# Daphnia Bioassay LD50

### Summary:

A bioassay involves the use of a biological organism to test for chemical toxicity. The oldest and most commonly known example is the canary in the coalmine. Traditionally, coal miners have taken caged canaries down into the mines to help ensure a safe air supply. Canaries are more sensitive to methane than humans, a colorless gas released during the mining process, so they were used to detect whether the gas levels were dangerous in the mines. If the canary died, then that signaled the coal miners to exit the mine as quickly as possible. Bioassays are used to test the effects of compounds considered for use in drugs and skin care products. Before a chemical compound receives FDA approval as an ingredient in products for human use, it must be thoroughly tested on laboratory animals.

In this laboratory, we will perform a bioassay on salts that are typically used for road de-icing. More and more towns are trying to steer away from using salt as a de-icer on roads during the winter because of the environmental effects when the snow melts and salty water runs off into streams, rivers, and lakes. One alternative to salt is sand, which does not melt highway ice but does help to increase traction. The disadvantage of sand is that unless it is cleaned up at the end of the season, it tends to clog drainage pipes, channels, and streams.[[1]](#footnote-1)

Another alternative is other chemical forms of salt. Normal road salt is sodium chloride (NaCl). Many homeowners buy de-icing products that claim to be less corrosive and more environmentally friendly than road salt. Usually these consist of either magnesium or calcium chloride (MgCl2 or CaCl2). Because of cost, these products generally are not feasible for widespread use on highways.

The green chemistry challenge is to identify a substance that can be used to coat the road to prevent highway ice related accidents keeping in mind the key criteria of safety, cost and performance. This lab consists of a bioassay using daphnia. The experiment will assess the toxicity of road salt and road salt substitutes.

**Chemistry Concepts:**

# Serial Dilution, Eco toxicity, Lethal Dose

# Green Chemistry Concepts:

Designing Safer Chemicals, Design for Degradation

# Contents:

# Background Hand-out Page 2

# Instructor Notes Page 3

# Student Worksheet Page 6Daphnia Bioassay LD50 Background

**Hand-out Page**

A bioassay involves the use of a biological organism to test for chemical toxicity. The oldest and most commonly known example is the canary in the coalmine. Traditionally, coal miners have taken caged canaries down into the mines to help ensure a safe air supply. Canaries are more sensitive to methane than humans, a colorless gas released during the mining process, so they were used to detect whether the gas levels were dangerous in the mines. If the canary died that signaled the coal miners to exit the mine as quickly as possible. Bioassays are used to test the effects of compounds considered for use in drugs and skin care products. Before a chemical compound receives FDA approval as an ingredient in products for human use, it must be thoroughly tested on laboratory animals.

More and more towns are trying to steer away from using salt as a de-icer on roads during the winter because of the environmental effects when the snow melts and salty water runs off into streams, rivers, and lakes. One alternative to salt is sand, which does not melt highway ice but does help to increase traction. The disadvantage of sand is that unless it is cleaned up at the end of the season, it tends to clog drainage pipes, channels, and streams.[[2]](#footnote-2)

Another alternative is other chemical forms of salt. Normal road salt is sodium chloride (NaCl). Many homeowners buy de-icing products that claim to be less corrosive and more environmentally friendly than road salt. Usually these consist of either magnesium or calcium chloride (MgCl2 or CaCl2). Because of cost, these products generally are not feasible for widespread use on highways.

The green chemistry challenge is to identify a substance that can be used to coat the road to prevent highway ice related accidents keeping in mind the key criteria of safety, cost and performance. In this experiment you will be performing a bioassay to determine the LD50 of road de-icers on a culture of Daphnia. The LD**50** is the lethal dose of a compound that will kill 50% of a group of animals when 100% of the organisms are exposed. The experiment will assess the toxicity of road salt and road salt substitutes.

# Daphnia Bioassay LD50

**Instructor Notes**

**Purpose:**

Perform serial dilutions to test the toxicity and evaluate the LD50 on Daphnia.

