Name _____*Teacher Version*_____ Date _____ Class_____

Activity 3.3: Brine Shrimp Toxicity Experiment

INTRODUCTION:

In this lab, we will use a small crustacean, the brine shrimp. It is normally found in brackish water and is a very hearty little organism and able to tolerate high salt concentrations. This lab will use herbal tea as a potential toxin.

Procedure:

A cup of tea contains 200 ml of water per teabag, so that would be considered a 1.0X dosage. You will prepare a 10X dosage by using 4 teabags in 80 ml of brine (seawater).

1. Place 4 teabags flatly onto the bottom of a beaker.
2. Place 80 ml of hot seawater into the beaker and let it seep for 10 minutes shaking gently every 2 minutes.
3. Squeeze each teabag between two forks. This solution is designated as the tea extract at a 10X solution.
4. Make a serial dilution of the 10X stock in order to make a 1X stock solution. Add 2 ml of 10X stock solution to 18 ml of room temperature seawater. The resulting 20 ml of solution will be 1X.
5. Make a serial dilution of the 1X stock in order to make a 0.1X stock solution. Add 2 ml of 1X stock solution to 18 ml of room temperature seawater. The resulting 20 ml of solution will be 0.1X.
6. Label 3 Petri dishes of each of the solutions: 10X, 1X, 0.1X and Control (12 total Petri dishes). Control is the brine salt water.
7. Using a pipette, transfer 10 shrimp to each Petri dish. **You must minimize the dilution error that occurs as a result of shrimp being in a salt solution, so just before you add the tea extract, decant the seawater (brine) from your vial of brine shrimp, leaving the brine shrimp in as little water as possible.**
8. **Set aside for 24 hours.**
9. Check on your Brine Shrimp. After 24 hours and 48 hours, count the surviving brine shrimp. Calculate the % death. Record results on the Student Data Sheet.
10. Graph the results.

DATA/OBSERVATIONS:

	24 hour			48 hour		
Concentration	**# Alive**	**# Dead**	**% Mortality**	**# Alive**	**# Dead**	**% Mortality**
10						
10						
10						
1						
1						
1						
0.1						
0.1						
0.1						
Control						
Control						
Control						

ANALYSIS:

1. Graph Your Results
    a. Plot a scatter graph of Mortality (Y axis) as a function of Concentration (X axis).
    b. In one or two sentences describe the trend or trends displayed in your graph:
    Sample student response:
    "The percent mortality remained relatively low and constant until the 10X solution at which time 50% of the population died."

    c. Extra Challenge, technology option: If you have computers available, use Excel, plot a scatter graph of Mortality (Y axis) as a function of Concentration (X axis).

d. You must attach **both** the hand drawn graph and the computer graph.

2. Although Brine Shrimp are hardy enough to withstand a wide range of salt concentrations, they are short-lived. Do you have any evidence of a background death rate independent of the addition of herbal teas? Explain.

Sample student response:
"By looking at the number of shrimp who died in both the control group and the ones with very low concentration we can get an estimate of the background mortality rate."

3. What is the LC_{50} for your tea on brine shrimp? In other words at what concentration did half of your brine shrimp population die?

Sample student response:
"At a concentration of 10X herbal tea, 50% of the brine shrimp died"

4. Based on your data in this lab what is the safe concentration for brine shrimp, that is the Lowest Observable Effect Concentration (LOEC)?

Sample student response:
"Since only the .1X solution appeared to be similar to the control, I would have to say that the lowest observable effect concentration (LOEC) was the 1X solution."

5. If you pursue this investigation further in order to publish your results in a scientific journal, what would you do to improve upon this lab?

Sample student response:
"I would do many more replicates and I would probably want a better way to establish exactly how much of the herbal tea was going into my initial solution. This could be done by getting an accurate dry weight of the tea bags before and after making the solution."

6. Brine Shrimp have a higher tolerance for many pollutants than another crustacean, the *Daphnia*, also called a water flea. Indicator species are used to study the overall health of an ecosystem. If you were to study an ecosystem would you use the Brine Shrimp or the Daphnia as indicator species? Explain your reasoning.

   Sample student response:
   "You would want to use the species which is most sensitive to environmental changes, which in this case is the Daphnia."

7. If your classmates chose a different toxin than you did, record their LC_{50} results below:
   Sample student response:

Name of toxin	LC_{50}

8. What problems are there in comparing one group's results with another? Can you say with any certainty which toxin is the most dangerous to brine shrimp?
   Sample student response:
   " unless you are comparing known quantities of a toxin it is really hard to make any meaningful comparison between different toxins. "

Concluding the lesson:
Share as a whole class the students responses to the analysis questions.
As Homework assign the Narrative reading for lesson 5: "Thinking about scale and the environment."