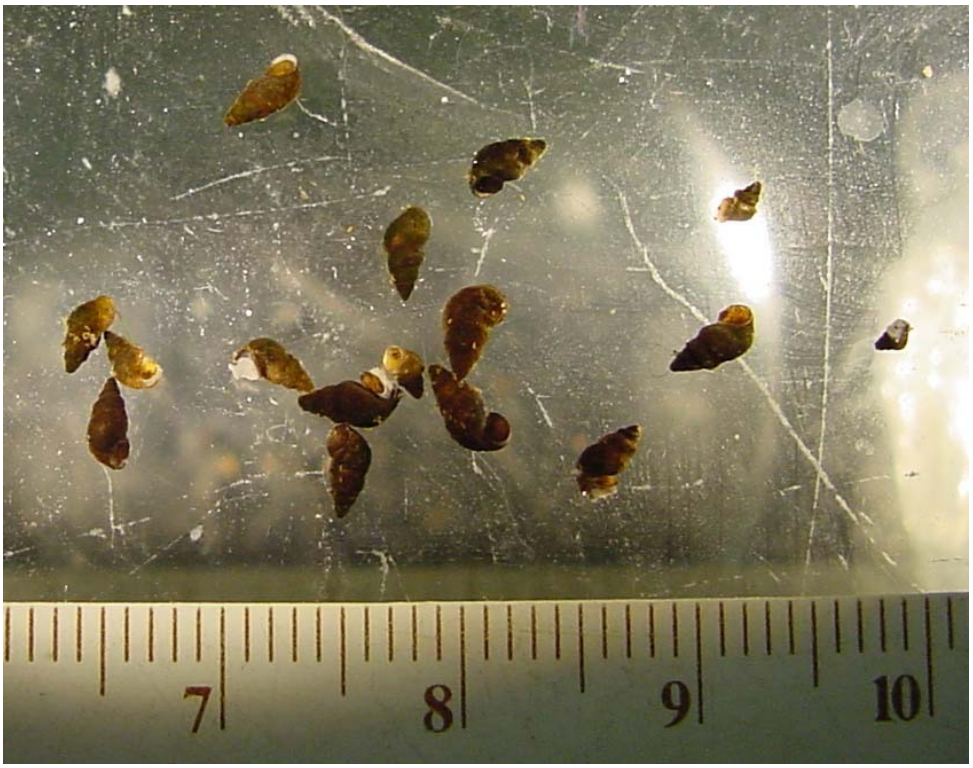

Figure 1. Some of the NZMS collected on 3/9/2009 (scale is in cm). (Photograph by Evan Newell.)

Relaxation Screen

As mentioned in the Methods section above, this test was performed in hopes of finding a compound to slow or prevent operculum closure in the NZMS. Results are shown in Table 3.

Table 3. Results from Relaxation Screen.

Test Solution	Number Relaxed (out of 10)
Control (Source Water)	none
30 g/L $MgCl_2$	10
65 g/L KCl + 30 g/L $MgCl_2$	none
130 g/L KCl + 30 g/L $MgCl_2$	1
195 g/L KCl + 30 g/L $MgCl_2$	none
260 g/L KCl + 30 g/L $MgCl_2$	none
36 g/L $MgSO_4$	8
MS-222	none
Clove Oil	10

Based on these results, we decided to include a 10-minute presoak in 30 g/L $MgCl_2$ followed by a 20-minute treatment in KCl as part of the screening test.

Toxicity Screen

Toxicity Screen results are shown in Figure 2.

The two groups exposed to the highest concentrations of KCl (260 g/L) and $MgCl_2$ (340 g/L) showed 100% mortality in this test. Note that these are extremely concentrated salt solutions – between one half and three quarters of a pound of salt per liter of water.

An interesting observation was made for the 30-minute exposure to 170 g/L $MgCl_2$. Snails in this exposure were extended outside of their shells and immobilized 48 hours after treatment. Their tissue seemed rigid. This treatment was very effective because the snails emerged from their shells into the treatment solution, avoiding the defense of the closable operculum. However, we could not verify that these snails had no possibility of recovery. Therefore, we decided to pursue the higher concentration treatments for this study, since the higher concentrations appeared to result in verifiable 100% lethality to the NZMS.

