

## Lesson 2

### Explore

# The Dose Makes the Poison

### Overview

Students observe beakers of water that contain different amounts of a mystery chemical. They discuss how each amount of the chemical might affect them if the chemical was beneficial or harmful to their bodies. Then, students set up investigations to test the effects of different doses of chemicals on seed germination and collect data for two consecutive days. Their investigations model the kinds of investigations toxicologists do to determine dose-response relationships in living systems.

### Major Concepts

The total amount of chemical administered to, or taken by, an organism is called a dose, and the effect a chemical has on a living organism is called the response. The effect a chemical has on a living organism is related to its dose and the resultant concentration of chemical in the organism. Toxicity tests enable toxicologists to learn about responses of living organisms to doses of chemicals.

### Objectives

After completing this lesson, students will

- recognize that the total amount of a chemical administered to, or taken by, the organism is called a dose,
- understand that the effect a chemical has on a living organism is called the response,
- recognize that the effect a chemical has on a living organism is related to its dose and the resultant concentration of chemical in the organism, and
- demonstrate how toxicity tests enable toxicologists to learn about responses of living organisms to doses of chemicals.

### Dose, Concentration, and Threshold

The beneficial and harmful effects that a chemical has on an organism depend, in part, on the amount of the chemical that gets into the organism. The total amount of a chemical that is administered to, or taken by, the organism is called the **dose**. The effect of a chemical depends not only on the amount of the chemical that gets into the organism but also on the resulting **concentration** of the chemical in the body (the amount of chemical compared with the body size), the length of exposure to the chemical, and the route of exposure.

The measure of dose in toxicology is important; a large dose of a beneficial chemical can have a harmful effect, and a small dose of a harmful chemical can have no adverse effect. In the words of the 16th-century physician Paracelsus, “All substances are poisons; there is none which is not a poison. The right dose differentiates a poison from a remedy.”<sup>1</sup>

### At a Glance

### Background Information

## Chemicals, the Environment, and You: Explorations in Science and Human Health

Approximate Lethal Doses of Common Chemicals (Calculated for a 160 lb. human from data on rats)	
Chemical	Lethal Dose
Sugar (sucrose)	3 quarts
Alcohol (ethyl alcohol)	3 quarts
Salt (sodium chloride)	1 quart
Herbicide (2,4-D)	1/2 cup
Arsenic (arsenic acid)	1–2 teaspoons
Nicotine	1/2 teaspoon
Food poison (botulism)	microscopic

Source: Marczewski, A.E., and Kamrin, M. *Toxicology for the citizen*. Retrieved August 17, 2000 from the World Wide Web: [www.iet.msu.edu/toxconcepts/toxconcepts.htm](http://www.iet.msu.edu/toxconcepts/toxconcepts.htm).

A chemical is considered toxic if it produces adverse effects in a living organism at levels of exposure that are likely to occur. These adverse effects can range from slight symptoms, such as headache, nausea, or rashes, to severe symptoms, such as coma, convulsions, and death. Toxicologists recognize that, for most types of toxic responses to a chemical, there exists a dose **threshold** below which no toxicity is evident. As the dose increases, more severe toxic responses occur.

### Toxicity Testing

How does a toxicologist know when a chemical is toxic to humans? When available, toxicologists study data from human populations that have been exposed to specific chemicals. The data usually come from studies of workplace exposure or incidents such as the Union Carbide chemical plant accident in India. In this way, toxicologists further their understanding of the effects of chemicals on humans. In the absence of human data, toxicologists test the toxicity of different doses of chemicals on cell and tissue cultures, plants, and other animals, such as rats and mice. These studies guide toxicologists in their understanding of which chemicals might be harmful to humans and in what amounts.

The use of animals in toxicology research is not taken lightly. Following is the Society of Toxicology's Animals in Research Policy Statement:

- Research involving laboratory animals is necessary to ensure and enhance human and animal health and protection of the environment.
- In the absence of human data, research with experimental animals is the most reliable means of detecting important toxic properties of chemical substances and for estimating risks to human and environmental health.
- Research animals must be used in a responsible manner.
- Scientifically valid research designed to reduce, refine, or replace the need for laboratory animals is encouraged.<sup>2</sup>

Toxicologists know that the kinds of questions they want to answer cannot always be answered by observing and describing humans exposed to chemicals.