**Time Required:** 1 x 60 minute period (48 Hour time period)

### Green Chemistry Principles:

* **Designing Safer Chemicals:** chemical products should be designed to effect their desired function while minimizing the toxicity conclusions can be drawn from this experiment about what concentrations of road salts are toxic to daphnia
* **Design for Degradation:** chemical products should be designed to break down into innocuous degradation products and not persist in the environment, can any conclusions be made about the degradation of road salts from this experiment

### Objectives:

* To learn how to prepare percent solutions
* To learn how to perform serial dilutions
* To determine the LD50 of road de-icers
* To reinforce the principles of green chemistry

**Materials:**

* 10ml—of **One** of the solutions below (evenly distribute solutions among lab groups)
* 10% MgCl2
* 10ml--10% CaCl2
* 10ml--10% NaCl
* 10ml 10% Green De-Icer Mix(see directions below)
* Daphnia cultures
* Small aquarium
* Fish net with very fine mesh
* 25 Petri dishes(15mm x 55mm) or five-12 well plates
* Permanent marker
* Distilled water
* 1-plastic zip lock bag gallon size
* Micro-pipetters (1ml and 200µl)
* Micro-pipette tips
* Stereo Microscopes or strong hand lenses

### Advance Preparation:

* Purchase a culture of *Daphnia magna* at least one to two weeks prior to the planned lab date
* Maintain and grow cultures;utilize the assistance of students if possible (aeration is key a small aquarium pump will help to keep the daphnia alive)
* Prepare 1 liter of the following 10% stock solutions for the class or have the students prepare them:
* Magnesium chloride MgCl2 (95.2 MW)
* Calcium chloride CaCl2 (110.98 MW)
* Sodium Chloride NaCl (58.44 MW)
* Make a 1 liter stock solution of Green De-Icer Mix using the following:

|  |  |
| --- | --- |
| MgCl2 | 15% |
| Corn meal | 3% |
| Protein (albumen, gluten, or egg whites) | 5% |
| Carbohydrates( corn syrup, molasses, simple syrup or honey) | 11% |

### Resources:

This lab was developed and adapted based on the Cornell University Environmental Inquiry Website link: Toxicology

[http://ei**.**cornell**.**edu**/**toxicology**/**](http://ei.cornell.edu/toxicology/)

**References:**

Athey, L.A., J.M. Thomas, W.E. Miller, and J.Q. Word. 1989. Evaluation of bioassays for designing sediment cleanup strategies at a wood treatment site. **Environmental Toxicology and Chemistry**, 8: 223-230.

Bowers, N., J.R. Pratt, D. Beeson, and M. Lewis. 1997. Comparative evaluation of soil toxicity using lettuce seeds and soil ciliates. **Environmental Toxicology and Chemistry**, 16: 207-213.

Chang, L.W., J.R. Meier, and M.K. Smith. 1997. Application of plant and earthworm bioassays to evaluate remediation of a lead-contaminated soil. **Arch. Environ. Contam. Toxicol.**, 32: 166-171.

Keddy, C.J., J.C. Greene, and M.A. Bonnell. 1995. Review of whole-organism bioassays: soil, freshwater sediment, and freshwater assessment in Canada. **Ecotoxicology and Environmental Safety**, 30: 221-251.

Thomas, J.M., J.R. Skalski, J.F. Cline, M.C. McShane, & J.C. Simpson. 1986. Characterization of chemical waste site contamination and determination of its extent using bioassays. **Environmental Toxicology and Chemistry**, 5: 487-501.

Wang, W. 1987. Root elongation method for toxicity testing of organic and inganic pollutants. **Environmental Toxicology and Chemistry**, 6: 409-414.

Wang, W. 1986. Comparative toxicology of phenolic compounds using root elongation method. **Environmental Toxicology and Chemistry**, 5: 891-896.

Wang, W., and J.M. Williams. 1988. Screening and biomonitoring of industrial effluents using phytotoxicity tests. **Environmental Toxicology and Chemistry**, 7: 645-652

### Safety information:

* No unusual safety concerns with this lab besides standard laboratory safety procedures.

### Disposal:

* No special hazards are associated with these materials check with local codes for disposal.

**Taking it Further:**

* Compare other formulations of road de-icers
* Do the experiment in triplicate and compare the results
* Compare other bioassay protocols
* Experiment with other variables other than salts

# Daphnia Bioassay LD50

Student Worksheet

The green chemistry challenge is to identify a substance that can be used to coat the road to prevent highway ice related accidents keeping in mind the key criteria of safety, cost and performance. In this experiment you will be performing a bioassay to determine the LD50 of road de-icers on a culture of Daphnia. The LD**50** is the lethal dose of a compound that will kill 50% of a group of animals when 100% of the organisms are exposed. The experiment will assess the toxicity of road salt and road salt substitutes.

