

APES LD-50/Bioassay Salinization Major Lab

Background Pre-lab – LD50 and MSDS

We handle many materials daily that are toxic. We are often unaware of the degree to which they are toxic. For a variety of reasons, different animals respond differently to the same toxin. Some animals may be very sensitive to a toxin, whereas others are relatively resistant to its effects. Because species of animals vary, it is important to understand that what is toxic to one organism may not necessarily be toxic to other kinds of organisms to the same extent.

Many household items that we deal with on a regular basis are toxic materials, but we don't usually think of them as being toxic. It can be instructive to examine several such materials to determine their toxicity.

The commonly used term to describe acute ingestion toxicity is LD₅₀. LD means Lethal Dose (deadly amount) and the subscript 50 means that the dose was acutely lethal to 50% of the animals to whom the chemical was administered under controlled laboratory conditions. The test animals (usually mice or rats) are given specific amounts of the chemical in either one oral dose or by a single injection and are then observed for 14 days.

Since LD₅₀ values are measured from zero up, the lower the LD₅₀ the more acutely toxic the chemical. Therefore, a chemical with an oral LD₅₀ of 500 would be much less toxic than a chemical with an LD₅₀ of 5. LD₅₀ values are expressed as milligrams per kilogram (mg/kg) which means mg of chemical per kg of body weight of the animal. mg/kg is the same as ppm. For example, if the oral LD₅₀ of the insecticide parathion is 4, a dose of 4 parts of parathion for every million parts of body weight would be lethal to at least half of the test animals.

An **MSDS** (Material Safety Data Sheet) is a document (for each chemical) with information on all the physical and chemical properties for that chemical, as well as information on reactions and safe disposal of the chemical waste. The following information can usually be found in a MSDS:

- Identity of the organization responsible for creating the sheet and the date of issue.
- The material's identity, including its chemical and common names.
- Hazardous ingredients.
- Exposure limits.
- Physical and chemical hazards and characteristics.
- Health hazards.
- Emergency and first aid procedures.
- Spill and disposal procedures.
- Precautions and safety equipment.

Instructions for Pre-lab – LD50 and MSDS

1. Using your OWN MASS in kg, figure out how many total g would be required to kill 50% of perfect duplicates of yourself. Be careful about units! For your reference, a penny weighs around 3000 mg or 3 g. You don't need to show work for all of these problems, but write out ONE complete example of your calculations and conversion to LD50/person below the table so that I know how you did it. Include your weight in pounds and kilograms. Remember, everyone's answers will be slightly different.

Note: 1 lb. = 0.45359 kg = 453.59 gm

2. Find a Material Safety Data Sheet (MSDS) for an ingredient in some household substance you have (e.g. toothpaste, shampoo, mouthwash, junk food additives, etc.) and give its LD for 50 the oral route for a person in g/person. Assume the LD50 of a rat or mouse will be the same as a human. Don't use any of the ones already listed below. Search for MSDS's at one of the following websites, and include the printed first page of the MSDS for the substance you have chosen.

<http://siri.uvm.edu/msds> or <http://www.msds.com/> or <http://www.setonresourcecenter.com/MSDSs/comply1.htm> as well as chem. Fact sheets (Not MSDS format) from the EPA at <http://www.epa.gov/chemfact/>

Name: _____ Your mass in lbs _____ Your mass in kg _____

Your substance: _____ Did you include the MSDS sheet? Yes No (circle)

Pre-Lab Data Table

| Substance (source or product) | LD50 (mouse or rat) mg/kg or g/kg | LD50 for you (g/person) |
|---|--------------------------------------|----------------------------|
| disodium EDTA (Secret | 2000. mg/kg | |
| benzaldehyde (Cherry Flavor)* | 4.8 mg/kg | |
| Tetrahydrocannabinol (THC from marijuana)* | 110 mg/kg | |
| Ethyl acetate (Cherry Flavor)* | 6100 mg/kg | |
| propylene glycol (Cherry Flavor) | 20 g/kg | |
| Caffeine (Mountain Dew)* | 0.13 g/kg | |
| malic acid (sour candy)* | 1.6 g/kg | |
| Methanol (wood alcohol)* | 5628 mg/kg | |
| Nicotine (through mouth)* | 190 mg/kg | |
| Botulinum toxin (bacteria)* | 3×10^{-8} mg/kg | |
| potassium nitrate (fertilizer) | 190 mg/kg | |
| sodium fluoride (toothpaste) | 52 mg/kg | |
| parathion (pesticide) | 6.0 mg/kg | |
| Vx (nerve gas) | 2×10^{-2} mg/kg | |
| tetrodotoxin (poison from puffer fish) | 334×10^{-6} g/kg | |
| diazinon (ant killer dust) | 0.076 g/kg | |
| amphetamine sulfate | 32 mg/kg | |
| Ephedrine | 0.600 g/kg | |
| gamma hydroxybutyrate (date rape drug) | 2.0 g/kg | |
| Your substance () | | |
| <i>* note: these are natural substances</i> | | |

Show calculations below and/or attach another sheet showing your sample calculations:

APES LD-50/Bioassay Salinization Experiment

Purpose:

The purpose of this lab is to study how a bioassay is used to determine the toxicity of a chemical. Students will determine the LD50 of salt on the growth of radish seeds.