They need to devise experiments that involve a system that resembles the human system. By studying a model of the human system, rats or mice, for example, toxicologists hope to apply the knowledge they gain to understanding the harmful effects of chemicals on humans. Although the basic tenet of toxicological studies is that “experimental results in animals, when properly qualified, are applicable to humans,”<sup>3</sup> toxicologists recognize that different species can respond to doses of toxic substances differently. For example, on the basis of dose per unit of body surface, toxic effects in humans are usually about the same as for experimental animals; but on a body weight basis, humans are about 10 times more vulnerable than small experimental animals, such as mice.<sup>3</sup>

Because of both practicality and ethics, scientists who use animals in research carefully select the species and design experiments to achieve scientifically valid results. They obey strict regulations about the use of animals in experiments.<sup>2</sup> Typically in these experiments, toxicologists expose experimental animals to high doses of toxic agents so that they minimize the number of animals they use. This experimental design assumes that the results of tests at high doses on a small number of animals can be extrapolated to estimate the risk of low doses to a large population of humans.

#### Guiding Principles in the Use of Animals in Toxicology

1. The use, care, and transportation of animals for training and for toxicological research and testing for the purpose of protecting human and animal health and the environment must comply with all applicable animal welfare laws.
2. When scientifically appropriate, alternative procedures that reduce the number of animals used, refine the use of whole animals, or replace whole animals (e.g., *in vitro* models, invertebrate organisms) should be considered.
3. For research requiring the use of animals, the species should be carefully selected and the number of animals kept to the minimum required to achieve scientifically valid results.
4. All reasonable steps should be taken to avoid or minimize discomfort, distress, or pain of animals.
5. Appropriate aseptic technique, anesthesia, and postoperative analgesia should be provided if a surgical procedure is required. Muscle relaxants or paralytics are not to be used in place of anesthetics.
6. Care and handling of all animals used for research purposes must be directed by veterinarians or other individuals trained and experienced in the proper care, handling, and use of the species being maintained or studied. Veterinary care is to be provided in a timely manner when needed.
7. Investigators and other personnel shall be qualified and trained appropriately for conducting procedures on living animals, including training in the proper and humane care and use of laboratory animals.
8. Protocols involving the use of animals are to be reviewed and approved by an institutional animal care and use committee before being initiated. The composition and function of the committee shall be in compliance with applicable animal welfare laws, regulations, guidelines, and policies.
9. Euthanasia shall be conducted according to the most current guidelines of the American Veterinary Medical Association (AVMA) Panel on Euthanasia or similar bodies in different countries.

Source: Society of Toxicology. March 1999. Retrieved August 14, 2000 from the World Wide Web: [www.toxicology.org/education/air/html](http://www.toxicology.org/education/air/html).

## Chemicals, the Environment, and You: Explorations in Science and Human Health

Toxicity testing is not designed to demonstrate that a chemical is safe for humans, but is used to identify the types of toxic effects a chemical can produce. One early test performed on a chemical is the Ames test, named after Bruce Ames of the University of California–Berkeley. In this test, specially engineered bacteria are exposed to a chemical. If the bacteria mutate, the chemical reacted with DNA and is a potential mutagen or carcinogen. Scientists use the Ames test to economically weed out mutagenic chemicals because it avoids testing on higher animals.

