LD_{50} Lab

The use of herbal products as medicinal remedies has increased greatly in the past decade. We are inundated with advertisements that tell us the natural remedies will accomplish cures and imply that the term "natural" means free of side effects. Many herbal products may do what they claim, and some do not. Any product that has a desired effect is also likely to have some undesired side effects. Unfortunately, only a few of the herbal remedies have scientific data to back up their clinical benefits, and some are known to be dangerous. Herbal teas remain an under investigated group of plant products.

Protocol

Tea used (species name) _____
(common name)_____
purported effects of tea:

You will make 3 vials (5 ml each) of the following solutions:
10x, 7.5x, 5x, 2.5x, 1x, .5x, .1x as well as a control of 0x.
Unless you are using the stock solution (10x, 1x or .1 x) directly on the shrimp, you must make each solution in a small beaker and stir it before adding the solution to the shrimp.

Preparation of tea extract. A cup of tea contains 200 ml of water per teabag, so that would be considered a 1.0x dosage. You will start with a 10x dosage by using 4 teabags in 80 ml of brine (seawater). This may be prepared for you when you arrive.

Part 1: making **stock** solutions for future dilutions

10X STOCK SOLUTION:

- Place 4 teabags flatly onto the bottom of a 100 ml beaker
- Place 80 ml of hot artificial seawater (brine) into the beaker and let it seep for 15 minutes, shaking gently every 5 minutes.
- Squeeze each teabag between two spoons. This solution is designated as the tea extract at a 10x solution.

1X STOCK SOLUTION:

- 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 seawater. The resulting 20 ml of solution will be 1x.

0.1X STOCK SOLUTION:

- 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 seawater. The resulting 20 ml of solution will be 0.1x.

Part 2: preparing the shrimp (this can be done while the dilutions are being made):

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. You will need to place 10 live shrimp in each of the 24 vials. Whether you add the shrimp to the dilutions or the dilutions to the shrimp is academic as long as a minimum of brine water is transferred with the shrimp. This is important because it helps to keep the dilutions close to the actual values you have created.

Part 3: making the remaining tea solutions

- To make a 7.5x solution, place 3.75 ml of the 10x stock solution and 1.25 ml of seawater into a vial containing 10 shrimp- label 7.5x. Repeat twice for the other two vials.
- To make a 5x solution, place 2.5 ml of stock solution and 2.5 ml of seawater into a vial containing 10 shrimp- label 5x. Repeat twice for the other two vials.
- How will you make a 2.5 x solution?

- Place 5 ml of the 1x stock solution into a vial containing 10 shrimp- label 1x. Repeat twice
- To make a 0.5x solution, place 2.5 ml of the 1x stock solution and 2.5 ml of seawater into a vial containing 10 shrimp- label 0.5x. Repeat twice for the other two vials.
- Place 5 ml of the 0.1x stock solution into a vial containing 10 shrimp- label 0.1x. Repeat twice
- Prepare a control of 10 shrimp in seawater. Repeat twice.

After 24 hours, count the surviving brine shrimp. Calculate the % death

Concentration	# dead	% mortality	Concentration	# dead	% mortality
10x			1		
10			1		
10			1		
7.5			.5		
7.5			.5		
7.5			.5		
5			0.1		
5			0.1		
5			0.1		
2.5			0		

2.5			0		
2.5			0		

Using Excel, plot a scatter graph of concentration (X axis) vs. mortality (Y axis) for any of the teas used by your class. Using a **logarithmic scale** for the x-axis does a good job of spreading out the lower concentrations. If you use a logarithmic scale, do not plot the 0 concentration data. You are probably better off drawing in a trendline of your own rather than having Excel plot one for you. Remember that it is possible for a natural die off and threshold response to affect your results. **Mark the LC-50's on your printed graphs**.

Compare the responses of the shrimp to the different tea solutions.

1. 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.

2. What is the LC-50 for your tea on brine shrimp?_____

3. Based on your data in this lab what is the safe concentration for brine shrimp--Lowest observable Effect Concentration (LOEC)?

4. 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?

5. Brine Shrimp have a higher tolerance for many pollutants than does 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.

6. In this lab, you are actually determining the LC-50 (Lethal concentration) rather than the LD-50. What is the difference, and why is the difference important