

APES LD-50 BIOASSAY LAB

Name _____

Purpose: The purpose of this lab is to determine the lethal dose of a chemical at which 50% of a test population would be killed by the chemical.

Materials:

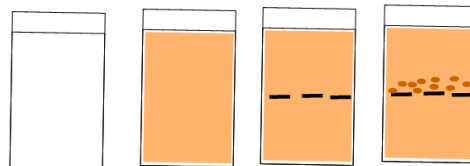
- Paper towels
- Pipettes
- Marker
- Beaker
- Salt
- Stapler
- 80 radish seeds
- 8 snack ziplock bags
- 8 test tubes
- Distilled water

Bag #	Concentration (g/ml)
1	0.100
2	0.050
3	0.025
4	0.013
5	0.006
6	0.003
7	0.002
Control	0.000

Setting up your bags:

1. Obtain 8 small snack ziplock bags. Label each bag with the concentrations from Table 1.
2. Cut pieces of paper towel and insert into bag.
3. Staple across each bag such that there is space for roots to grow.
4. Add 10 seeds to each bag.

Get 2 x 3 inch jewelry bags Cut piece of paper towel and insert into bag Staple across the bag such that there is space for roots to grow Add 10 seeds to **each** bag



Creating your stock solution:

1. Using the electronic balance and a weighing boat, mass out 2 g (**½ tsp for virtual**) of salt.
2. Add 2 g (**½ tsp for virtual**) of salt to a beaker and add 10 mL (**2 tsp for virtual**) of distilled water.
3. Stir solution.
4. This creates a 0.2g/mL NaCl stock solution

Creating your experimental solutions:

5. Obtain 8 test tubes (mini cup for virtual). Label each test tube (mini cup for virtual) with the concentrations from Table 1.
6. Fill each test tube with 5 mL (1 tsp for virtual) of distilled water.
7. Add 5 mL (1 tsp for virtual) of stock solution to Test Tube 1 (mini cup for virtual) labeled "0.1 g/mL". Mix these two solutions by swirling.
8. Using a pipette remove 5 mL (1 tsp for virtual) of solution from Test Tube 1 (mini cup for virtual) and put it into Test Tube 2 (mini cup for virtual) labeled "0.05 g/mL". Mix these two solutions by swirling.
9. Using a pipette remove 5 mL (1 tsp for virtual) of solution from Test Tube 2 (mini cup for virtual) and put it into Test Tube 3 (mini cup for virtual) labeled "0.025 g/mL". Mix these two solutions by swirling.
10. Using a pipette remove 5 mL (1 tsp for virtual) of solution from Test Tube 3 (mini cup for virtual) and put it into Test Tube 4 (mini cup for virtual) labeled "0.0125 g/mL". Mix these two solutions by swirling.
11. Using a pipette remove 5 mL (1 tsp for virtual) of solution from Test Tube 4 (mini cup for virtual) and put it into Test Tube 5 (mini cup for virtual) labeled "0.0063 g/mL". Mix these two solutions by swirling.
12. Using a pipette remove 5 mL (1 tsp for virtual) of solution from Test Tube 5 (mini cup for virtual) and put it into Test Tube 6 (mini cup for virtual) labeled "0.0031 g/mL". Mix these two solutions by swirling.
13. Using a pipette remove 5 mL (1 tsp for virtual) of solution from Test Tube 6 (mini cup for virtual) and put it into Test Tube 7 (mini cup for virtual) labeled "0.0016 g/mL". Mix these two solutions by swirling.
14. Using a pipette remove 5 mL (1 tsp for virtual) of solution from Test Tube 7 (mini cup for virtual) and squirt this into the sink. By doing this we are ensuring all test tubes have 5 mL of solution in them.
15. This is a serial dilution. Each successive test tube of solution is half the strength of the previous test tube.

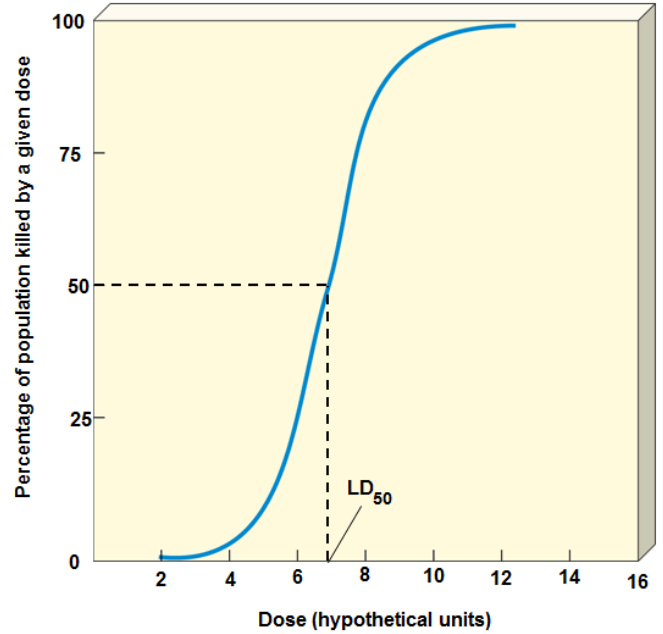
Adding the solution to the bags:

16. Using a pipette, add 3 mL ($\frac{1}{2}$ tsp for virtual) of each solution to the appropriate bag. Your control solution will come from the beaker of distilled water. Close the top of the bag.
17. Place all bags into a beaker and put in the tray designated by your teacher.

Data Analysis:

18. After 2 days, determine the percent dead and/or ungerminated for each concentration. Record this data in Table 2.
19. Graph the % mortality on the y-axis and the salt concentrations on the x-axis.
20. Determine the LD50 which is the concentration where 50% of the seeds did not germinate or died & label on graph.
21. Make another graph to present the CLASS % mortality. Determine the LD50 of the class data & label on graph.
22. See the example LD50 graph below.

Table 2			
Concentration (g/ml)	# dead or ungerminated (out of 10 seeds)	Your Group % Mortality	Class % Mortality (copy from board)
0.100			
0.050			
0.025			
0.013			
0.006			
0.003			
0.002			
0.000 (control)			



Conclusions:

1. Your teacher performed a demonstration at the beginning of class. What did you learn from that demonstration about chemicals in water?
2. What is the control in this experiment? What is the dependent variable? What is the independent variable?
3. Based on your results from this lab, what is the safest concentration (Lowest Observable Effect Concentration- LOEC) of salt for seeds?
4. Compare the class data with your data. Which do you think is more accurate? Why?
5. Suppose you were testing the LD₅₀ of arsenic on animals. How would the results you get for a small perch (a type of fish) be different from the results you would get for a human? Why would they be different?

6. What is meant by the term “threshold of toxicity”? Label this threshold on each line on your graph.
7. What is meant by LD50? Infer what LD25 and LD75 are based on your knowledge of LD50.
8. Discuss 3 environmental effects of using NaCl on roads and highways during ice and snow storms.
9. Define salinization and describe how it affects agricultural land on developing crops.
10. What are sources of salt on salinized lands?
11. What is a method of getting salt out of soil?
 - a. What is a disadvantage of using this method?
12. Why do only a certain percentage of organisms die in a given population when exposed to a certain concentration of a toxic chemical?
13. What possible sources of error were present in this experiment? How might they have influenced your data and how would you resolve those in future experiments?