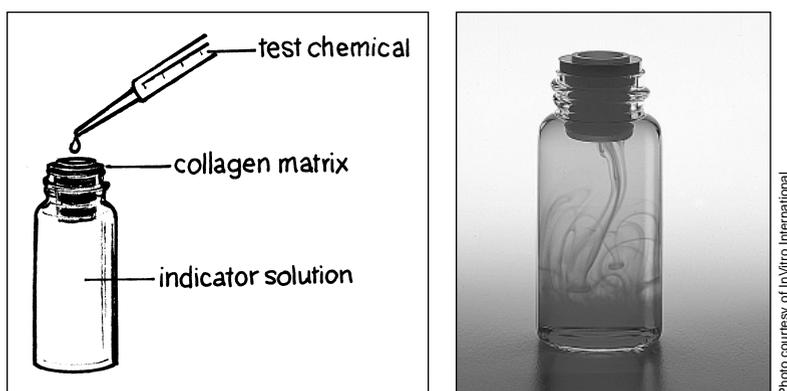
Often, scientists use cell cultures in toxicology testing. Scientists expose isolated cells to a chemical and observe the response. If the cells die during the experiment, the chemical may be too toxic for use by humans. As with the Ames test, tests on cell cultures help scientists narrow the list of chemicals they need to test further on animals by eliminating those that are clearly too toxic.

If these preliminary tests suggest a chemical might be used safely with humans, scientists consider testing with animals. One of the first animal tests that scientists perform on a new chemical determines its acute toxicity. Toxicologists determine what dose of the chemical, under the intended route of exposure, causes 50 percent of the animals (mice or rats) to die (lethal dose, or LD<sub>50</sub>). Toxicologists also determine the effective concentration at which 50 percent of the animals exhibit a measurable response (EC<sub>50</sub>).

Scientists perform subacute toxicity tests to learn about the toxicity of a chemical after repeated doses. To test a chemical that is likely to enter the body through ingestion, scientists add doses of a chemical (high, low, and intermediate) to the feed for the experimental animals, usually rats or mice. Each animal receives a specified dose over the course of 90 days. Scientists observe the animals once or twice daily for signs of toxicity, including changes in body weight, diet consumption, changes in fur color or texture, respiratory or cardiovascular distress, motor and behavioral abnormalities, or palpable masses. They record premature death and collect blood and tissue samples from all animals for further study. If the chemical is likely to pose a risk to humans through skin contact or inhalation, scientists perform tests that incorporate those routes of exposure. They conduct long-term or chronic exposure studies in a similar manner, but the exposure time is increased to a time period that can range from six months to two years.

Efforts are under way to reduce the use of animals in some kinds of toxicity testing.<sup>4</sup> For example, researchers have developed a collagen matrix barrier that serves as a kind of artificial skin. If a chemical or chemical mixture penetrates the artificial skin, it is likely to irritate, corrode, or burn human skin.

For example, in the illustration on page 43, the drawing shows how a chemical is tested using the collagen matrix barrier. A sample of the test chemical is dropped onto the matrix. If no chemical penetrates the matrix, the solution in the bottle below the matrix remains clear. If the chemical penetrates the matrix, it will cause a color change in the solution in the bottle below. The photo on the



right shows the indicator solution changing color after the test chemical has penetrated the matrix. This method, using artificial skin, can replace the current practice of using three animals to test every new chemical. Because more than 2,000 chemicals are introduced each year and many are tested before they are introduced on the market, this replacement means a significant reduction in the number of experimental animals used in toxicity testing.<sup>4</sup>

Researchers also are developing techniques that are more accurate than the traditional methods. In the past, when researchers wanted to know if a blood pressure medicine was working in an animal, they inserted a catheter into an artery in the animal's leg. The animal then needed to be restrained so that scientists could take readings over a four- to five-hour period. Today, a sensor implanted in the animal's abdominal cavity allows researchers to continually measure results while the animal can move freely and remain with its family. Its heart rate is more relaxed and normal, so the results do not mirror the compounding effect of stress.

The rate at which new technology is being used to help researchers reduce their reliance on laboratory animals is accelerating. People who are concerned about animal welfare are working with researchers to encourage better experimental design and more humane techniques. Together, they are working to replace laboratory animals with scientifically valid alternatives, reduce their numbers, and refine techniques to minimize pain and suffering.