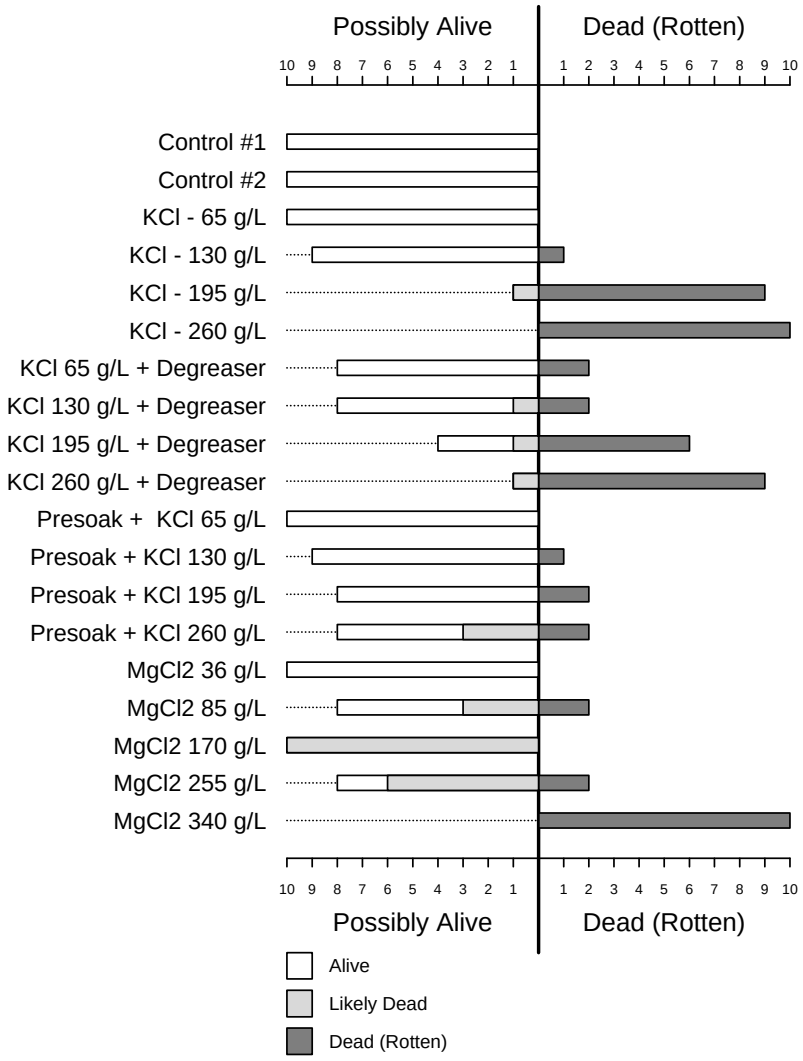

Figure 2. Results from the Toxicity Screen (10 snails in each test group).

Definitive Toxicity Test

Definitive test results are shown in Figure 3.

None of the groups in the replicate test showed 100% mortality. This was surprising since the screening test found 100% mortality for both 260 g/L KCl and 340 g/L $MgCl_2$.

The reason for the difference between the screening test and replicate test is uncertain; possible explanations follow. First, we lowered the KCl concentration for the replicate test due to the difficulty experienced in dissolving so much salt; however, the $MgCl_2$ concentration remained constant. Second, the temperature change between collection and testing was slightly greater for the screening test compared to the replicate test (temperature changed 1.9°C vs. 1.4°C,

respectively), which possibly stressed the snails prior to their chemical exposure. Third, the slightly warmer temperature in the replicate test may have allowed the snails to close their operculum more quickly. Specific conductivity of the canal water was similar for both collections; this does not appear to offer further explanation based on salinity in the canal.

