Module 1: Everyday Toxicology
Lesson 2: How Can We Measure the Effects of Salt Contamination on Crop Plants?

Lesson Overview

Summary:

At the conclusion of this lesson, students will have designed an experiment that they will carry out during the next lesson. The results of this experiment will be observed and interpreted in the final lesson (Lesson 6), three to five days afterward. In this experiment, students will measure a dose/response curve that will allow them to evaluate the effect of salt on crop plants.

Pedagogically and chronologically, then, setting up the experiment follows the main objective of this lesson, which is for students to construct an experimental design by thinking very rigorously, very deliberately, very critically—and very creatively. As such, this lesson is devoted to activities and discussion within small groups where this thinking takes place.

The homework assignment is designed to extend the thinking on the students' experimental designs. Each group will have the opportunity to have their experimental designs critiqued by their peers, and to critique the other groups' designs, perhaps using the insights gained to refine their own designs before actually carrying out the experiment in the next lesson.

Objectives:

By the end of the lesson, students will be able to:

1. plan an experimental design for measuring a dose/response curve that evaluates the effects of salt on crop plants.
2. describe the importance of effective laboratory notebooks to instructor satisfaction.
3. design an observation table to record results in laboratory notebooks.
4. utilize a laboratory notebook in the practice of experimental science.
5. evaluate peer developed experimental designs to instructor satisfaction.

Grades:

9th through 12th

Prepping the Lesson

Instructions:

Materials/Technical Resources:

It is our recommendation that you walk through the teacher and student materials for this lesson to ensure that students will be able to receive the information through the modes of delivery that we intended prior to using the material in the classroom. If you or your school does not have the resources needed, you may need to make some modifications depending on the resources you have available.

The following materials/technical resources will be needed to complete the lesson. We recommend using Option #1 to provide the materials to your students in the manner in which they were intended to be delivered.

Option #1 (Preferred Requirement)
You will need to have access to a computer, the Internet, and a projection device during the entire lesson. Your students will need to have access to computers and the Internet. You and your students will simultaneously step through the module while using their given computers. You may have to make special arrangements for all of your students to have a computer. Be sure you and your students will not be blocked from Google Documents, YouTube, and your selected online collaborative tool. You may be currently using an online collaborative tool but if not, we recommend Facebook groups, Edmodo, or eChalk.

Option #2 (Minimum Technology Requirement)
If you do not have a way for your students to access the Internet individually, then you will need to facilitate their access to the information. You will need access to a computer, the Internet, and a projection device during the entire lesson. You will step through the module as your students watch and complete the presented activities. There may be modifications to the delivery of the materials that you will need to make, depending upon the resources you have available. Be sure you have access to Google Documents, You Tube, and your selected online collaborative tool. You may be currently using an online collaborative tool but if not, we recommend Facebook groups, Edmodo, or eChalk.

Lesson Time and Supply List:

This document will provide you with information on prep time needed, a list of supplies, and total lesson time for this particular lesson. See appendix for the Lesson Time and Supply List document.

Student Homework: Prior to starting this module, it is important to determine which online collaborative tool you and your students will be using. Once you have had an opportunity to review all of the lessons, decide how you will facilitate the homework discussions and submissions using your selected online collaborative tool. Be sure to give your students clear directions and objectives on your expectations of the use of this tool and their participation in their homework activities. We highly encourage you to participate with your students in their homework discussions to enhance the quality of the experience.

Essential Vocabulary: acute effects, calcium crystals, cardiovascular, cholesterol, chronic effects, continuous exposure, coronary disease, environmental concentration, experimental systems, germinate, long-term responses, median germination failure concentration or GFC_{50}, neurological disorders, pharmaceutical, plasma membrane, regulator, short-term responses, tubules, vitamin D (See Lesson 1 for additional vocabulary words.)

Student Notebook: Laboratory notebooks are arguably the most useful tool at an experimenter's bench. Remind students of the critical importance of recording all experimental observations, and especially recording all experimental conditions (e.g., the type of chemical treatment, the range of concentrations, number and species of seeds, etc.) when starting an experiment so that they can maintain comprehensive, unambiguous control over those variables when they follow up with subsequent experiments.