Even as progress is made in the name of animal welfare, however, conflicting pressures arise from the public's interest in knowing more about the health and safety data on major industrial chemicals. For example, in October 1998, Vice President Al Gore announced his plan to collect data on 2,800 high-production-volume chemicals. Animal rights groups recognized that such testing would require the destruction of more than 1 million animals. For a year, animal welfare activists lobbied to halt or modify the plan to minimize the number of animals and avoid needless testing. One year later, the U.S. Environmental Protection Agency (EPA) made new recommendations for high-production-volume chemical testing that should reduce animal use. They now will consider previous results from chemical safety databases to ensure that testing is not redundant and will postpone the testing of some chemicals in the hope that nonanimal tests will become available. To that end, the

## Chemicals, the Environment, and You: Explorations in Science and Human Health

National Institute of Environmental Health Sciences (NIEHS) will invest at least \$4.5 million over two years to develop and validate nonanimal protocols, and the EPA will contribute \$250,000 each year for two years.<sup>5</sup>

### Notes About Lesson 2

In this lesson, students perform toxicity tests on seeds, paying careful attention to the dose and concentration of chemicals. Students might not be aware that plants differ from animals in many ways: They have no nervous system or efficient circulatory system, and they have a photosynthetic mechanism and cell walls that animals do not. Therefore, the students' results from toxicity tests on seeds cannot be extrapolated to suggest a chemical's risk or safety to humans without further testing on animal systems, which is inappropriate for the classroom. However, students can understand the importance of using model systems in science when human subjects cannot be used because of the potential risk. Students can understand that many questions in science suggest a variety of investigation methods and that their use of models in scientific inquiry can help them establish relationships based on evidence from their own observations.

### In Advance

CD-ROM Activities	
Activity Number	CD-ROM
Activity 1	no
Activity 2	optional
Activity 3	no
Activity 4	optional
Extension Activity	no

Photocopies		
Activity Number	Master Number	Number of Copies
Activity 1	Master 2.1, <i>Opening Questions</i>	1 transparency
Activity 2	Master 2.2, <i>Making Solutions for Toxicity Testing</i>	1 for each student <sup>a</sup>
Activity 3	Master 2.3, <i>Toxicity Testing on Seeds</i>	1 for each student
Activity 4	none	none
Extension Activity	none	none

<sup>a</sup> If you want students to calculate percent concentration on their own, mask the numbers in the concentration column before copying.

<b>Materials</b>	
<b>Activity 1</b>	
<p><b>For the class:</b></p> <ul style="list-style-type: none"> <li>• overhead projector</li> <li>• transparency of Master 2.1, <i>Opening Questions</i></li> <li>• shoe box from Lesson 1</li> <li>• 1 small jar containing the mystery chemical from Lesson 1<sup>a</sup></li> <li>• 1 eyedropper</li> <li>• 1 pair of safety glasses</li> <li>• 1 pair of latex gloves</li> </ul>	<ul style="list-style-type: none"> <li>• 3 1,000-mL beakers or 3 large jars of the same size, each containing 500 mL of water</li> <li>• 1 piece of white poster board to use as a backdrop for the demonstration</li> <li>• 1 resealable plastic sandwich bag containing radish seeds</li> <li>• 1 beaker containing 250 mL of water (optional)</li> </ul> <p><b>For each student:</b></p> <ul style="list-style-type: none"> <li>• science notebook</li> </ul>
<b>Activity 2</b>	
<p><b>For the class:</b></p> <ul style="list-style-type: none"> <li>• CD-ROM</li> <li>• computer</li> <li>• chemicals from Lesson 1</li> <li>• mystery chemical from Lesson 1</li> <li>• 1 resealable plastic sandwich bag containing radish seeds</li> </ul>	<p><b>For each student:</b></p> <ul style="list-style-type: none"> <li>• 1 copy of Master 2.2, <i>Making Solutions for Toxicity Testing</i></li> <li>• science notebook</li> </ul>
<b>Activity 3</b>	
<p><b>For each team of 3 students:</b></p> <ul style="list-style-type: none"> <li>• 3 copies of Master 2.2, <i>Making Solutions for Toxicity Testing</i></li> <li>• 3 copies of Master 2.3, <i>Toxicity Testing on Seeds</i></li> <li>• 3 pairs of safety glasses</li> <li>• 3 pairs of latex gloves<sup>b</sup></li> <li>• 1 100-mL beaker filled with 50 mL of a chemical; see <i>Preparation</i> for Activity 3</li> <li>• 1 permanent marker</li> <li>• length of masking tape</li> <li>• 6 50-mL beakers<sup>c</sup></li> </ul>	<ul style="list-style-type: none"> <li>• 1 50-mL graduated cylinder</li> <li>• 1 10-mL graduated cylinder</li> <li>• 100 mL of purified water in a beaker</li> <li>• 1 eyedropper</li> <li>• 6 resealable plastic sandwich bags</li> <li>• 12 paper napkins<sup>d</sup></li> <li>• 60 radish seeds in a resealable plastic sandwich bag</li> <li>• 1 tray</li> </ul> <p><b>For each student:</b></p> <ul style="list-style-type: none"> <li>• science notebook</li> </ul>
<b>Activity 4</b>	
<p><b>For each team of 3 students:</b></p> <ul style="list-style-type: none"> <li>• bags of seeds treated with chemicals from Activity 3</li> <li>• 1 copy of Master 2.3, <i>Toxicity Testing on Seeds</i>, from Activity 3</li> </ul>	<ul style="list-style-type: none"> <li>• CD-ROM version of data for Day 2 (optional; see <i>Preparation</i> for Activity 4)</li> </ul> <p><b>For each student:</b></p> <ul style="list-style-type: none"> <li>• science notebook</li> </ul>
<b>Extension Activity</b>	
<p><b>For the class:</b></p> <ul style="list-style-type: none"> <li>• computers</li> <li>• access to the World Wide Web</li> <li>• materials for designing a bulletin board display</li> </ul>	

