Lesson 3:

Measuring Toxicity - Lettuce Seed Assay

9-12 Grade | **90 Minutes**

Subjects:
Life, Physical, and Earth science

Setting:
Classroom, Lab with students working in small groups

Pre-Homework:
Read article "What does ppm or ppb mean?" from *On Tap*:

http://bit.ly/1MLqJek

Develop a procedure to prepare a 100 percent solution of an assigned chemical.

Create data table for recording results.

INTRODUCTION

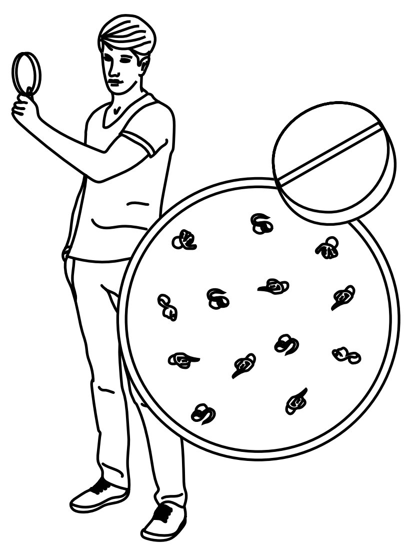
Pharmaceuticals and personal care products that we ingest or use on our bodies every day are excreted or washed off and ultimately enter the wastewater stream. In this lesson, students will conduct dose/response experiments to assess the effect of common household substances on the germination of lettuce seeds.

STUDENT OBECTIVES

1. Conduct a serial dilution to prepare solutions of a chemical over a range of concentrations.
2. Conduct a dose/response experiment with the solutions prepared in the serial dilution to determine the chemical's toxicity to lettuce or radish seeds.
3. Work collaboratively to conduct the experiment, analyze data, and interpret results. Document their work by writing a lab report.

DAILY ASSESSMENT

Students should be able to prepare a 100 percent solution and calculate the dilution's concentrations in terms of ppm. They should also create a data table with detailed observations and accurate results.



Materials:

- ❑ Lettuce/Radish Seeds
- ❑ Lab Equipment/Supplies (See next page for detailed list)

DSRP Vocabulary:

- ▹ ppm (parts per million)
- ▹ Serial dilution
- ▹ Toxicology
- ▹ TC_{50} (toxic concentration 50)

STATE AND NATIONAL STANDARDS

COMMON CORE

Writing: W.5.8, W.5.9, WHST.9-12.2

RI.5.1, RI.5.7, RI.5.9

Speech: SL.11-12.5

Math: MP.2, MP.4

Literacy: RST.9-10.7, RST.11-12.1 *(cont.)*

NAAEE GUIDELINES

1.C
1.D
1.E
1.G
2.1.A
2.4.A
2.4.B
2.4.C

NGSS

HS-LS1-A
HS-LS2.A
HS-LS2.C
HS-PS1.A
HS-PS1.B
HS-ESS3.C

The Take-Away

An understanding that "the dose" makes the poison, and that the sensitivity of an organism to a chemical depends on the identity of both the chemical and the organism.

Materials:

- Aspirin and/or acetaminophen tablets
- Balance
- Beakers
- Beral pipets
- Bleach
- $CaCl_2$ or ice melting salt
- Coffee filters
- $CuSO_4$ or Root Kill
- Distilled or deionized water (tap water is OK, but introduces more variability)
- Filter paper
- Funnels
- Graduated cylinders
- Lettuce or radish seeds
- Markers
- $MgSO_4$ or Epsom salts
- Mortar and pestle
- Parafilm or plastic baggies
- Plastic cups
- Polystyrene petri dishes
- Sodium lauryl sulfate
- Stirring rods
- Trays
- Tweezers
- Ringstands
- Ruler

Teacher Notes:

All instructions that begin with an * are found on the Illinois-Indiana Sea Grant Resource or YouTube page

Chemicals for Testing

It is recommended that each student group tests a single chemical on a single organism, and that all of that data is shared with the class. In this way, students have the opportunity to conduct the experiment and also think about the bigger picture, i.e. the effect of different chemicals on the same organisms or the effect of the same chemical on different organisms.

Depending on available resources, students can test readily available household chemicals or chemicals from a scientific supply company. A list of potential chemicals to test along with their solubility and recommended 100 percent concentration is given in Table 1.