### GREEN CHEMISTY PRINCIPLES

* **Designing Safer Chemicals:** chemical products should be designed to effect their desired function while minimizing the toxicity conclusions can be drawn from this experiment about what concentrations of road salts are toxic to daphnia
* **Design for Degradation:** chemical products should be designed to break down into innocuous degradation products and not persist in the environment, can any conclusions be made about the degradation of road salts from this experiment

### OBJECTIVES

* To learn how to prepare percent solutions
* To learn how to perform serial dilutions
* To determine the LD50 of road de-icers
* To reinforce the principles of green chemistry

### Materials

* 10ml—of **One** of the solutions below (evenly distribute solutions among lab groups)
* 10% MgCl2
* 10ml--10% CaCl2
* 10ml--10% NaCl
* 10ml 10% Green De-Icer Mix
* Daphnia cultures
* Small aquarium
* Fish net with very fine mesh
* 25 Petri dishes(15mm x 55mm) or five-12 well plates
* Permanent marker
* Distilled water
* 1-plastic zip lock bag gallon size
* Micro-pipetters (1ml and 200µl)
* Micro-pipette tips
* Stereo Microscopes or strong hand lenses

### Procedure

1. Obtain a culture of Daphnia
2. Prepare 6 petri dishes by labeling each with type of salt and amount of concentration on the lid
3. Examine the daphnia under the microscope and determine how many you have and remove any discarded shells or dead organisms
4. **Determine the concentrations of salt solutions you wish to test on your Daphnia ( a recommended starting point for salts are 1/100 (1%) or 1/1000 (0.1%).**
5. Construct a data table to record your observations, numbers of Daphnia, concentrations of substances tested, time intervals (1 hour, one class, 24 hours, 48 hours etc.) and the LD50 of Daphnia for each concentration.
6. To prepare your dilutions, mass an empty Petri dish. Tare the balance. Place the desired amount of daphnia in the Petri dish, including water from culture in the transfer. It is a good idea to have 10ml as the amount of water in each dish
7. Using the mass of the water (1g/ml), you can determine the volume of liquid in your Petri dish. The dilutions can then be calculated. Example: 10g H2O in Petri dish = 10ml. To make a 1/100 dilutions remove 0.1ml of water with a micropipette. Add 0.1ml of 10% stock salt solution. Serially dilute each subsequent Petri dish
8. Place in a prepared location, out of light.
9. Observe your Daphnia for the time periods selected and record percent of organisms dead at each observation on your data table. **Be sure to maintain adequate water levels in dish for long-term experiments!**
10. Determine the LD 50 by calculating which concentration causes death in 50% of the Daphnia

POSSIBLE DATA TABLE TO SUGGEST

***Daphnia* Dose/Response Bioassay**

**Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Chemical tested \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**100% concentration \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** mg/L

**Length of experiment \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** days

Constants (such as temperature and light) \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 **Table 4a. *Daphnia* Bioassay Results**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Concen-****tration****(%)** | **# of starting Daphnia** | **Concen-tration****(mg/L)** | **# Dead after** **1 hour** | **# Dead after** **24 hours** | **# Dead after** **48 hours** | **Average****# Dead****after 48 hours** |
|  Control |  |  |  |  |  |  |
| 0.001% |  |  |  |  |  |  |
|  0.01% |  |  |  |  |  |  |
|  0.1% |  |  |  |  |  |  |
| 1% |  |  |  |  |  |  |
| 10% |  |  |  |  |  |  |
| 100% |  |  |  |  |  |  |

Make a bar graph showing the average # dead Daphnia after 48 hours:

**Draw conclusions**

1. Did at least 80% of the *Daphnia* in the control beakers survive? If not, what would you recommend doing differently next time to try to get a better survival rate? Does this affect your final result?

2. Did the rate of *Daphnia* survival respond in a predictable way to concentration? Describe any trends you observed.

3. What can you conclude about the toxicity of the substances you tested? Is this what you expected? Was your hypothesis supported by the data?

4. How does this experiment connect to the green chemistry principles? Explain your answer.

5. Think about whether any of the *Daphnia* might have died for reasons other than poisoning by the chemical you tested. What other factors do you think might possibly have killed some of them?

6.Using class data, compare results, data and trends. What overall conclusions can be reached from the class data?

7. Based on this experiment, would you say *Daphnia* would provide a useful bioassay organism for water samples from the environment? Why or why not?

8. If you were going to repeat this experiment, what would you do differently? How might you improve the experimental design to reduce the variability of your data or lead to more reliable results?

9. Can conclusions be made about what road salt would be environmentally preferable? If so explain. What additional information would you need to make recommendations to your town about what road salt to use?

1. http://ei.cornell.edu/toxicology/bioassays [↑](#footnote-ref-1)
2. http://ei.cornell.edu/toxicology/bioassays [↑](#footnote-ref-2)