DOSE/RESPONSE EXPERIMENTS USING LETTUCE SEEDS

(*Adapted from:* Trautmann, NM, WC Carlsen, ME Krasny, and CM Cunningham. 2001. Assessing Toxic Risk. Teachers' Manual and Student Edition. Cornell Scientific Inquiry Series. Arlington, VA: National Science Teachers Association. 156 pp.)

DESCRIPTION:
Students conduct dose-response experiments in which they test the response of lettuce seeds to varying concentrations of salt (NaCl). They will measure the toxic effects of salt on plant growth by measuring two end points: seed germination and root growth.

RATIONALE:
In the scenario, students will determine the potential risk that Hydroville residents face when exposed to toxins and understand the relationship between the dose of a toxin and the magnitude and type of biological response that would be expected to occur based on the toxicity of the chemical or biological agent.

PURPOSE/GOALS:
Students will:
- Conduct an experiment using lettuce seeds to test the toxicity of salt and to understand the dose-response relationship.
- Perform serial dilutions to become familiar with the concepts of chemical concentrations and dilutions, and to prepare solutions they can use with dose-response experiments.
- Work collaboratively to implement experiments, interpret results, and analyze data and conclusions.
- Write a laboratory report which includes: problem and purpose, hypothesis, experimental methods, results, analysis, and conclusion.

PREREQUISITE KNOWLEDGE:
- Knowledge of the scientific method.

TIME ESTIMATE:
Prep: 60 minutes

Activity: Two to three 55-minute class periods
- **Part 1: Preparing Serial Dilutions of a Salt Solution – 55 minutes** (can be pre-made to save time)
- **Part 2: Setting up a Dose-Response Experiment – 55 minutes**
- **Part 3: Collecting Data and Plotting Results – 55 minutes**
- **Part 4: Laboratory Report - homework**

MATERIALS:
- 1 packet lettuce seeds: *Buttercrunch* (*Lactuca sativa*) (600/class)
- distilled water
- table salt (sodium chloride - NaCl)
- balance
- 1000 mL graduated cylinder
- stir bar

- 1000 mL beaker

MATERIALS (PER GROUP OF 3-4 STUDENTS):

Part 1: Preparing Serial Dilutions of a Salt Solution
- 6 – test tubes (size: 20 X 150 mm)
- tape or wax pencil for labeling test tubes
- test tube rack
- 10 mL graduated cylinder or pipette
- distilled water
- "concentrated salt solution" = 12.0 grams table salt (sodium chloride - NaCl)/1 Liter distilled water

Part 2: Setting up a Dose-Response Experiment
- 6 – test tubes with serial dilutions of salt solutions (from Part 1)
- 6 - 90 mm petri dishes with lids
- 3 pieces of unbleached paper towel or 6 coffee filters
- pencil
- scissors
- 10 mL graduated cylinder or syringe
- tape and permanent marker
- 60 lettuce seeds (10/petri dish)
- tweezers
- plastic bag

Part 3: Collecting Data and Plotting Results
- metric ruler
- calculator
- 2 sheets graph paper (75 squares X 100 squares)

MATERIALS TO PHOTOCOPY:
(1copy/student)
- Student Handout 1: Some Tips for Planning an Experiment
- Student Handout 2: Laboratory Report Outline
- Laboratory Report Scoring Guide

(These sheets can be laminated to use in all of your classes.)
- Lab Protocol #1 - Part 1: Preparing Serial Dilutions of Salt Solutions
- Lab Protocol #2 - Part 2: Setting up a Dose-Response Experiment
- Lab Protocol #3 - Part 3: Collecting Data and Plotting Results

BACKGROUND INFORMATION:
For centuries, humans have observed that some chemicals produced by nature were poisonous. Poisonous chemicals produced by living organisms are called toxins. Knowledge of toxins has proved useful in killing animals for food or getting rid of enemies. The 16th century physician, Paracelsus, recognized that the same chemical could have both therapeutic and toxic properties depending upon how much of it was used. He wrote, "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy."

The beneficial or harmful effects of a chemical are dependent upon the amount of the chemical that gets into an organism. The absolute amount of a chemical is called the *dose*. The toxicity or harmful effects from a chemical depends upon the dose, the exposure (route, frequency, and duration), individual susceptibility, properties of the chemical, and exposure of the organism to other substances.