Chemical name	**Rationale for inclusion**	**Solubility in water**	**Recommended 100% concentration**	**Other comments**
NaCl	Readily available household chemical	357 g/L at 25°C	12 g/L	Regular table salt
KCl	Alkali metal beneath Na in periodic table	360 g/L at 25°C	12 g/L	Sold as salt substitute
$CaCl_2$	Readily available household chemical	740 g/L at 20°C	12 g/L	Sold for deicing
$MgCl_2$	Alkaline earth metal above Ca in periodic table	400 g/L at 20°C	12 g/L	Sold for deicing
$KAl(SO_4)_2 \cdot 12(H_2O)$	Readily available; used in deodorants	140 g/L at 20°C		Sold as alum for baking
$CuSO_4 \bullet 6H_2O$	Readily available household chemical	320 g/L at 20°C	500 mg/L	Sold as Root Kill
$MgSO_4 \bullet 7H_2O$	Readily available household chemical; compare with $CuSO_4$	335 g/L at 20°C	500 mg/L or greater	Sold as Epsom salt
Sodium dodecyl sulfate	Used in many hygiene and cleaning products	~150 g/L at 20°C	800 mg/L	Also called sodium lauryl sulfate; purchase from lab supply
Aspirin	Readily available household chemical; OTC analgesic	3 g/L at 25°C	3 g/L	Try to obtain uncoated or minimally-coated tablets
Acetaminophen	Readily available household chemical; OTC analgesic	12.78 g/L at 20°C	13 g/L	Try to obtain uncoated or minimally-coated tablets

To prepare the 100 percent solutions from tablets of aspirin or acetaminophen, grind uncoated or minimally-coated tablets using a mortar and pestle, and weigh out the appropriate amount of powder. These organic molecules have much lower solubility than the inorganic salts, so it may be necessary to heat solutions to get the maximum amount dissolved. Since tablets for human consumption contain a small amount of fillers and other agents, it will be necessary to run the solutions through a coffee filter or filter paper. Even then, the solutions may appear cloudy.

Teachers can ask students to research their assigned chemicals to learn about their structures, properties, uses, handling, and toxicity. Safety data sheets (SDS, formerly known as MSDS) are a good source of information and are readily downloadable for no cost. Teachers can also ask students to make predictions about the effects of the chemicals on seed germination. Explanations for proposed effects could consider factors such as polarity, solubility, counter ion, cation location in the periodic table, molecular size, etc.

Part 1: Serial Dilution

Optional: * Watch serial dilution demo video.

*Optional: **To visualize the serial dilutions, students can use food coloring or powdered dye as outlined in the video on the Illinois-Indiana Sea Grant YouTube page.

Note: To save time, the teacher can prepare all the solutions before class.

Pre-work: Assign a certain chemical to each student group, give them the 100 percent concentration (e.g. 4 mg/L) and ask them to create a procedure for preparing the 100 percent solution. Tell them that they will dilute the 100 percent solution 10-fold, 5 times and ask them to calculate the concentrations of the resulting solutions (6 total including the 100 percent solution) in units of ppm. Determining concentrations in ppm could also be assigned as part of the lab report.

1. Set up 6 plastic cups or beakers and label them with the following percent concentrations and the chemical name: 100% (#1), 10% (#2), 1% (#3), 0.1% (#4), 0.01% (#5), 0.001% (#6) and control.

2. Add 90 mL of distilled water to test tubes #2 – 6 and the control.

3. Measure 100 mL of the 100% solution and pour into #1.

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

5. Gently swirl or stir #2 to mix the solution.

6. Repeat steps 4 and 5 for #3 – 6 measuring 10 mL each time. DO NOT add any solution to the control.

NOTE: The total remaining solution in each test tube is 90 mL; #6 will have a volume of 100 mL. If the solutions will not be used until the next class period, cover them with parafilm or plastic wrap.

Part 2: Set Up Dose/Response Assay

1. Treat the lettuce or radish seeds in a 10 percent bleach solution for 20 minutes, then rinse 5 times with deionized or distilled water to kill fungi or bacteria that can interfere with seed germination.

2. In each of the seven petri dishes, place a piece of filter paper cut to fit the dish. Label the dishes according to the identity of the chemical being tested and its concentration. *Note: Absorbent paper towels or coffee filters can be substituted for the filter paper – avoid bleached products as they may contain chlorine or dyes that can interfere with the experiment.*

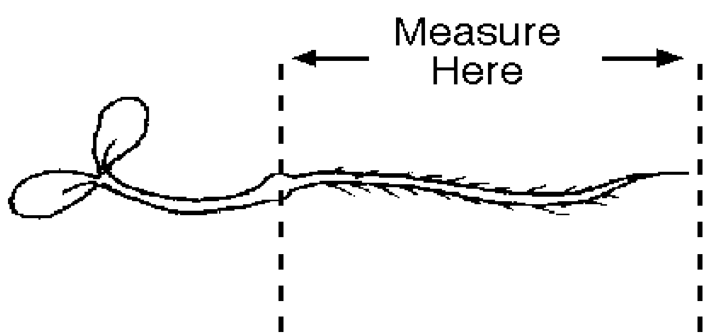
3. To each petri dish, add 2 mL of the appropriate test solution. In the control dish, use 2 mL deionized or distilled water (whichever was used to prepare the solutions).