Implementing the Lesson

Instructions:

1. Guided Discussion (10 Minutes)

What Is the Experiment?

Start by conducting your normal opening remarks to your students and explain that students will **design and carry out** an experiment to measure dose/response curves for the effects of salt on crop plants. Today, they will design and set up the experiment, and in three to five days they will record their observations and draw conclusions.

In their homework after lesson 1, students should have **invented experimental systems** to measure the effect of salt on plants. Using the online collaborative tool, they should have answered the following questions and **critiqued one another's responses**:

What dose/response curve would you draw in order to evaluate the effects of salt on crops? In particular,

- Which variable(s) would you manipulate experimentally? In other words: **what specific quantity** would you measure and plot on the X-axis of the dose/response curve?

- Which biological response(s) would you measure? In other words: **what specific quantity** would you measure and plot on the Y-axis of the dose/response curve?

Consider how you will conduct the experiment:

- Compared with others, are these methods more or less **practical**? (Think: cost, time, resources?)

- Compared with others, are these methods more or less **sensitive**?

Here are some representative answers:

Practicality: *Experiments that require seeds or small sprouts will obviously be cheaper and easier than those that require full-size plants or trees in the field. For instance, analysis of fruit or nut yield (while providing potentially critical data) would require a long-term commitment of fairly large resources of test plots, labor, and so forth.*

Sensitivity: *There is a great deal of variation in salt tolerance among different plant species, or even among different cultivars and varieties of a particular crop species. Plants with low salt tolerance will, in general, serve as more sensitive indicators. Many plants will have different sensitivities at different developmental stages; in general, very young seedlings will be more sensitive than established plants.*

Summarize (or have students summarize) the ideas and insights that emerged from the responses and discussion on the online collaborative tool.

2. Guided Discussion (5 Minutes)

Why Use Concentration in Place of Dose?
Practical constraints on this experimental system force us to use **concentration as a proxy for dose**, and **failure to germinate as a proxy for lethality**. These constraints, like many encountered in the

Teacher Tip: How can a seed be dead? It's a fine semantic point, perhaps, but let's define a "dead" seed as one that will never germinate under any conditions.

Then how would you distinguish, for example, a seed that is dead from salt contamination from a seed that has merely failed to germinate due to salt contamination?

You could isolate seeds that have failed to germinate in salt, rinse the salt away with a thorough wash in distilled water, and test to see whether those seeds now germinate in distilled water. Those that do not are "dead," by our definition, and in this way we could calculate a true LD_{50}.

desing of experiments, reflect the ease and reliability of measurements of key variables.

- While it is very **difficult to measure the amount of salt absorbed** by an individual plant or seed, it is very **easy to expose them to defined concentrations** of salt.

- While it is more **time consuming to demonstrate that a seed is "dead,"** it is relatively **quick to determine whether a seed simply has not germinated**.

Furthermore, plants are different from animals and humans in the following important way. Plants **continuously take in substances from their environment** through their roots and leaves—both nutrients and contaminants. Plants' **continuous exposure** to environmental chemicals is thus different from a single exposure event, such as a person drinking a cup of coffee or taking an aspirin. For plants, it is less meaningful to consider a single dose of a certain substance, and more meaningful to consider the **concentration of that substance in the immediate environment**.

Thus in cases like this one, in which we **consider environmental concentration as an indirect measure of dose**, we measure dose/response curves in which the X-axis is concentration rather than dose. Therefore we would, for example, calculate a median lethal concentration (LC_{50}) in place of a median lethal dose (LD_{50}).

Moreover, since we cannot immediately tell whether a seed is "dead" (see sidebar), and because the distinction between "did not germinate" and "will never germinate" means little to a farmer with a salt-contaminated field full of failed crops, we **consider failure to germinate instead of lethality**.

