Name _____ Date _____

**How much salt?**

**Background:** You are a resident of a town where the local stream has a concentration of sodium chloride of 50 mg/L. You want to find out whether this amount of pollution affects the aquatic life in the stream, and consequently, what should be done about the pollution. In this activity, you will try and discover at what level of salt pollution aquatic organisms respond negatively.

**Before you begin:** Develop a hypothesis that explains what you think will happen to either an aquatic organism, *Daphnia magna*, or aquatic plant, duckweed, at different concentrations of salt.

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**Materials:**

| ***Daphnia* experiment** | **Plant experiment** |
|---|---|
| *Daphnia* – enough so that you can put 5-10 organisms in each solution | Duckweed- you will need 5 plants for each container |
| Analytical balance | |
| *Daphnia* – enough so that you can put 5-10 organisms in each solution | tweezers |
| Thermometer | |
| Cups or beakers to hold the *Daphnia*, duckweed, and solution (14 per experimental group) | |
| 10 mol pipette | Plastic wrap |
| Spring water | |
| Salt- deionized | |
| Optional: Water chemistry kits; Microscopes or magnifying glasses | |

**Preparation:** Make the following salt solutions, or use the ones prepared by your teacher. *If you are using the solutions prepared by your teacher, skip to the procedures.*

a. Measure out the required amount of deionized water in a beaker.
b. Measure out the required amount of salt using the balance.
c. Once the salt has dissolved, divide your stock solution into two replicate solutions. Do this by pouring 250mL into each of two beakers. Label each beaker with the following information: ***A or B, the solution number (1-6, or control), your initials.***

1. Stock solution #1 of 9.5 grams salt for 500mL of water = 19,000 mg/L
2. Stock solution #2 of 2 grams salt for 500mL of water = 4000 mg/L
3. Stock solution #3 of 1 gram salt for 500mL of water = 2000 mg/L
4. Stock solution #4 of 1 gram salt for 1000mL of water = 1000 mg/L
5. Stock solution #5 of 0.5 gram salt for 1000mL of water = 500 mg/L
6. Stock solution #6 of 0.1 gram salt for 1000mL of water, then take 500 ml of this water and add another 500 mL of regular water = 50 mg/L
7. Control solution- water only.

**Part 1: Procedure for *Daphnia magna* experiment:**

1. Obtain the beakers that your group is testing. You should have at least one replicate for each salt solution.
2. Get enough *Daphnia* so that you will have 5-10 test organisms in each of your containers.
3. Label each beaker or cup with the appropriate concentration of salt. Put the same amount of your salt solutions in labeled beakers or cups. Make sure you have a control.
4. Place same number of *Daphnia* in each of the beakers. Record the time and number of *Daphnia* in each beaker.
5. Monitor the *Daphnia* over the next 48 hours, checking as often as possible, or at least once a day. Remove any dead *Daphnia* as well as the molt castings from the container.
6. If desired, test the water in each beaker and record the water chemistry.

**Data: Record the date and time and number of *Daphnia* that died each day for the next 48 hours. Remember that the hour that you start the experiment is time zero. When you record data tomorrow you will probably be close to 24 hours.**

DAPHNIA DATA TABLE

| Salt concentration | Time (hours) | Total *Daphnia* dead | Average # of *Daphnia* dead | Water Chemistry |
|---|---|---|---|---|
| 1A- 19000 mg/L | | | | |
| | | | | |
| | | | | |
| 1B- 19000 mg/L | | | | |
| | | | | |
| | | | | |
| 2A- 4000 mg/L | | | | |
| | | | | |
| | | | | |
| 2B- 4000 mg/L | | | | |
| | | | | |
| | | | | |
| 3A-2000 mg/L | | | | |
| | | | | |
| | | | | |
| 3B- 2000 mg/L | | | | |
| | | | | |
| | | | | |
| 4A- 1000 mg/L | | | | |
| | | | | |

| | | | | |
|---|---|---|---|---|
| 4B- 1000 mg/L | | | | |
| | | | | |
| | | | | |
| 5A – 500 mg/L | | | | |
| | | | | |
| | | | | |
| 5B- 500 mg/L | | | | |
| | | | | |
| | | | | |
| 6A- 50 mg/L | | | | |
| | | | | |
| | | | | |
| 6B- 50 mg/L | | | | |
| | | | | |
| | | | | |
| Control A | | | | |
| | | | | |
| | | | | |
| Control B | | | | |
| | | | | |
| | | | | |

**Part 2: Procedure for duckweed experiment:**

Now that you have completed the first part of this experiment you can continue with the duckweed experiment to see how salt may affect aquatic plants. Use the same stock solutions that you prepared for the *Daphnia* experiment. You may be recording date for both duckweed and *Daphnia* at the same time. Check with your instructor for direction.

1. Get enough containers to have a replicate for each level of salt concentration, as well as a control. For example, if you are testing four different concentrations, plus a control, you will need 10 containers.
2. Label your containers with the appropriate salt concentration.
3. Place five duckweed plants in each beaker (don't use your fingers). Each plant should have two fronds and should look healthy.
4. Cover the containers with plastic wrap, and place them under grow lights (natural light is an alternative but the results will take longer).
5. Let the containers sit for 5 days. Do not add water or disturb the containers.
6. At the end of 5 days, count the number of fronds in each container.
7. If desired, test the water in each beaker and record the water chemistry.
8. Record your data.

DUCKWEED DATA TABLE

| Salt concentration | # duckweed fronds/container | Average # of fronds | Observations | Water Chemistry |
|---|---|---|---|---|
| 1A- 19,000 mg/L | | | | |
| 1B- 19,000 mg/L | | | | |
| 2A- 4,000 mg/L | | | | |
| 2B- 4,000 mg/L | | | | |
| 3A- 2,000 mg/L | | | | |
| 3B- 2,000 mg/L | | | | |
| 4A- 1,000 mg/L | | | | |
| 4B- 1,000 mg/L | | | | |
| 5A – 500 mg/L | | | | |
| 5B – 500 mg/L | | | | |
| 6A – 50 mg/L | | | | |
| 6B – 50 mg/L | | | | |
| Control A | | | | |
| Control B | | | | |

**Analysis:**

Share results with the class.

Create a graph for the class data for Duckweed and *Daphnia.*

**Discussion Questions**

1. Looking at your data, did you notice any trends?
2. Are there any data that don't make sense, or did something happen that was different from what you anticipated? If so, try to explain why these data are different from your expectations.
3. Since everyone took an average from two tests, you may be missing some information about the variability between the test samples. Look back at your data and that from the class and decide whether the variability is reasonable, or whether you think there may have been a problem with the way you (or others) conducted the experiment. Explain any problems you might have had. What could you do in the future to reduce the variability in the experiment?
4. If you tested the water quality of any of your samples, what did you find? Why do you think this happened?
5. Based on what you've learned from this experiment, at what concentration of salt do aquatic organisms begin to respond? Is the 50 mg/L level that was found in the stream a problem? Why or why not?
6. Where do you think the salt in the stream comes from? What do you think should be done about this problem?
7. Could the high levels of salt affect humans as well as aquatic organisms?

Adapted from: Environmental Inquiry, Cornell University and Penn State University, 2006.
http://ei.cornell.edu/toxicology/bioassays/