How do we know a chemical is toxic?
Toxicologists are scientists that study the toxicity of substances. Toxicologists use plant and animal models to determine the toxicity of a chemical. With pharmaceutical drugs, there is the additional advantage of data from clinical trails in humans prior to marketing. Animal studies are done first. The clinical trials provide an opportunity to correlate toxicity seen in animal models to humans. Correlation of animal results to humans is good but not perfect. People who are more sensitive to the drug than the general population may emerge when large numbers of people are exposed. With non-drug chemicals such as pesticides, we have little or no human data. The only human toxicity data on pesticides and industrial chemicals come from accidents, suicides, or occupational exposure when good exposure measurements are taken.

Chemical Toxicity
Two terms that are commonly used when referring to the toxicity of a chemical are NOEL (NOAEL) and LD_{50}. NOEL (No Observed Effect Level) is the maximum daily dose at which the response is zero. This means that the experimental animals do not appear to have any adverse effects with exposure to the chemical at the specific NOEL concentration. LD_{50} (Lethal Dose 50) is the dose of the chemical at which 50% of the animals or plants die. For other effects such as tumor development or, in this experiment, seed germination and root length, the term TD_{50} (Toxic Dose 50) is used. TD_{50} is the dose at which 50% of t6he population shows the toxic effect.

Why conduct toxicology experiments?
"One of the reasons for studying toxicology at the high school level is its relevance to everyday life. On a daily basis we are confronted with news reports about toxic chemicals in our food, water, and environment. How do we decide which of these are worth worrying about? Each of us must make individual decisions about questions such as, "Should I buy bottled water, or is it safe to drink water from the tap?" We also can exert political pressure to influence broader societal questions such as, "Should the federal government ban sales of saccharin?" or "Should the town spray herbicides to control weed growth on the highways?" Too often these decisions are based on misconceptions about what is "safe" and what involves too great a risk. In learning the basic concepts of toxicology, students will become better prepared to make reasoned decisions about issues such as these."

Excerpted from: Trautmann, N. et al. 2001. Assessing Toxic Risk. National Science Teachers Association Press. Arlington, VA.

What do dose-response experiments tell us?
Dose-response bioassays are experiments where a population of test organisms is exposed to increasing doses of a single chemical and the range of responses of the test organism to the chemical is recorded. In dose-response experiments, the composition and concentrations of chemical solutions are known. Often in dose-response experiments, chemical solutions that could be lethal at high concentrations may be harmless or possibly even beneficial at low concentrations. Overall, dose-

response experiments demonstrate the toxicological principal that "the dose makes the poison." The resulting data can also be used to determine the LD_{50} or $\mathbf{TD_{50}}$.

Bioassays: Laboratory mice vs. Other Organisms in Toxicology Experiments
Dose-response bioassays provide information about the acute toxicity of a single chemical to the bioassay organism from short-term, acute exposures. Dose-response experiments can demonstrate the range of effects of chemicals on bioassay organisms, from no observable effect to extreme toxicity. Since other types of organisms might respond differently to the same chemical, it is a good idea to try bioassays using several different species.

When scientists want to determine the possible impacts of a substance on human health, they start their investigations by conducting dose-response bioassays using rats, mice, or other laboratory animals. Laboratory mice are used for bioassays related to human health because they provide a reasonable model of human response to chemicals. The results of these experiments are LD_{50} values. Scientists use these bioassay results to compare the toxicity of various compounds and then to predict the potential effects of these same chemical on human health.

Bioassays are also used to test the toxicity of environmental samples, and in these types of bioassays we are interested in the response of organisms other than humans. In environmental sampling, bioassays can indicate the toxicity of an unknown solution or environmental sample. In these cases instead of using laboratory rats or mice, it makes sense to conduct bioassays with organisms that are typical of the environment being tested. Herbaceous/aquatic plants, aquatic invertebrates, fish, worms, single-celled algae and fungi are all useful for bioassays because they are representative of the types of organisms found in freshwater or terrestrial ecosystems, and they are responsive to many types of environmental contaminants. Dose-response experiments provide a useful frame of reference for bioassays because these experiments test mixtures of unknown chemical found in environmental samples and can be compared to laboratory run dose-response experiments as a reference of response for specific doses of known chemicals.

So how do scientists choose what types of bioassay organisms to use in their experiments? The species used for bioassays should be sensitive to various types of chemicals and chemical mixtures. The bioassay organism should also be relatively easy to keep alive in the laboratory. No single species provides the perfect bioassay. Each responds in its own way, so toxicity testing usually includes more than one species in order to provide a more complete picture of toxicity. The species of choice may also depend on the type of chemicals being studied and the purpose of the experiments. For example, suppose that you want to investigate the impact of household cleaners advertised to be effective in killing molds on non-porous hard surfaces. Using bioassays with yeast or other fungi could help determine whether a specific household chemical is toxic to molds and at what concentrations.