We might even coin a term like **median germination failure concentration**, or GFC_{50}, as an analogue to LC_{50} (and thus also to LD_{50}).

3. Guided Discussion (5 Minutes)

<u>Materials and Methods</u>

Explain that the experimental system that students will in fact use to measure the effects of salt on crop plants is:

- germinating garden seeds
- in aqueous solutions of NaCl
- in petri dishes.

It is an important point for students to understand that in order for them to **construct an experimental design**, they must first consider the materials and methods that are available to them. Convey the following ideas as you show students the materials that you have prepared:

- You will be provided with a stock solution of salt water (3.50% NaCl, equivalent to sea water).
- You will be provided with 80 seeds.
- You will be provided with 8 petri dishes.
- To help retain moisture and keep the seeds from rolling around, cut paper towels into circles to line the bottoms of the dishes.
- Make sure to label each petri dish and its cover, so they don't get separated and mixed up!

Now ask students to discuss the following question in their groups: "What are the advantages and disadvantages of this system?" After students have had a couple of minutes to discuss the questions, facilitate a class discussion amplifying the following concepts:

> ***Ideal Answers:*** *advantages—seeds are cheap, easy to grow in the lab, no need for fieldwork, results available in days, rather than weeks or months; disadvantages—only observing effects in one developmental stage of the life cycle, no way to observe effect on crop yield, etc.*

4. Guided Discussion (7 Minutes)

Serial Dilution

Direct students to think carefully about what they are actually going to do in order to design and set up their experiments.

Ask students to consider again the questions they had answered in their homework, but this time focus on this experimental system: germinating seeds in petri dishes. Direct students to answer these questions within the group. Once all students have had a chance to work in their groups, call on a few students for their responses.

- Which variable(s) would you manipulate experimentally? (X-axis)
- Which biological response(s) would you measure? (Y-axis)

> ***Ideal Answers:*** *1. (only one correct answer) measure*

responses over a range of salt concentrations; 2. (can be multiple answers) measure percent germinating/ sprouting, length of spouts/roots, rate of growth, color, other indicators of overall health

After the class discussion, provide students with the following directions:

- You will vary the concentration of salt in which the seeds are incubated.

- You will measure the number of seeds that fail to germinate at each concentration.

- You must select and measure at least one other biological response.

By this point, students will probably have at least attempted to formulate a technique for making different concentrations of salt. Explain that they will use a technique called serial dilution. Introduce the concept of serial dilutions with these concepts about experimental controls:

- What makes an experiment an experiment is the use of a control condition in addition to an experimental condition.

- The control condition allows the experimenter to confirm the expected outcome. Validation comes from comparing the control and the experimental results.

- There are two types of controls, a positive control and a negative control.

- A positive control is one where you expect to observe a positive result, in this case failure to germinate. What would serve as a positive control in this case? [You would expect ALL the seeds to fail to germinate in the stock solution, equivalent to sea water.]

- A negative control is one where you expect to observe a negative result, in this case germination. What would serve as a negative control in this case? [You would expect NONE of the seeds to fail to germinate in distilled water.]

- This design is typical in that the positive and negative controls set the outer boundaries of the parameter we plan to vary.

5. Video (3 Minutes)

Serial Dilution

Prompt students to view the **"Serial Dilution"** video, which explains the technique for diluting the stock solution into the appropriate range of concentrations of salt solutions for the experiment.

See the appendix for the **"Mixing Dilutions"** document that will help you guide your students in making their serial dilutions. You may choose to laminate it in order that students can use it to follow the schema outlined in the video.

6. Group Activity (10 Minutes)

Notebooks and Observation Table

This is a two-part activity.

1. reviewing the importance of the laboratory notebook

2. guiding students to design a detailed observation table in their laboratory notebook

The laboratory notebook. It is impossible to overemphasize the central importance of the laboratory notebook in all fields of experimental science. Communicate these key concepts:

- The notebook is arguably the most crucial laboratory tool. Results and insights that **are not recorded might as well have never happened**. Results and insights that are not immediately clear to the reader (including, after time has passed, the experimenter herself!) might as well have never been recorded.

