$\qquad$ Date $\qquad$

## How much salt? - Hydrofracking

Background: You are a resident of a town where the local stream has a sodium chloride concentration of $50 \mathrm{mg} / \mathrm{L}$ as a result of some salt pollution - potentially from a local hydrofracking operation. 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 predicts at what concentration of chloride you think an aquatic animal or plant will begin to feel the effects.

## Materials:

| Animal Experiment | Plant Experiment |
| :--- | :--- |
| Cladocerans or copepods- students will need 5- <br> 10 organisms per container | Duckweed plants -students will need 5-10 plants <br> per container |
| Rock salt (deionized) <br> Deionized water <br> Balance |  |
| Beakers or cups (7 per group) |  |
| Pipette | Tweezers, filter paper, petri dish |

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 procedure.

| Stock <br> solution \# | Amount of salt <br> needed | Amount of water | Concentration of salt |
| :--- | :--- | :--- | :--- |
| 1 | 9.5 g | 500 ml | $19,000 \mathrm{mg} / \mathrm{L}$ |
| 2 | 1 g | 500 ml | $4,000 \mathrm{mg} / \mathrm{L}$ |
| 3 | 0.5 g | $1,000 \mathrm{ml}$ | $1,000 \mathrm{mg} / \mathrm{L}$ |
| 4 | 0.1 g | $1,000 \mathrm{ml}$ <br> and add 500 ml of distilled water <br> $1,000 \mathrm{ml}-$ then take 500 ml of this water <br> and add 500 ml of distilled water <br> 5 | $25 \mathrm{mg} / \mathrm{L}$ |
| 6 | 0.05 | Water only | $50 \mathrm{mg} / \mathrm{L}$ |
| Control | None | $0 \mathrm{mg} / \mathrm{L}$ |  |

## Part 1: Procedure for the animal experiment:

1. Get 7 containers, and label them \#1-6 and "control". You will not conduct replicates within your group; however, as a class, each group will act as a replicate.
2. Put the same amount of salt solutions in the containers ( 100 mL is plenty).
3. Get enough copepods/cladocerans so that you have 5-10 test organisms in each of your containers. Use only young animals that do not have eggs.
4. Place the animals in the beakers.
5. Monitor the animals over the next 3-5 days, checking at least once a day. Remove any dead animals as well as the molt castings from the container.

Your Data: Record the number of animals that are dead each day for the next 3 days.

| Salt <br> concentration | \# dead <br> after 1 <br> hour | \# dead <br> between <br> $1-24$ <br> hours | \# dead <br> between <br> $25-48$ <br> hours | \# dead <br> between <br> $49-72$ <br> hours | Cumulative \# dead (add up your <br> dead!) |
| :--- | :---: | :---: | :---: | :---: | :--- |
| $1-19000 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $2-4,000 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $3-1,000 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $4-500 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $5-50 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $6-25 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| Control |  |  |  |  |  |

Class Data: Compare your data with your classmates' data. How many organisms were dead after 3 days at each concentration?

|  | 19,000 <br> $\mathrm{mg} / \mathrm{L}$ | 4,000 <br> $\mathrm{mg} / \mathrm{L}$ | 1,000 <br> $\mathrm{mg} / \mathrm{L}$ | 500 <br> $\mathrm{mg} / \mathrm{L}$ | $50 \mathrm{mg} / \mathrm{L}$ | $25 \mathrm{mg} / \mathrm{L}$ | Control |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Group 1 |  |  |  |  |  |  |  |
| Group 2 |  |  |  |  |  |  |  |
| Group 3 |  |  |  |  |  |  |  |
| Group 4 |  |  |  |  |  |  |  |
| Group 5 |  |  |  |  |  |  |  |
| Group 6 |  |  |  |  |  |  |  |
| Group 7 |  |  |  |  |  |  |  |
| Group 8 |  |  |  |  |  |  |  |
| Average |  |  |  |  |  |  |  |

Make two graphs; one showing the change in survival over time, and a second showing the total number of surviving animals after 3 days, based on your class averages.

## Part 2: Procedure for duckweed experiment:

1. Get 7 containers, and label them \#1-6 and "control". You will not conduct replicates within your group; however, as a class, each group will act as a replicate.
2. Put the same amount of salt solution in each container ( 100 mL is plenty).
3. Get enough duckweed plants so that you have 10 plants in each of your container. Each plant should have two fronds and should look healthy.
4. Cover the beakers with plastic wrap, and place them under grow lights (natural light is an alternative but the results will take longer).
5. Check the beakers after 1, 3, and 5 days. Do not add water or disturb the beakers. Count the number of fronds in each beaker that are damaged. Before you begin, decide what it means for a plant to be "damaged" - will the plant be brown, white, etc? Make sure to leave your plants in an area with enough light.
6. Record your data.

## DUCKWEED DATA TABLE

| Salt concentration | \# damaged <br> after 1 <br> hour | \# damaged <br> between 1- <br> 24 hours | \# damaged <br> between 25 <br> hours -2 <br> days | \# damaged <br> between 2 - <br> 5 days | Cumulative \# damaged <br> (add up all your <br> damaged/dead plants) |
| :--- | :--- | :--- | :--- | :--- | :--- |
| $1-19,000 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $2-4,000 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $3-1,000 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $4-500 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $5-50 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| $6-25 \mathrm{mg} / \mathrm{L}$ |  |  |  |  |  |
| Control |  |  |  |  |  |

Class Average: Compare your data with your classmates.

|  | 19,000 <br> $\mathrm{mg} / \mathrm{L}$ | 4,000 <br> $\mathrm{mg} / \mathrm{L}$ | 1,000 <br> $\mathrm{mg} / \mathrm{L}$ | 500 <br> $\mathrm{mg} / \mathrm{L}$ | $50 \mathrm{mg} / \mathrm{L}$ | $25 \mathrm{mg} / \mathrm{L}$ | Control |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Group 1 |  |  |  |  |  |  |  |
| Group 2 |  |  |  |  |  |  |  |
| Group 3 |  |  |  |  |  |  |  |
| Group 4 |  |  |  |  |  |  |  |
| Group 5 |  |  |  |  |  |  |  |
| Group 6 |  |  |  |  |  |  |  |
| Group 7 |  |  |  |  |  |  |  |
| Group 8 |  |  |  |  |  |  |  |
| Average |  |  |  |  |  |  |  |

Make two graphs; one showing the change in survival over time, and a second showing the total number of damaged duckweed after 5 days.

## Analysis:

1. Looking at your individual group data, do you notice any trends?
2. Looking at your individual group data, 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.
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3. Using the class data, at what concentration(s) were the organisms affected immediately?
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4. Using the class data, at what concentration(s) did the greatest number of organisms die?
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5. Why do organisms die when they are exposed to too much salt? Explain what happens inside of an organisms' cells that cause it to be affected by the salt.
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$\qquad$
6. What kind of graph did you make for your averaged class data? $\qquad$
7. Why?
8. How do your own group's data contrast with the average (this is called variability)? In other words, are your group data close to the average or do they vary greatly from one another? Why do you think this?
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9. If you wanted to repeat this experiment, explain what you would do to reduce the variability?
10. Based on what you've learned from this experiment, at what concentration of salt do aquatic organisms begin to respond? Is the $50 \mathrm{mg} / \mathrm{L}$ level that was found in the stream a problem? Why or why not?
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11. Make a claim, based on the evidence you found and the information in the graph provided from the experimental forest, about whether the concentrations that were found in the experimental forest are a problem. (Note: $\mathrm{mg} / \mathrm{kg}$ and $\mathrm{mg} / \mathrm{L}$ are the same.)
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