Conducting Dose-Response Experiments Using Lettuce Seeds – Why Lettuce Seeds?
Using lettuce seeds, student can carry out the same types of toxicity tests used by scientists in universities, government, and industry. Lettuce seeds are used in bioassay of water and other environmental samples because of their sensitivity to environmental contaminants such as heavy metals and some pesticides, solvents, and other organic compounds. Lettuce seeds are also inexpensive, easy to store, and don't require maintenance. (*Excerpted from: Trautmann, N. et al. 2001. Assessing Toxic Risk. National Science Teachers Association Press. Arlington, VA. pp.20)*

TERMINOLOGY:

Hypothesis	Toxicity
Dose-Response	Serial dilution
Independent variable	Dependent variable
Radicle	Control
Mean / average	Concentration
LD_{50} (Lethal Dose 50)	TD_{50} (Toxic Dose 50)

SUGGESTED LESSON PLAN:

Getting Started

1. Purchase lettuce seed varieties: *Buttercrunch* (*Lactuca sativa*) or *Black-Seeded Simpson* since they are the standard species recommended for bioassays. Approximately 600 seeds (one packet) are required for a class of 30 students. Seeds may be stored in a refrigerator for months.
2. Prepare a 0.2M NaCl solution or "concentrated salt solution" from which students will make their serial dilutions. Measure 12.0 grams of table salt and add enough distilled water to make 1 liter of the concentrated salt solution. Stir solution until all of the salt is dissolved. Decant the concentrated salt solution into smaller beakers for student use.
3. To save time, you can prepare the serial dilutions of the various salt solutions for the class to use. Each group of students (working in threes) needs 5 mL of the various serial dilutes. Students would then proceed to Part 2. Setting up a Dose-Response Experiment.
4. Remember that it takes 4-5 days for the lettuce seeds to grow and before data can be collected. Plan to incorporate other activities during this time.

Part 1. Preparing Serial Dilutions of a Salt Solution – 55 minutes (can be pre-made to save time)
Doing the Activity

1. Display all of the materials needed for the experiment including concentrated salt solution, petri dishes, lettuce seeds, etc.
2. Discuss with the class, "When you want to conduct an experiment, a good way to start is by asking a question for a specific purpose. Brainstorm some experimental possibilities, e.g., 'Is sugar good for your teeth?' or 'Will fertilizers help plants grow' Looking at these materials, what types of test could we run?" Decide on one **Question**, "How do variations in salt solutions affect the root growth of lettuce seeds?"
3. Discuss the possible reasons or purpose for testing the effects of salt on lettuce seed growth. The intended **Purpose** is as follows:

   Answer: *In many areas of the country, road salt is used to melt the snow on roads. Use of salt on the roads is a concern to some scientists who want to know how it affects the nearby vegetation. Lettuce seeds are used in bioassay of water and other environmental samples because of their sensitivity to environmental contaminants such as heavy metals and some pesticides, solvents, and other organic compounds. This type of experiment can also be repeated on aquatic organisms, such as Daphina, or brine shrimp to predict what would happen if the salt solution got into a water system.*

4. Have students develop their own **Hypotheses**. "Write your hypothesis to the research question that you agreed on in your class."
   Answers will vary.

5. Distribute a back-to-back copy of *Student Handout 1: Some Tips for Planning an Experiment and Student Handout 2: Laboratory Report* to each student. Discuss each of the aspects of an experiment and a lab report with the class.
6. Students work in groups of three and should follow instruction on Lab Protocol #1 Part 1: Preparing Serial Dilutions of Salt Solutions to make five serial dilutions from the concentrated solution. Making their own serial dilutions helps students become familiar with the concepts of chemical concentrations and dilutions.
7. Ask students to write out their **Experimental Method** section for homework.

Part 2. Setting up a Dose-Response Experiment – 55 minutes

1. Students will have work in same groups to complete Lab Protocol #2 – Part 2: Setting up a Dose-Response Experiment. See student worksheets for specific instructions.
2. If petri dishes are not available, sandwich-size, resealable plastic bags can be used for incubating the seed samples.
3. *NOTE: Absorbent paper towels or coffee filters can be substituted for the filter paper, as long as they are unbleached. Bleached paper may contain dyes or chlorine that inhibits seed growth.*
4. The lettuce seeds need to be incubated for 4-5 days in the dark at a constant room temperature. Lettuce seed germination can exceed 5 days, up to 10 days if needed.
5. Inspect the lettuce seeds during incubation. If the paper seems dry, add a few more milliliters of the appropriate concentration of salt solution onto the paper. Supplemental watering of salt solutions on day 5 or 6 is recommended.