- **Before you begin an experiment**, start by jotting down your experimental objectives and design:

  1. Why are you doing this experiment, and what justifies this particular approach?

  2. What you are measuring, and why?

- As you conduct your experiment, it is crucial to record careful observations in your notebook. **Anticipate** any information or ideas that you may need later.

- A supervisor or colleague who reads your notebook should be able to **immediately see what you were doing and why**, and put it into context with the other experiments in the notebook. In particular, they should be able to find all of the following easily:

a. the experimental objective (best expressed as the "title" of the experiment)

b. the hypothesis, explicitly stated

c. all of the methods, explained in detail

d. all of the results and observations (including calculations)

e. all of the conclusions (best expressed as a summary at the end)

• Finally, **when in doubt, write it down**.

Designing an observation table in advance. Guide students in designing their own data collection tool for their particular experiment. Prompt them to think about **what data they will need to collect in order to draw their dose/response curves** (remembering that each group will measure at least two curves: failure to germinate, and another response).

Circulate through their groups and help guide each group as they work on their own tables. We provide a sample table, but do not distribute that table to the students! Let them produce their own design.

This data collection chart should be part of the notebook. It can be drawn onto a page directly, printed from a spreadsheet and punched into a binder, or whatever technique a student prefers.

*[**Answer:** Students should produce something like the sample Observation Table found in the appendix, but do not distribute this table to the students; let them produce their own design.] (Each student can take a different approach. What they should have in common is a range of dilutions from 0.00% salt (distilled water) to 3.50% salt (salinity equivalent to sea water), measuring percentage of seeds failing to germinate, and at least one additional response.)*

7. Group Activity (15 Minutes)

<u>What Is Your Experimental System?</u>
The culture of research science is built on continuous public critique. From the casual proposal of an experiment to a colleague in the hallway to the process of peer review of a completed manuscript for publication in a scientific journal, the expectation and the reality is that a scientist's ideas and work will come under withering scrutiny.

In fact, some of the key skills scientists learn during their doctoral training include anticipating criticism of their ideas and refining them in advance (and learning not to take criticism personally).

On the other hand, high school students will not necessarily have developed these skills and will likely not be accustomed to having their ideas come under sharp critique. Please contextualize the following assignment in terms of the accepted practice of science as outlined above.

Each group should provide you with **a summary of their design before they proceed to the assignment** and finish the lesson today. This summary can take a variety of forms—a written description of objective, hypothesis, methods, and observation table; a written protocol of all the steps they will take to mix dilutions and arrange seeds in petri dishes; a flowchart of experimental steps, including contingency plans; or many others—or some combination.

The designs need not be perfect. The standard for the summary is that you can clearly evaluate each design and determine that each group is indeed ready to carry out the experiment during the next lesson **in a timely way without further logistical planning**. For experimental designs that are not up to that standard, asking probing, leading questions can help students refine their designs. Critiques from their peers in the homework assignment will give students the opportunity to make design refinements at the beginning of the next lesson.

Next, each group will **describe their experimental design briefly in a 2-3 minute video**. Students can use whatever tools are available: laptop or desktop webcams, digital cameras, cell phone cameras—anything that will produce digital video that can be **easily uploaded to the online collaborative tool for comments** from students outside their group.

Prior to recording their video, students will do well to discuss the main points that they want to convey, composing an outline or perhaps even a script for their remarks. They can use whatever form and style their creativity permits, as long as their description of their experimental design is concise and clear.

Each group's video should be uploaded to the online collaborative tool (or provided to the instructor) by the end of the lesson.

8. Guided Discussion (1 Minute)

Conclusion

As you check experimental designs, and see to it that the videos are completed, address any student questions or concerns. Finally, introduce the homework assignment.