Objectives:

1. Conduct a controlled experiment to test the toxicity of salt on the growth of radish seeds
2. Apply the experimental results to environmental problems

Introduction:

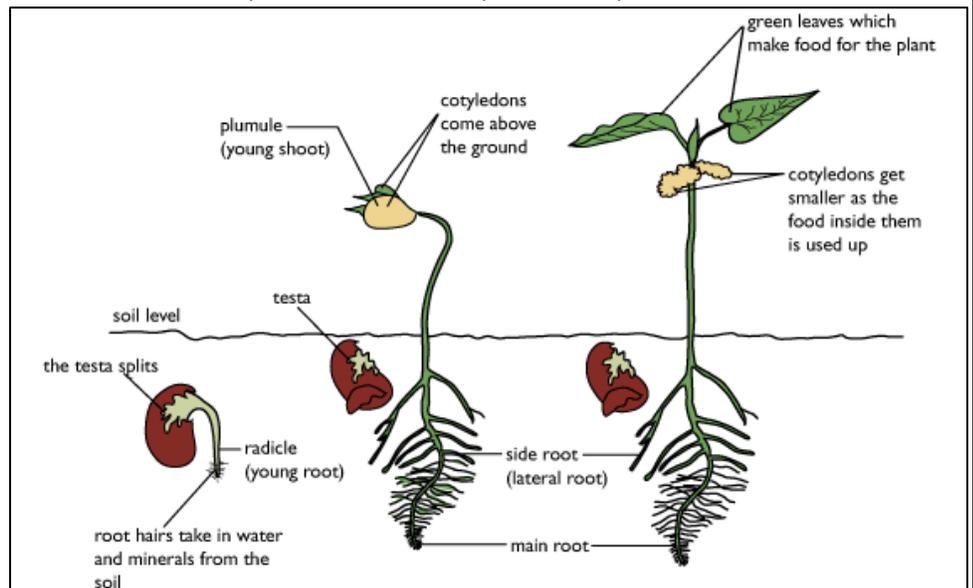
Over 15% of the earth's agricultural land is irrigated in order to maximize crop yields. Irrigation water contains a variety of dissolved salts including NaCl, MgCl₂, CaCl₂, Na₂SO₄, CaSO₄, CaCl, KCl, MgSO₄, Na₂CO₃, CaCO₃, and MgCO₃ among others. These salts are leached from soil and rocks during percolation or runoff of surface water. Groundwater can have significant amounts of salts, leached from sedimentary rocks comprising the aquifer. When a field is irrigated, much of the water can evaporate, leaving these salts behind as a thin layer on top of the soil. If you take a glass of tap water and leave it in the sun until all the water evaporates, a film will be left on the glass. These are salts. Soil salinity can affect crop germination levels and yields. Over time, salts build up on fields until the soil is so salty (salinized) that seeds will no longer germinate in the soil. Excessive salinity costs the United States billions of dollars each year. As new land comes into use, it is often in arid areas, which are highly susceptible to the problems associated with soil salinization.

The use of a biological organism to test the toxicity of a chemical compound is termed **bioassay**. In this method it is assumed that a tested organism will react in a predictable way to increasing amounts of a particular chemical compound. Bioassay has been used by drug companies to test new products on laboratory animals before humans. Bioassays are also used in environmental testing. They can determine the degree of harm to be expected from toxic soil, industrial effluents, agricultural runoffs, dredge spoils, and drilling and mining wastes, as well as to test for the effectiveness of the clean-up of a contaminated site.

In this investigation, you will perform what is called a **dose/response experiment**. This method requires you to increase the dose of a chemical incrementally and record how the organism responds to the exposures. For a test organism, you will use radish seeds. Radishes are commonly used in bioassays because their root growth, rather than just germination rate, is especially sensitive to many chemicals. For a variety of reasons salt solution will be the toxin. Salt is inexpensive and safe to use, and is a widespread environmental problem for plants.