Part 3: Collecting Data and Plotting Results – 55 minutes

1. Before the students start Part 3: Collecting Data and Plotting Results, have them reexamine the hypothesis they wrote before they set up the experiment. Ask them to reconsider or form a new hypothesis before and after they analyze the **results** of the experiment.
2. Announce to students that the radicles are very delicate and that they must be removed from the paper towel and measured very carefully.
3. Students measure the lettuce seeds to determine two endpoints: percentage of seeds germinated and the length of the radicle (embryonic root).
   NOTE: Students calculate the mean (average) based on germinating lettuce seeds only; they should not factor in the non-germinating seeds.
4. Students create two graphs: dose vs. response of lettuce seeds and percent of germinating seeds. The percentage of seed germination is graphed to determine if the seeds were viable and the study valid. If fewer than 80% of the seeds in your control dishes sprouted, something may have gone wrong in your experiment or concentration of salt is the cause.
5. Students must also calculate the "Difference on Radicle Length" which compares the mean radicle length for each treatment (T) to the mean control (C) to determine if the radicle growth was inhibited (negative change), promoted (positive change), or not affected (no change).

Wrap-up

1. Have students answer the conclusion questions under the headings **Analysis** and **Conclusion** in their Lab Reports.

ASSESSMENT:

- Students complete a written lab report. Students can have peers review each other's lab reports using the scoring guide before a teacher evaluation. (See *Laboratory Report Scoring Guide* on the next page.)

EXTENSIONS:

- Students calculate standard deviation for each treatment of salt concentration.
- Students use a computer application to create spreadsheets and graph dose-response results.
- Students test other chemicals following the same lab protocol.
- Students conduct bioassays of unknown salt solutions found in environmental samples such as stream water, parking lot runoff, etc.
- For additional information or other research extensions on lettuce seeds, refer to the website by Cornell University. < http://ei.cornell.edu/toxicology/bioassays/lettuce/>.

RESOURCES:

- Assessing Toxic Risk. Environmental Inquiry. Cornell University. <http://ei.cornell.edu/toxicology/>.
- Rathbun, Joe. The Volunteer Monitor. A Simple Bioassay Using Lettuce Seeds. Vol.8. No.1. Spring 1996. <www.epa.gov/owowwtr1/monitoring/volunteer/spring96/proman21.html>.
- Trautmann, NM, WC Carlsen, ME Krasny, and CM Cunningham. 2001. Assessing Toxic Risk. Teachers' Manual and Student Edition. Cornell Scientific Inquiry Series. Arlington, VA: National Science Teachers Association. 156 pp.