Homework

9. Reflection Questions (20 Minutes)

Teacher Tip: *If students cannot easily post their videos to the online collaborative tool directly, you may need to collect them and post them on their behalf.*

Comparing Experimental Designs

Select 2 or 3 other groups' experimental design videos to watch and answer the following questions on the online collaborative tool:

- What part of this group's experimental design is particularly strong?
- What part of this group's experimental design could use some improvement?
- What part of this group's experimental design includes an idea that your group did not think of?

Appendix

Prep Time, Supply List, and Total Lesson Time

Prep Time:

We recommend 30-45 minutes depending on your expertise level with the content. Each module will vary depending on your previous experience with the content and technology.

Materials:

Each student will need

- access to a computer to view the "Serial Dilution" video (unless you decide to show the video through the class projector unit).
- a "Mixing Dilutions" handout.
- their laboratory notebook that they began to use in Lesson 1.
- resources to video record their group's experimental design and a means to post the video to their class's online collaborative tool.
- their homework added to the online collaborative tool.

You will need

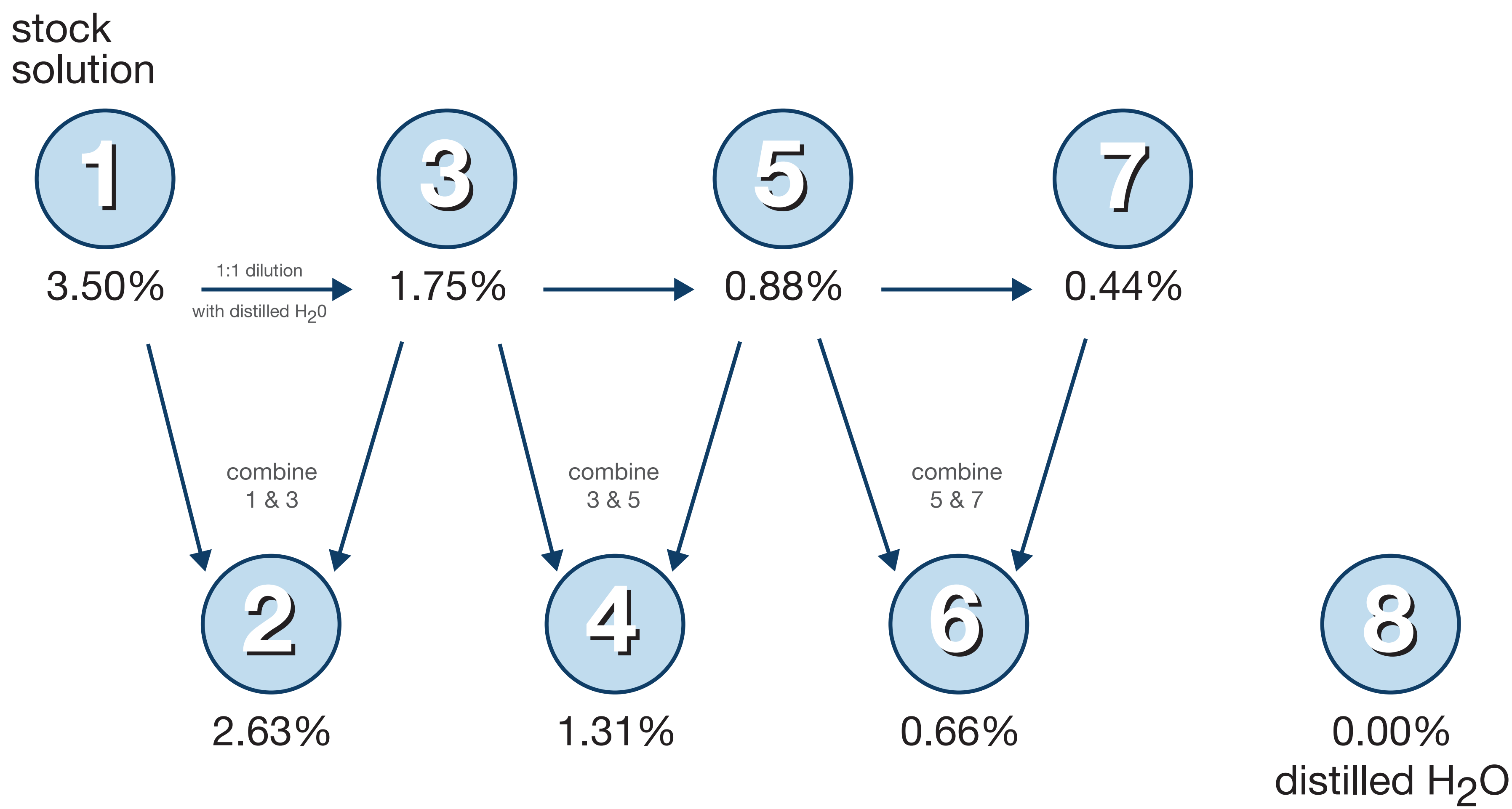
- to print and (laminate if you choose) "Mixing Dilutions" handouts.
- a copy of the "Observation Table" sample to help guide your students in the development of their own.
- to determine the tools/resources your student groups will use to video record their experimental designs.
- to decide whether your classroom will provide ample support for students to upload their videos or whether you will do it (this decision will dictate the tools/resources your students will use to video record).
- to post your students' homework to your class's online collaborative tool.