4. To each dish, use tweezers or a spatula tip to add five lettuce seeds. Space them out evenly on the filter paper so that they do not touch each other or the sides of the dish.

5. Place the lids on the dishes, and seal the edges with parafilm to retain moisture. If parafilm is not available, dishes can be placed in a sealed baggie. Incubate the seeds in the dark at constant temperature (preferably 24.5°C) for five days (120 hours).

Part 3: Measurements and Data Processing

After seeds have incubated in the dark for five days, count how many seeds in each dish have germinated, and measure the root length of each to the nearest millimeter using a ruler. Create a data table to record the results and any other important observations. Use a paper towel to *gently* blot the seedlings dry prior to measurement. Look carefully at the plants to make sure you are measuring just the root, not the shoot as well (see Figure 1). Roots may curl so every effort should be made to straighten out the root for measurement without breaking it.



http://ei.cornell.edu/toxicology/bioassays/lettuce/data.asp

Factors to consider for the interpretation of results:

- Comparison of germination and root length data between the tested chemical and the control
- Comparison of control data across groups
- Identification of trends in germination and root length data
- Variability in the data
- Estimation of the TC_{50} for germination rate and root length

Data should be displayed in the lab report in both tabular and graphical formats.

Potential Questions for Students to Address:

These questions can be addressed during classroom discussion, student presentations or in lab reports.

- Which value TC_{50} value (germination rate or root length) showed a greater response to the chemical being tested? Which would you use in a bioassay to test environmental water samples for the chemical?
- For a given chemical, how did the TC_{50} values compare to the LD_{50} values from the SDS?
- Was the model used for the dose/response assay a good representation of an ecosystem? How could it be improved?

Resources:

Bleam, W., Cooper, S., Goode, R., McKinney, D., Pages, P., Sitzman, B., & Tempest, R. (2015, April). *April/May 2015 teacher's guide for Parabens: source of concern?* Retrieved from http://www.acs.org/content/acs/en/education/resources/highschool/chemmatters/teachers-guide.html

Bleam, W., Cooper, S., Goode, R., McKinney, D., Pages, P., Sitzman, B., & Tempest, R. (2014, December). *December 2014 teacher's guide for How toxic is toxic?* Retrieved from: http://www.acs.org/content/acs/en/education/resources/highschool/chemmatters/teachers-guide.html

Franzen, P. (1996, October 18). Investigating parts per million [newsletter]. Retrieved from http://ed.fnal.gov/trc_new/sciencelines_online/fall96/activities.html.

Gmurczyk, M. (2015). Parabens: source of concern? *ChemMatters, April/May 2015,* pages 8-9.

Rohrig, B. (2015). How toxic is toxic? *ChemMatters, December 2015/January 2015,* pages 5-7.

Satterfield, Z. (2004, Fall). What does ppm or ppb mean? On Tap, 38-40. Retrieved from http://www.nesc.wvu.edu/ontap.cfm.

SLS toxicology test. (2010). Retrieved from http://www.beyondbenign.org/K12education/middleschool.html

Trautmann, N.M., Carlsen, W.S., Krasny, M.E., & Cunningham, C.M. (2001). *Assessing toxic risk: Teacher edition.* Arlington, VA: NSTA Press.

Trautmann, N.M., Carlsen, W.S., Krasny, M.E., & Cunningham, C.M. (2001). *Assessing toxic risk: Student edition.* Arlington, VA: NSTA Press.

Extensions or Possible Student Projects:

- Allow students to conduct another bioassay using a chemical/substance of their choice or a different type of organism (such as *Daphnia,* see *Assessing toxic risk).* Students should submit proposals so that the teacher can approve their choices before they begin.
- Allow students to conduct another bioassay using the same organism and chemical, but using a narrower concentration range in order to better define the TC_{50} value.
- Obtain copies of the article *How Toxic is Toxic?* from *ChemMatters* for students to read and discuss. A free teacher's guide with activities is available.
- Obtain copies of the article *Parabens: Source of Concern?* from *ChemMatters* for students to read and discuss. A free teacher's guide with activities is available.