<sup>a</sup> The mystery chemical is the solution of blue food coloring and water used in Lesson 1.

<sup>b</sup> Check that no students are allergic to latex. If any are, assign their team a chemical that you know will not irritate the skin, such as sugar water or cola. The team members with the latex allergy then can work safely without gloves. Alternatively, they can use vinyl gloves, if available.

<sup>c</sup> Alternatively, you could use 6 clean baby food jars or 6 test tubes set up in a rack made out of a shoe box.

<sup>d</sup> Use regular, white, one-ply napkins (12 x 11 <sup>5</sup>/<sub>8</sub> inches, unfolded) that you can buy in bulk at the grocery store. If you use something different, test your setup to make sure that the napkins or paper towels you use can absorb 20 mL of liquid in a plastic bag.

## Chemicals, the Environment, and You: Explorations in Science and Human Health

### PREPARATION

#### Activity 1

Pour 500 mL of water into each of the 3 1,000-mL beakers. Label the beakers #1, #2, and #3.

Put a handful of radish seeds in a resealable plastic sandwich bag.

Gather the materials you need for the demonstration.

Make a transparency of Master 2.1, *Opening Questions*.

#### Activity 2

Gather the materials you will need for this activity.

Duplicate Master 2.2, *Making Solutions for Toxicity Testing*, 1 copy for each team.

Set up a computer center at which students can view the CD-ROM.

#### Activity 3

Decide whether you will use the print or CD-ROM version of the laboratory investigation. It is tempting to avoid the preparation and materials that a laboratory investigation requires, but students benefit from conducting a scientific investigation, using tools to gather data, and developing a hands-on understanding of the use of models in scientific inquiry. The simulation presented on the CD-ROM enables teachers and students without access to laboratory equipment to gather data to use in Lesson 3, but it should not replace actual laboratory experience. In addition, Lesson 2's laboratory investigation provides students with an opportunity to meet Content Standard A of the *National Science Education Standards*: All students should develop abilities necessary to do scientific inquiry and understandings about scientific inquiry.<sup>6</sup>

#### For the print version of the laboratory investigation:

Prepare the chemicals, 1 chemical for each team of 3 students:

- Choose a wide variety of chemicals for testing:

water-soluble plant food	artificial sweetener
liquid detergent	shampoo
soft drink	window cleaner
instant coffee	salt
nontoxic environmental cleaner	sugar
tempera paints	fruit and vegetable rinse (Fit)
all-purpose disinfectant cleaner (Lysol)	

**Tip from the field test:** During the field test, the following chemicals yielded data that made the most interesting dose-response curves for students to graph in Lesson 3: salt, Miracle Gro, fruit punch soft drinks, window cleaner, and Lysol. The results from other chemicals also were of interest to students, so be sure to include a wide variety of familiar chemicals, such as shampoo, soft drinks, coffee, and sweetener, even if the dose-response curves generated

from the use of these chemicals in the investigation are less exciting. One of the reasons to use a variety of chemicals is to demonstrate the range of responses that are possible.

