Name _______________________ Date _________

How much salt? -Hydrofracking

Background: You are a resident of a town where the local stream has a sodium chloride concentration of 50 mg/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.

Based on the information on the Salt Pollution Tolerance Chart, I think that animals will start to die in water with 250 mg/L of chloride.

Materials:

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

<u>Preparation:</u> 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 solution #	Amount of salt needed	Amount of water	Concentration of salt
1	9.5g	500 ml	19,000 mg/L
2	2 g	500 ml	4,000 mg/L
3	1 g	1,000 ml	1,000 mg/L
4	0.5 g	1,000 ml	500 mg/L
5	0.1 g	1,000 ml – then take 500 ml of this water and add 500 ml of distilled water	50 mg/L
6	0.05	1, 000 ml – then take 500 ml of this water and add 500 ml of distilled water	25 mg/L

Control	None	Water only	0 mg/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 concentration	# dead after 1 hour	# dead between 1- 24 hours	# dead between 25- 48 hours	# dead between 49- 72 hours	Cumulative # dead
1- 19000 mg/L	10	10	10	10	10
2- 4,000 mg/L	1	3	5	9	9
3-1,000 mg/L	0	0	2	4	6
4- 500 mg/L	0	1	3	3	7
5 – 50 mg/L	0	2	2	3	6
6- 25 mg/L	0	1	1	1	3
Control	0	0	2	2	2

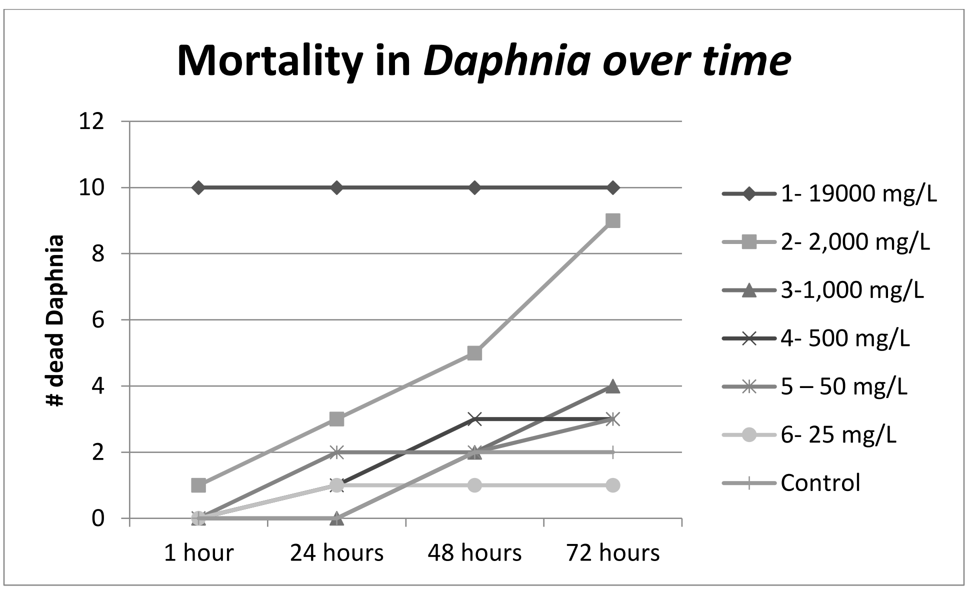
Class Data: Compare your data with your classmates' data. How many organisms were dead after 3 days at each concentration?

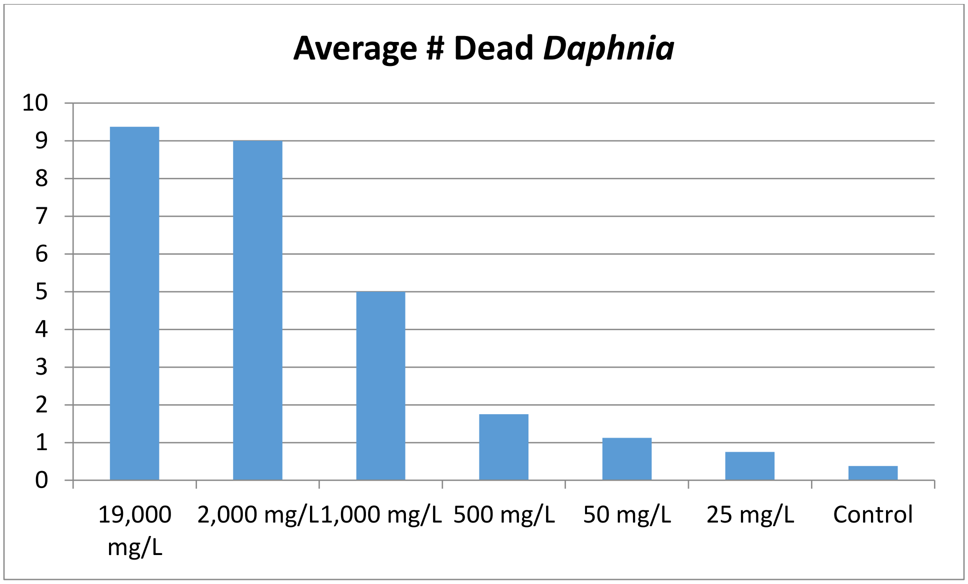
	19,000 mg/L	4,000 mg/L	1,000 mg/L	500 mg/L	50 mg/L	25 mg/L	Control
Group 1	10	9	9	3	2	1	1
Group 2	10	9	8	2	1	0	0

Group 3	9	8	4	1	2	1	2
Group 4	9	9	3	1	1	1	0
Group 5	10	9	3	2	1	1	0
Group 6	10	10	7	1	0	0	0
Group 7	9	9	4	3	2	1	0
Group 8	8	9	2	1	0	1	0
Average	**9.4**	**9**	**5**	**1.75**	**1.125**	**0.75**	**0.375**

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.

Note: only Daphnia graphs are shown below, but duckweed graphs will look similar.





Note: if students used Excel to graph their data, they can try adding error bars (after calculating the standard error).

Analysis:

1. Looking at your individual group data, do you notice any trends?

   Students may have noticed that more of the organisms died at higher concentrations of chloride.

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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.
   Student answers will vary.

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3. Using the class data, at what concentration(s) were the organisms affected immediately?
   _____19,000 mg/L____________
4. Using the class data, at what concentration(s) did the greatest number of organisms die?
   _____19,000 mg/L____________

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.

   Freshwater organisms die because when they are exposed to salt, the water leaves their cells through osmosis (from the cytoplasm). Eventually there wouldn't be enough water in the organisms' cells to allow it to survive. (In animals, this "shrinkage" is called crenation, while in plants, the cells are called plasmolysed.

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6. What kind of graph did you make for your averaged class data? *Most students will likely make a bar graph.*
7. Why?
   Bar graphs are easy to read to see an average.

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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?
   Student answers will vary. Students should pay attention to whether their individual data are close to the average or not – in statistics, if one data set has a lot of data that are far away from the mean, then the standard deviation will be much higher than another data set that has data which are closer to the mean (or, less variable).

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9. If you wanted to repeat this experiment, explain what you would do to reduce the variability?
   Students may refer to being more careful about counting organisms; setting criteria for when duckweed plants are damaged; picking only young Daphnia to ensure their survival.

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10. 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?
    Although answers may vary, most students will find that organisms can survive in 50 mg/L. Students should note, however, that salt concentrations are continuing to increase.

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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: mg/kg and mg/L are the same.)*
    Answers may vary; but students should note that in the experimental forest, the chloride levels ranged between about 380 mg/L and 1200 mg/L, which is definitely a cause for concern.
