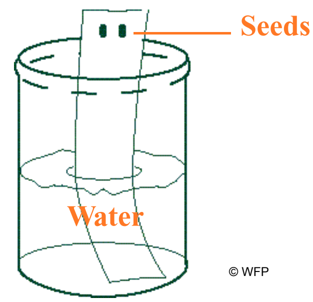
FIND THE TOXIC DOSE

This exercise will allow *you* to become a toxicologist. You will determine the toxic dose of a chemical that will inhibit seed germination. The living system in which you will perform this exercise is the rapid-cycling *Brassica rapa*, a plant related to cabbages and mustards.

These instructions provide a guide, but you will need to do much of the experimental planning on your own. You may even want to perform the experiment more than once, to verify your results, or to narrow the toxic dose range that you determine. Read all the instructions first, and write your own detailed experimental plan.



1. Choose a chemical.

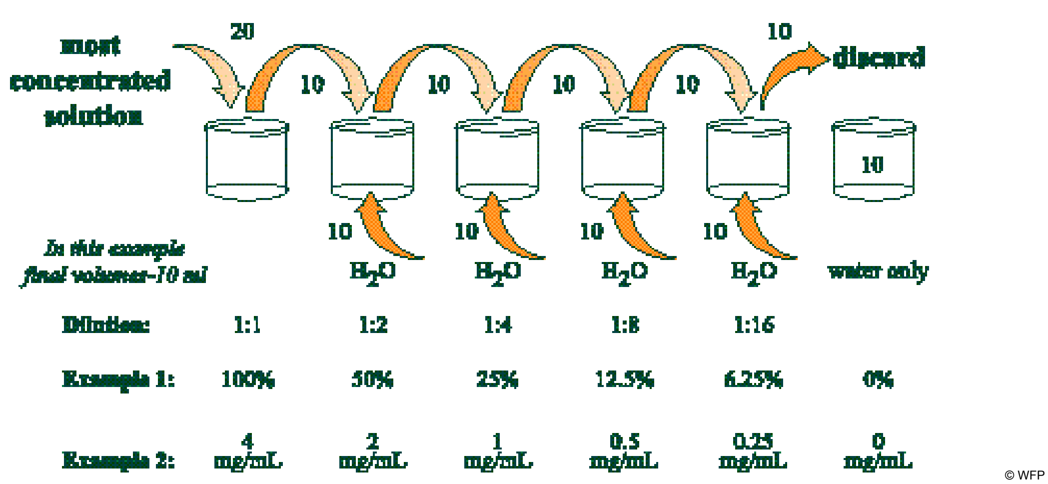
Use your imagination! Remember the famous quote from Paracelsus. Think about the kitchen cupboard, the cleaning products, the medicine cabinet, a local stream, and your classroom chemical shelf. The only limitations— it must be safe for you to handle, and be water soluble.

2. Plan and make dilutions.

This part is very important. (You may want to check your plan with your teacher before beginning.) First, choose the most concentrated dose you wish to test. This may be the full strength cleaner, or the melted snow from a newly salted roadway. Make at least 4 "serial" dilutions of this starting solution. (The more dilutions you use, the more precise your results will be.) Use water to make your dilutions, and include water (only) as a "toxin-free" control. This will give you a total of at least 6 treatments. Examples follow.

2-fold serial dilutions

Start with the most concentrated solution. Decide what final volume you will need for each treatment solution, and begin with twice that. (In this example, you need 10 mL for each treatment, so start with 20 mL of your most concentrated solution.) Take half this amount (10 mL) and add it to the same volume of water (10 mL). This is your first dilution, and its concentration is half that of the starting solution. Repeat by removing half the volume of this solution, and again, adding it to the same volume of water. Repeat until all the dilutions are made. You will have to discard half of the volume of your last dilution to make the final volumes all equal. (Try 5-fold or 10-fold serial dilutions to cover a broader range of doses.)



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3. Set up germination chambers.

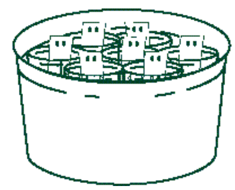
There are several options for germination chambers. Empty plastic film canisters work great. About 10 mL final volume will be necessary for each chamber. Other containers would work equally as well, just be sure that they are all the same, and adjust the final volume of solution and wick size as appropriate. Wash all containers prior to use.