- Measure 50 mL of each liquid chemical into a 100-mL beaker. Label the beaker with the name of the chemical (for example, window cleaner).
- Make solutions of nonliquid chemicals by mixing them with water. Then, measure 50 mL of each liquid solution into a 100-mL beaker. Label the beaker with the name of the chemical.

*When available, follow directions on the container to make solutions of nonliquid chemicals, such as plant food or instant coffee. When no directions are available, make as saturated a solution as possible: Heat the water and slowly stir in a small amount of the chemical until it no longer dissolves easily in the water. In pilot testing this activity, we made a sugar solution with 40 g of sugar in 100 mL of water and a salt solution with 24 g of salt in 100 mL of water. Be sure to make enough solution for all your classes.*

- Place each chemical on a tray, 1 tray and chemical per team.

Purchase radish seeds. Put 60 radish seeds into a resealable plastic sandwich bag. Continue until you have a bag for each team of 3 students.

*Radish seeds found in a local garden store work well for this investigation. They will germinate in 1 to 3 days. If you prefer faster germination (6–24 hours), you can purchase Wisconsin Fast Plants™, Brassica rapa seeds (which are close relatives of the radish) from Carolina Biological Supply (1-800-334-5551). Be aware that the Brassica rapa seeds are quite a bit smaller than radish seeds, so consider your students' dexterity when deciding which seeds to use.*

**Tip from the field test:** Counting the 60 seeds can be time consuming. Estimate the number of seeds by measuring approximately  $\frac{1}{4}$  teaspoon of regular radish seeds (less if you use *Brassica rapa* seeds) for each bag. There will be a little more than 60 seeds in each bag. Students tend to lose a few as they set up the investigation, so it doesn't hurt to have a few extra seeds in the bag or on hand at the materials table.

#### Activity 4

If your students conducted the CD-ROM version of Activity 3, arrange for students to have access to computer for this activity.

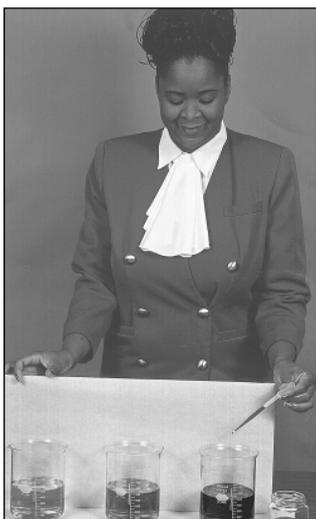
#### Extension Activity

Arrange for students to have access to the World Wide Web. Gather materials needed to design a bulletin board display or ask students to provide them.

### ACTIVITY 1: DOSE AND CONCENTRATION

1. Place the shoe box with the mystery chemical from Lesson 1 in it and the three beakers of water on a table in the front of the room.
2. Display a transparency of Master 2.1, *Opening Questions*.

3. Allow the students a few minutes to puzzle through the questions on the transparency. Assure them that it is perfectly acceptable not to know the answers to all the questions right now. Remind students that toxicologists study chemicals and perform toxicity tests because they don't know the answers to questions like these about every chemical.
4. Once students have answered the opening questions, draw their attention to the shoe box that holds the mystery chemical from Lesson 1. Announce to the students that a toxicologist partially analyzed the mystery chemical and informed you that the chemical would not enter your body through inhalation or contact with the skin, so it is safe to handle the chemical as long as you don't ingest it.
5. Demonstrate appropriate laboratory safety measures by putting on safety glasses and a pair of latex gloves. Open the shoe box and remove the jar of mystery chemical.
6. Direct students' attention to the three large beakers of water. Let students know that each beaker holds exactly the same amount of water as the other two. As the students watch, use the eyedropper to add
  - 1 drop of mystery chemical into Beaker #1,
  - 4 drops into Beaker #2, and
  - 16 drops into Beaker #3.