TEACHER KEY

Part 3: Collecting Data and Plotting Results

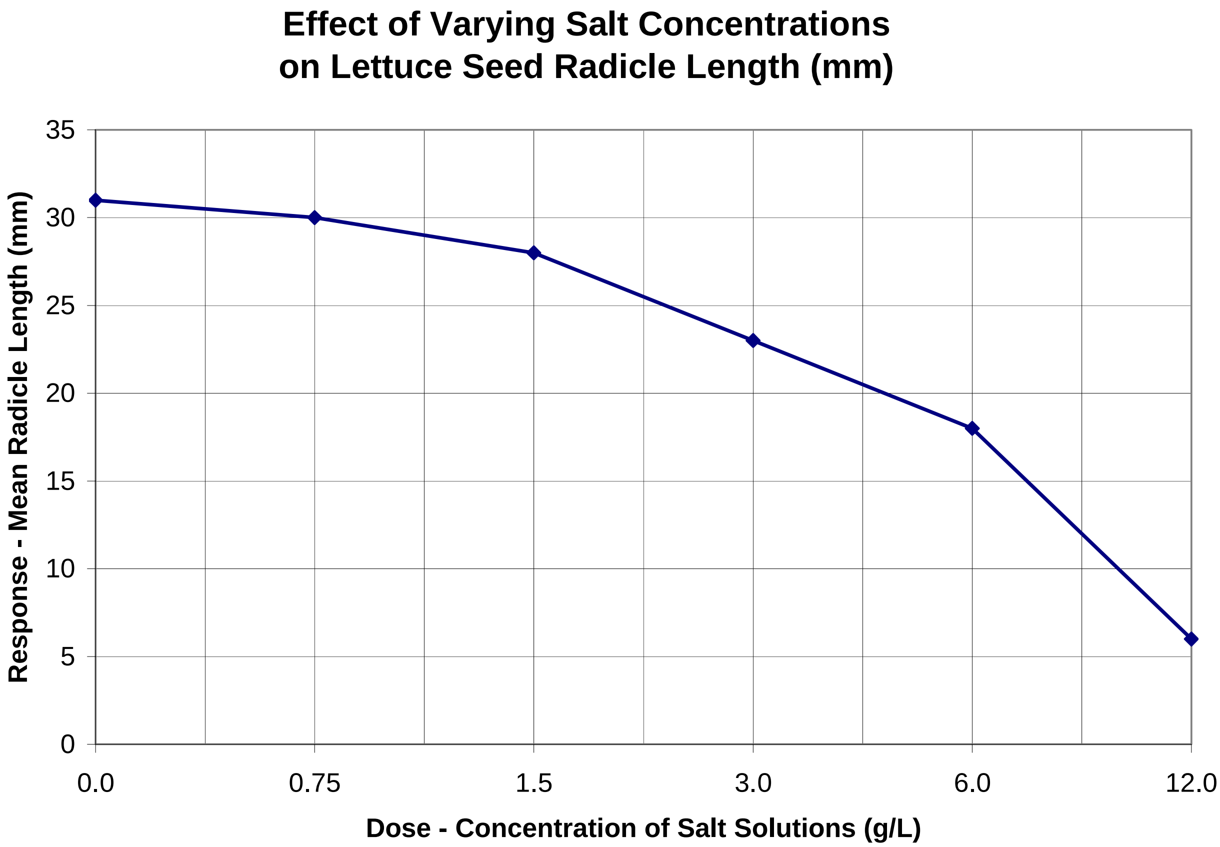


Table 2: Lettuce Seed Results

Treatments: Concentration of Salt Solution (mg/L)	% Seeds Germinated	Replicates - Radicle Length (mm)											Difference in Radicle Length: C – T
		1	2	3	4	5	6	7	8	9	10	T = Mean Radicle Length (mm)	
C = Control	100	34	30	30	27	31	32	30	29	32	30	31	**0**
0.75	90	30	35	34	20	28	35	30	35	25		30	1
1.5	100	32	25	31	35	30	27	28	23	21	24	28	3
3.0	90	27	26	26	25	25	25	9	26	19		23	8
6.0	70	24	16	10	23	14	19	20				18	13
12.0	50	7	5	8	5	3	-	-	-	-	-	6	25

TEACHER KEY

Concentration of Salt Solutions (g/L)	Germinating Seeds (%)
Control	100
0.75	90
1.5	100
3.0	90
6.0	70
12.0	50

Insert Table 2: Lettuce Seeds Result Overhead

Hydroville, USA

STUDENT PAGES
FOR

BACKGROUND ACTIVITY 5
DOSE-RESPONSE EXPERIMENTS
USING LETTUCE SEEDS

FOLLOW THIS PAGE

STUDENT HANDOUT 1: SOME TIPS FOR PLANNING AN EXPERIMENT

Ask a question and describe the purpose of the experiment
When you want to do an experiment, a good way to start is by asking a *question.* For a toxicity test, the question might be "How does salt used on roads to control ice effect nearby vegetation?" Or more specifically, "Does salt inhibit germination seeds?" or "Does salt have an effect on plant growth?" Explain why your question may be important to answer to define the *purpose* of the experiment.

Form your hypothesis
After you decide on a research question, then you are ready to state your *hypothesis* or prediction of what you think will happen. An example hypothesis: "The number of seeds that germinate will decrease with increasing concentration of salt."

Experimental Method:
Plan your experiment before you do it. These are some factors to keep in mind:

Choose the variables
Your *independent variable* is the factor that you will change in your experiment. For example, in a toxicity test, the independent variable is usually *dose* – the concentration of chemical to which the organism is exposed. In this experiment, you will test five different concentrations of salt solutions.

The *dependent variable* is the factor that you predict will change or have a *response* as a result of variation in your independent variable. In this experiment, the number of seeds that germinate and radicle length are examples of two different dependent variables.

All other variables need to be controlled, e.g., light, temperature, volume of salt solution, etc. Can you think of any others?

Plan the treatments
A treatment is a factor that affects the outcome of a scientific experiment. In a toxicity test, the experimental treatment usually is the concentration of the solution to which the seeds or organisms are exposed. In this experiment, you will test varying salt concentration starting with a concentrated slat solution 12.0 grams of NaCl/1 liter of distilled water.