4. Make wicks.

Use water mat (a felt-like material designed for use with plants) or pellon or felt (both available at fabric stores). Pellon and felt must be washed and double rinsed before being used. Washing removes chemicals used in the manufacturing process which are toxic to the germinating seed. If using film canisters as containers, wicks should be approximately 1.5 cm wide and 6 cm long.

5. Put it all together.

Be sure all containers are well labeled. If you use a wick of the size mentioned above, you can place 2 seeds on each wick. (If you use smaller wicks and smaller containers, set up 2 per treatment.) Place treatment solutions in the containers, and place the wicks in the solutions. Allow the wick to absorb the solution. Once moist, seeds can be placed on the wicks. Seal it all in an airtight container (like one from cottage cheese), and leave it undisturbed.



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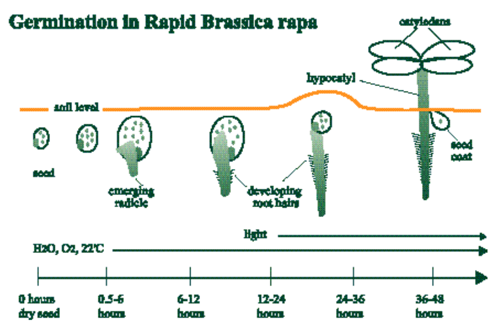
6. Observe.

Germination will occur over the next 3–4 days. Some suggestions for data collection include: Germination achievement (measured in "yes" or "no"). Time to germinate (measured in hours or days). Length of radicle and/or hypocotyl each day (measured in millimeters). Emergence of cotyledons and/or root hairs (measured in hours or days). Make drawings or take pictures of the developing plants for presentation.



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7. Make conclusions.

What numerical conclusions can you make from your data? (For example, "the toxic dose to inhibit germination for chemical X is between 5 and 10 mg/mL.") Think also about other factors, besides chemical influence, that might affect germination. Could these have had an effect on your results? What problems did you encounter with the experiment or its design? How would you fix these?

8. Keep asking questions.

Graph your results to create a dose vs. response curve, using the numerical data you obtained to describe germination. How might these results relate to the toxicity of your chemical on other living things? Design an experiment to test your chemical on other phases of the *Brassica rapa* life cycle. What features of the life cycle could you measure? How could you apply the chemical to the growing plant? Can you isolate effects on specific stages of plant development? What related chemicals might you test?

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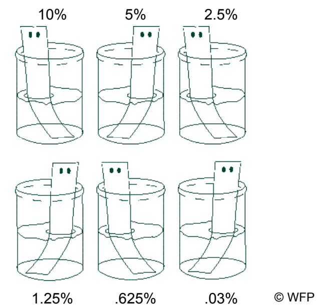
TEACHER'S NOTES



Introduction:

This laboratory exercise will help introduce your biology, chemistry or physiology class to the science behind many contemporary issues. Testing new drugs and determining cause and effect relationships between the presence of chemicals in the environment and detrimental effects on wildlife are examples which draw on the basic principles of toxicology. This lab will help students understand the concept of dose *vs.* response.

Features:

As we know from the famous quote from Paracelsus, all chemicals are poisonous. The students will be allowed to choose their own chemical to deduce its toxicity to a germinating seed. The materials are readily available, the experiment takes a small amount of space, and it takes a limited amount of time over the course of a week. Mathematical principles are emphasized when they use serial dilutions to establish a dose range. The experiment can be adapted to emphasize botany, algebra, graphing or the relationship between different chemicals (for example, comparing different acids or bases).

Scientific Writing:

The exercise is not written with "cookbook" instructions. Instead, only guidelines are provided. To emphasize scientific writing, have students write their results in the style of a scientific paper.

Introduction:

– purpose of this type of experiment
– choice of chemical and dose range
– type of information hoped to gain

Materials and methods:

– source of chemical
– treatments, dilutions
– seeds, containers, wicks, etc.

Results

– organized presentation of all observations
– tables, graphs, drawings

Discussion

– conclusions
– significance of results (relate back to introduction)
– proposal of further experiments

Materials:

Brassica rapa seeds are available from Carolina Biological Supply (as are other Wisconsin Fast Plants materials) or adapt the experiment to use seeds from other plants. Seeds that are relatively large will probably fall off the wicks as they are used in this experiment. You will need to set up a horizontal, rather than vertical, wick arrangement.

This laboratory exercise was adapted from materials written by Wisconsin Fast Plants Program, College of Agriculture and Life Science, University of Wisconsin-Madison.
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