Total Lesson Time:

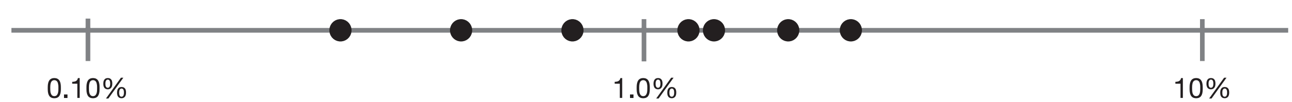
Lesson Activity	**Amount of Time in Class**
Guided Discussion: What Is the Experiment?	10 Minutes
Guided Discussion: Why Use Concentration in Place of Dose?	5 Minutes
Guided Discussion: Materials and Methods	5 Minutes
Guided Discussion: Serial Dilution	7 Minutes
Video: Serial Dilution	3 Minutes
Group Activity: Notebooks and Observation Table	10 Minutes
Group Activity: What is Your Experimental System?	15 Minutes
Guided Discussion: Conclusion	1 Minute
Total Time	**56 Minutes**

Lesson Activity	Amount of Time Out of Class
Reflective Questions: Comparing Experimental Designs	20 Minutes
Total Time	**20 Minutes**

Mixing Dilutions



These eight concentrations of salt should cover a sufficient range to reveal effects in most garden seeds. Plotted on a logrithmic scale, they might look like this:



Observation Table

Salt Solution	Number of Germinated Seeds	Individual Sprout Lengths (mm)	Average Sprout Length (mm)	Comments/Notes
3.50% w/v (ocean water)		seed #1 seed #2 seed #3 seed #4 seed #5 seed #6 seed #7 seed #8 seed #9 seed #10		
2.63%		seed #1 seed #2 seed #3 seed #4 seed #5 seed #6 seed #7 seed #8 seed #9 seed #10		
1.75%		seed #1 seed #2 seed #3 seed #4 seed #5 seed #6 seed #7 seed #8 seed #9 seed #10		
1.31%		seed #1 seed #2 seed #3 seed #4 seed #5 seed #6 seed #7 seed #8 seed #9 seed #10		

Salt Solution	Number of Germinated Seeds	Individual Sprout Lengths (mm)	Average Sprout Length (mm)	Comments/Notes
0.875%		seed #1 seed #2 seed #3 seed #4 seed #5 seed #6 seed #7 seed #8 seed #9 seed #10		
0.656%		seed #1 seed #2 seed #3 seed #4 seed #5 seed #6 seed #7 seed #8 seed #9 seed #10		
0.438%		seed #1 seed #2 seed #3 seed #4 seed #5 seed #6 seed #7 seed #8 seed #9 seed #10		
0.00% (distilled water)		seed #1 seed #2 seed #3 seed #4 seed #5 seed #6 seed #7 seed #8 seed #9 seed #10		