Materials:

- Radish seeds
- Salt water solutions
- Distilled water
- Petri Dishes (10)
- Paper towel
- Salt Water Concentrations:
 - 3.0%
 - 2.5%
 - 2.0%
 - 1.5%
 - 1.0%
 - 0.5%
 - 0.1%
 - 0.05%
 - 0.01%



Note (1% = 1 gram of salt to 100 mL of water)

Bioassay Procedures – Day 1

- Step 1)** Prepare your salt solutions, then label 10 Petri Dishes with your name and the pre-selected salt concentrations. Nine will be test solutions and one will be a control with distilled water.
- Step 2)** Soak a paper towel with in your first salt solution (Just enough to moisten the paper towel- not dripping wet) and place in the appropriately labeled Petri Dish.
- Step 3)** Complete step 2 for the remainder of the test solutions. (Remember your control group)
- Step 4)** Place 10 radish seeds into the Petri Dish. Make sure there is a separation between each seed. Seal your dish with Parafilm
- Step 5)** Place the Petri Dishes in a dark place at room temperature for at least 4 days.
- Step 6)** Make a table of data. Be sure to include:
- The type of radish seed you used
 - The number of seeds you started with
 - The number of seeds that did not germinate
 - The percent non-germinating seeds
 - The average length of the roots called *radicle*.
 - Percent mortality (this is shown in analysis)

Bioassay Procedures – Day

- Step 7)** After at least 4 days, start to collect your data. Count how many seeds in each bag germinated and measure the radicle in millimeters. Be sure that you measure only the root, from the seed remnant to the tip of the root, not the shoot and beginnings of leaves.
- Step 8)** Calculate the average length of the radicle and record on data table. (show calculations)

Data Analysis

- Step 1)** Graph the average length of the radicle and the percent non-germinated versus salt solution strength on excel using a scatter plot with only markers.
- Step 2)** Right click on the X axis and format the axis to be a logarithmic scale on the base of 10.
- Step 3)** Right click on each graphed data set and add a polynomial trendline
- Step 4)** Print your graph and paste into your lab notebook.
- Step 5)** Calculations will be performed using the following formula:

$$\text{Percent Mortality (PM)} = \frac{(I - S)}{I} \times 100$$

- Step 6)** Calculate the percent mortality using the formula listed above. Include in your data table.
- Step 7)** Plot the concentration of the pollutant (salt) and percent mortality on a graph. The graph should fill an entire page of your lab notebook. Percent Mortality should be plotted on the Y-axis 0 to 100 and the Concentration of Pollutant (mg/L) should be plotted on the X-axis. It should be a log scale that runs from 0 to 1.0 to 100 to 1000.
- Step 8)** Draw a line through the plotted points on your graph and estimate the LD50 by drawing a horizontal line from the 50 percent mortality point on the graph to the plotted line and then a vertical line from that point down to the concentration of the pollutant scale on the X-axis. The intersecting point on your plotted line is the LD50 estimate.
- Step 9)** Using the same procedure used above, determine the LD25 and LD75.

Analysis/Conclusion:

- 1a. What is meant by the term *threshold of toxicity*? In what other contexts have you seen this term used?
- 1b. On your graph of percent non-germination vs. solution strength, label the Threshold of Toxicity on your excel graph.
- 2a. What is meant by *LD-50*? Describe some situations in which it is used. What do the LD25 and LD75 represent?
- 2b. Label LD-50 on your excel graph of percent non-germination vs. solution strength.
3. Discuss three environmental effects of using sodium chloride (NaCl) on roads and highways during ice and snow storms.
4. Define salinization and describe how it affects agricultural land on developing crops.
5. What are the sources of salts on the salinized lands?
6. Discuss three specific ways that farmers can remediate the salinization of their cropland.
7. Looking at your answers to number 6, give one negative aspect to each of the remediation techniques listed above.
8. Why is it that only a certain percentage of organisms die in a given population when exposed to a certain concentration of a toxic chemical?
9. What are your variables in this experiment (independent, dependant, control)?
10. What is the purpose of the Petri Dish where only distilled water was used?
11. Based on your data in this lab, what is the safe concentration of salt for radish seed germination? What is the Lowest Observable Effect Concentration (LOEC)? Explain your answer.
12. Often indicator species are used to study the overall health of an ecosystem. If you were to study an ecosystem containing radish plants, would you use it as an indicator species, why or why not? Explain your reasoning.

Sources of Error:

What possible sources of error were present? How might they have specifically influenced your data and how would you resolve them in future experiments?

Suggestions for Future Research:

What changes would you make to this lab to advance your studies on this LD50 & bioassays?
What changes would you make to this lab to advance your studies on this salinization?

LD50/Salinization Lab Scoring Sheet

Name _____

| | |
|--|--|
| _____/10 Table of Contents | _____/30 Data Table |
| _____/50 Pre-Lab (including MSDS & calculations) | _____/50 Graph 1 completed and labeled |
| _____/25 Materials, Procedures & Introduction | _____/50 Graph 2 completed and labeled |
| _____/10 Calculations Shown | _____/240 Discussion |
| _____/20 Sources of Error | _____/20 Suggestions |

_____/500 Total

***Please cut out scoring sheet and place in your lab notebook. Use it as a checklist!
This is a MAJOR lab – worth as much as a unit exam! Take your time with it!***