Choose a control
In a scientific experiment, the *control* is the group that serves as a standard of comparison. It is exposed to the same conditions as the treatment groups, except for the variable being tested. In toxicity tests, the control group is the set of seeds grown in distilled water rather than in a salt solution.

Decide the number of replicates
Replicates are individuals or groups that are exposed to exactly the same conditions in an experiment. In a lettuce seed experiment, you will test six different salt solutions, plus a distilled water control. For each of these concentrations, you will have a petri dish containing ten lettuce seeds.

STUDENT HANDOUT 2: LABORATORY REPORT OUTLINE

After you discuss *"Tips for Planning an Experiment"*, review the sections of a laboratory report. Before you begin the experiment, you should have completed these sections of your lab report.

- ☑ Title
- ☑ Question and Purpose
- ☑ Background Information
- ☑ Hypothesis
- ☐ Experimental Method
- ☐ Results
- ☐ Analysis
- ☐ Conclusion

Title: The title describes what the lab was about.

Question and Purpose: State the problem that you are testing to solve and give a reason why this experiment would be useful.

Background Information: This section provides background information that is necessary for understanding what the lab is trying to find out. It may explain a scientific principle related to the problem or explain the findings of a similar experiment. The goal of this section is to help someone who has never done the lab before to understand the data being collected and analyzed. *This section is information you have before you start your lab!*

Hypothesis: A statement explaining your prediction or the outcome of the testing.

Experimental Method: This section contains step-by-step instructions describing the lab set up and methods used to design your experiment. The directions must be clear and concise so that someone else can repeat your experiment.

- Describe your dependent and independent variables and the purpose of a control. Discuss how you controlled all of the other variables.

Results: This section contains whatever data you collected and want to display. The data may be a table, graph, diagrams, and/or observations.

Analysis: Analyzing the data collected is the most important part of a lab report. The analysis involves interpreting what your data is telling you – it provides a thorough explanation of what you have discovered from you data results. Be sure to address the following questions in this section:

- Looking at your data, do you notice any trends? Do any of your data not fit the trends you observed? If so, can you think of any reasons why these data might lie outside the range you would expect?
- What can you conclude about the toxicity of the substance you tested on lettuce seeds? Is this what you expected? Reexamine your hypothesis. Was it supported by your data?
- If you were going to repeat this experiment, what would you do differently? How might you improve the experimental design to reduce the variability of your data or lead to more reliable results?
- What additional experiment(s) would you conduct based on the results of this experiment?

Conclusion: Provide a brief summary about what you did, major results, and why your results are important.

LAB PROTOCOL #1:

Part 1: Preparing Serial Dilutions of a Salt Solution

It is suspected that salt (NaCl - sodium chloride) applied to highways for deicing may affect the growth of vegetation along the roadside and aquatic plants in nearby streams. Therefore, you will conduct a dose-response experiment to determine how lettuce seeds will respond to various concentrations of salt. Your teacher will provide you with a "concentrated salt solution" so that you can make serial dilutions - a series of solutions, each of which are half as concentrated (or two times more dilute) than the one from which it is made. You will set up a wide range of concentrations of salt solutions to test on lettuce seed growth.

Materials:

- 6 – test tubes (size: 20 X 150 mm)
- tape or wax pencil for labeling test tubes
- test tube rack
- 10 mL graduated cylinder or pipette
- distilled water
- "concentrated salt solution" = 12.0 grams table salt (sodium chloride)/1 Liter distilled water

Lab Set Up:

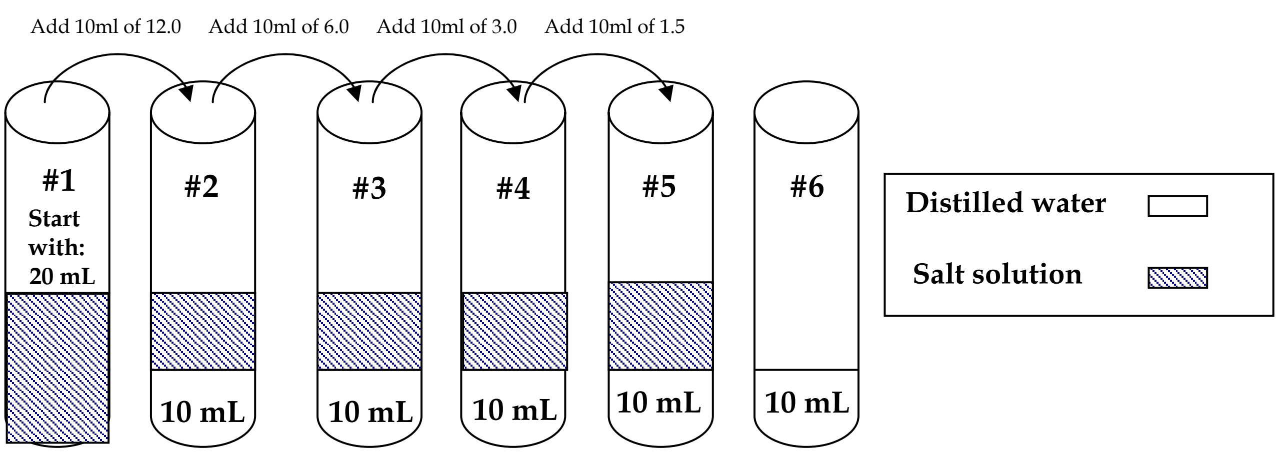


Table 1: Salt Solution Concentrations

Test Tube	Salt Concentration (g NaCl/L H_2O)
#1	12.0
#2	6.0
#3	3.0
#4	1.5
#5	0.75

#6	control

LAB PROTOCOL #1: CONTINUED

Part 1: Preparing Serial Dilutions of a Salt Solution – continued

Procedure:

1. Set up 6 test tubes in a test tube rack and label the tubes with the following salt concentrations: 12.0 g/L, 6.0 g/L, 3.0 g/L, 1.5 g/L, 0.75 g/L and Control. See Table 1. Salt Solutions.

2. Add 10 mL of distilled water to test tubes #2 - 6.

3. Measure 20 mL of the concentrated solution (12.0 g/L) and pour into test tube #1.

4. Transfer 10 mL of salt solution from test tube #1 to test tube #2.

5. Gently swirl test tube #2 to mix the salt solution.

6. Repeat steps 4 and 5 for test tubes #3-5 measuring 10 mL each time. ***DO NOT*** add any salt solution to test tube #6.

7. Measure 10 mL of distilled water into test tube #6 to serve as the control. The control will indicate whether or not your seeds are viable (capable of growing or developing).

   NOTE: The total remaining solution in each test tube is 10 mL, except for test tube #5 will have 20 mL.

8. Unless you will be using the solutions right away, cover them tightly with plastic wrap to prevent water loss through evaporation.

LAB PROTOCOL #2:

Part 2: Setting up a Dose-Response Experiment
In this protocol, you will carry out a dose-response experiment to test the sensitivity of lettuce seeds to the serial dilutions that were created in Protocol #1. This is called a dose-response experiment because you will expose lettuce seeds to various doses of a chemical (salt) and then measure the response (seed growth). You will be testing lettuce seeds since they have been proven to be an easy and inexpensive means of testing the toxicity of some types of chemicals or contaminants that may be found in water and sediments, including heavy metals, and some pesticides.

Materials:

- 6 test tubes with serial dilutions of salt solutions (from Part 1)
- 6 - 90 mm petri dishes with lids
- 3 pieces of unbleached paper towel
- pencil
- scissors
- 10 mL graduated cylinder or syringe
- tape and permanent marker
- 60 lettuce seeds (10/petri dish)
- tweezers
- plastic bag

Laboratory Protocol 2: Continued

Procedure:

1. Obtain six petri dishes. Label each dish according to the concentration of salt solution to be tested. See Table 1. Salt Solution Concentrations.

2. Fold a half of sheet of paper towel or coffee filter into quarters. Cut it out so that it fits into the bottom of the petri dish.

3. Measure 6 ml of salt solution and pour onto the paper towel in the appropriate petri dish. In the control dish, add 5 mL of distilled water. The purpose of a control is to identify how well the seeds will grow without any salt.
   NOTE: *If using the same graduated cylinder, start with the lowest salt solution (distilled water) to the highest salt solution so that the graduated cylinder is not contaminated with a higher salt solution.*

4. Add 10 lettuce seeds to each petri dish. Space the seeds out evenly on the paper towel so that they do not touch each other or the sides of the dish.

5. Place the dishes in a plastic bag and seal it to retain moisture. Label your group's name on the outside of the bag.

6. Incubate the seeds in a dark place at a constant temperature (preferably 24.5^{O}C) for 4-5 days.

7. Inspect lettuce seeds during incubation period. Record any observations. If the paper seems dry, add a 1 or 2 more millimeters of the appropriate salt solution or distilled water (control).