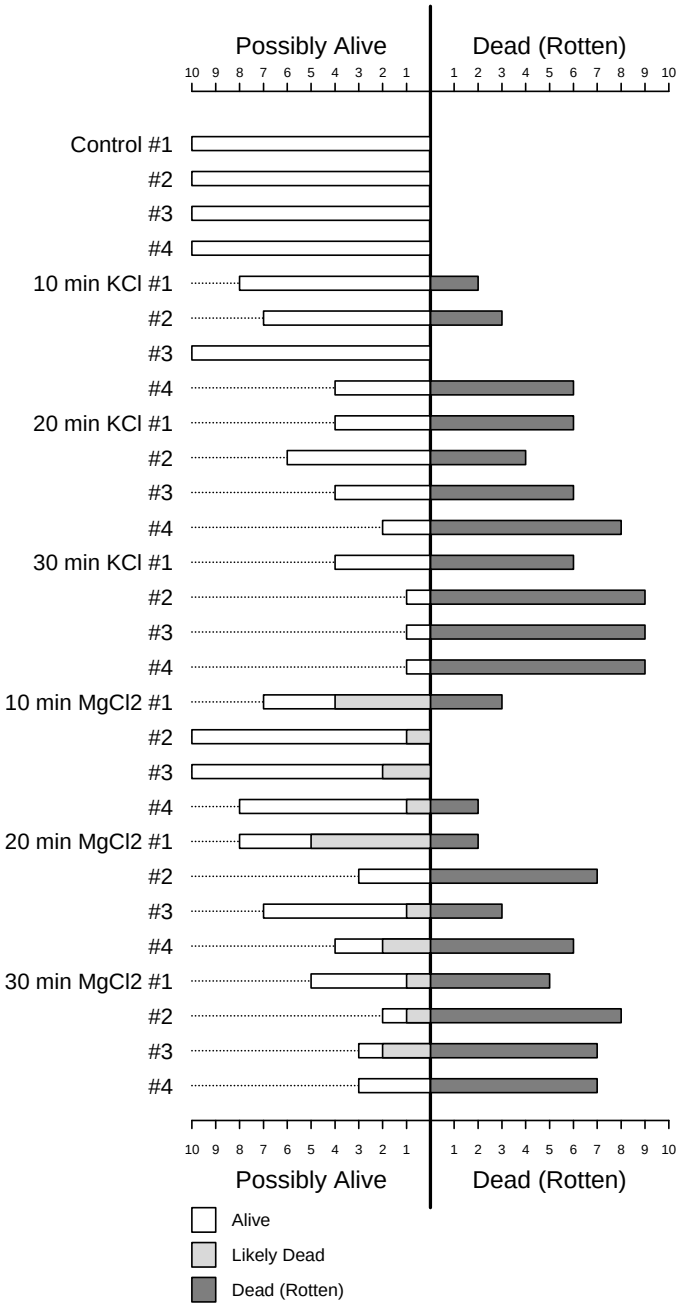

Figure 3. Results from the Definitive Toxicity Test (10 snails in each test group).

Conclusions and Recommendations

None of the solution concentrations and exposure times we tested resulted in 100% lethality to the New Zealand Mud Snail (NZMS). The tested concentrations were near the solubility limit for these salts. The ability of the NZMS to tightly seal themselves inside their shell allowed at least some specimens to survive these exposures to extremely high salt concentrations. This is consistent with other researchers' findings, such as the NZMS surviving passage through fish digestive tracts.

Given that our tests were conducted in relatively cool conditions, it is possible that the salt solutions we tested may be even less lethal in warmer temperatures (such as those found in summer). Our tests were conducted at approximately 10-11°C. A previous study reports that chemical exposures made at low temperature (5°C) were more lethal than those performed at higher temperature (15°C), due to slower operculum closure (Hosea and Finlayson, 2005).

Magnesium chloride was found to be an effective chemical relaxant for the NZMS. Clove oil was also an effective relaxant, but it was not included in our further testing since it is significantly more expensive.

We observed an interesting result for the 30- minute exposure to 170 g/L $MgCl_2$. Snails in this exposure were fully extended outside of their shells and immobilized 48 hours after treatment. This differed from most other treatments where the snails had retracted and closed their operculum. Using a 48-hour recovery period, we could not determine whether snails from this treatment were dead. Longer recovery periods might allow this determination.

Because our tests, using the highest achievable concentrations, did not reliably kill 100% of the NZMS, we do not recommend using either potassium chloride or magnesium chloride as a decontamination solution for field equipment exposed to NZMS.

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Appendix: Acronyms and Abbreviations

Ecology	Washington State Department of Ecology
KCl	Potassium Chloride
$MgCl_2$	Magnesium Chloride
$MgSO_4$	Magnesium Sulfate
NZMS	New Zealand Mud Snail
WDFW	Washington Department of Fish and Wildlife

Units of Measurement

°C	degrees centigrade
g/L	grams per liter
mm	millimeter
uS/cm	microsiemens per centimeter