LAB PROTOCOL #3:

Part 3: Collecting Data and Plotting Results
In this protocol, you will measure the response of the lettuce seeds to each of the various salt concentrations. After the lettuce seeds have germinated, count the number of seed that germinated and measure the length of each radicle (embryonic root). After recording your results, you will create two graphs (% seed germination and dose-response curve) to help you analyze the data collected.

Materials:
- metric ruler
- calculator
- 2 sheets graph paper (75 squares X 100 squares)

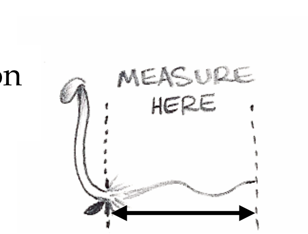
NOTE: *The lettuce seed radicles are very delicate. Handle with extreme care.*

Procedure:
1. Remove the lid of the control dish. Count the number of seeds that germinated (sprouted). Calculate the percentage of seeds that germinated and record in Table 2. Lettuce Seed Results. ***Note: If fewer than 80% of the seeds in the control sample germinate, this indicates a problem with the experiment, e.g., bad seeds, poor incubation conditions, etc. The results discarded or the test rerun.***

2. To measure the length of the radicle (embryonic root), carefully remove the germinating lettuce seed from the paper towel in one piece. The radicle may be growing into the layers of towel and can break if you pull too hard.

3. Measure the length of the radicle for each of the germinating lettuce seeds to the nearest millimeter (mm). Look carefully at each sprout to make sure you are measuring just the root, not the shoot as well. In the picture below, you would measure just the part between the two arrows, not the shoot and cotyledons to the left. Record data in Table 2. Lettuce Seed Results.

cotyledon



radicle length (mm)

4. Repeat steps 1-3 for each petri dish.

5. For each treatment, calculate the mean (arithmetic average) radicle length for each salt solution. Add the total radicle lengths for each salt solution and divide by the

total number of seeds that germinated. ***DO NOT INCLUDE data from seeds that did not germinate.*** Record data in column labeled, "Mean Radicle Length (mm)" in Table 2. Lettuce Seed Results.

LAB PROTOCOL #3:

Part 3: Collecting Data and Plotting Results - continued

Table 2: Lettuce Seed Results

Treatments: Concentration of Salt Solution (mg/L)	% Seeds Germinated	Replicates - Radicle Length (mm)											Difference in Radicle Length: C – T
		1	2	3	4	5	6	7	8	9	10	T = Mean Radicle Length (mm)	
C = Control													0
0.75													
1.5													
3.0													
6.0													
12.0													

6. Make a line graph from the data collected to show a dose-response curve. The horizontal axis should be for the independent variable, **dose** (concentration of salt solutions). The vertical axis should be for the dependent variable, **response** (mean radicle length). Draw and label the axes. In your group, you will need to come to some agreement about scales for these by looking at your data. Remember to give your graph a title.

7. To help you answer the following question: “Did the radicle length increase or decrease in length as compared to the control?” subtract the mean radicle length of each treatment **(T)** from the mean radicle length of the control **(C)**. Record your answers in the column, “Difference in Radicle Length” on Table 2.

   Difference in Radicle Length = C (control) - T (treatment mean radicle length)
   For example, calculate 0.75 mg/L: 15 mm – 5 mm = 10 mm (decrease in length)

8. Make a line graph to show the percentage of seeds that germinated for each salt solution.

9. Complete your lab report. *See Student Handout 2: Laboratory Report Outline*.

Student Name ____________________________

LABORTORY REPORT SCORING GUIDE

Possible Points	Your Points	Criteria
3	_______	You stated the *question* and explained the *purpose* of your experiment
3	_______	You included a section on *background information* of the problem
3	_______	You included your *hypothesis*
3	_______	You explained your *experimental method* in step-by-step instructions
3	_______	You explained your independent, dependent variables and control
3	_______	You explained how you will evaluate your results
3	_______	You have included your *results*, such as in data tables, graphs, etc.
3	_______	Your *analysis* section is complete and answers all of the questions
3	_______	You included a *conclusion* summarizing what you learned from your results
3	_______	Your work is generally free of errors in spelling, and you used appropriate grammar and punctuation

30 POSSIBLE POINTS _______ **YOUR TOTAL POINTS**

Grade:

A (90%) = 27-30 points

B (80%) = 24-26 points

C (70%) = 21-23 points

D (60%) = 18-20 points