Gently swirl or stir each beaker until the drops of mystery chemical are totally distributed in the water. Use the white poster board as a backdrop and ask students to tell you what they can observe.

Students will observe that the water in the three beakers has turned a shade of blue. In Beaker #1, the color of the water is a very pale blue. In Beaker #2, the water is a darker blue. In Beaker #3, the water is the deepest blue.

7. Suggest that the beakers of water represent human bodies. Ask students to tell you what they know about the relative sizes of the three "bodies." Once they recognize that all the bodies are the same size, discuss the added chemicals using questions such as these:
    - If we found out that the mystery chemical is harmful to people, which of these beakers would you rather be right now and why?
- Students probably will say that they would rather be Beaker #1 because it contains the smallest amount of the harmful chemical.
- Are you sure that the amount of chemical in Beaker #2 is enough to cause harm? Is there enough in Beaker #3 to cause harm?

Students may realize that they do not know how much of the chemical must be present to cause harm, but they would want to err on the safe side and choose Beaker #1. Some students may wonder if even the small amount in Beaker #1 is harmful.

- **What if we learned that the chemical is good for you? Which beaker would you choose to be and why?**

Students probably will choose Beaker #3 because it has more of the beneficial chemical in it. Some students may recognize that they don't know enough about the chemical to know how much of it is good for a person. They may wonder if it is possible for too much of even a good chemical to be harmful.

- **Some chemicals are good for you in small doses but bad for you in large doses. If that were the case here, which beaker would you want to be?**

Some students may opt for Beaker #1 because the amount of chemical is small. Others may choose the middle road, Beaker #2, so that there is enough of the chemical to be beneficial but not enough to do harm. Others may say that they cannot choose because they do not know enough about the amount of chemical that is beneficial or the amount of chemical that is harmful.

- 8. Point out to students that the questions they have about how much of a chemical is harmful and how much is beneficial are the same kinds of questions that toxicologists study. Help students understand some basic vocabulary of toxicology by asking them these questions and writing the terms on the board.**

- **When toxicologists look at the total amount of chemical that is given to, or taken by, an organism, they talk about *dose*. In which beaker did the body receive the largest dose of chemical?**

Beaker #3 received the largest dose: 16 drops of the chemical.

- **Toxicologists study the *concentration* of a chemical in an organism by comparing the dose of chemical to the size of the organism. In which of these three beakers is the concentration of chemical highest?**

Beaker #3 has the highest concentration of all the beakers because it has the largest dose of chemical compared with the constant volume of water. For the same-size person, ingesting more of a chemical results in a higher concentration of the chemical in that person's body. It follows that if several people of various sizes take the same amount of a chemical, the final concentration to which each person is exposed can vary, depending on the size of the person.

To help clarify the difference between dose and concentration, you can use a fourth beaker. This time, fill it with only 250 mL of water, half the amount of the other three beakers. Ask students to describe to you the relative size of this new "body" compared with the other beakers. Tell students that you are going to give this new body the same dose as you gave Beaker #2: 4 drops. Add that dose to the beaker. Ask students to observe the color and compare the concentration of chemical in the

## Chemicals, the Environment, and You: Explorations in Science and Human Health

fourth beaker with the concentrations in the other three beakers. Is this concentration more or less than the others? Students should see that the water in the fourth beaker does not look like the water in Beaker #2, even though the dose was the same. The color is deeper, showing that the concentration of chemical in the smaller body is greater for the same dose than it is in the larger body.

### 9. Ask students to come up with ways to find out if the mystery chemical at different doses (1, 4, and 16 drops) is harmful or beneficial to living things.

Students might suggest that someone could drink the three solutions of chemical and see what happens. If you ask for volunteers, however, you probably will not get any; you can discuss with students why this method may provide information but would not be a responsible way to test the toxicity of a particular dose of chemical. Ask students to think of other living things that they could use for testing. Use the *Background Information* in this lesson to discuss how animals are used in research. Be sure to discuss the Society of Toxicology's policies and guidelines for animals in research.



Photo courtesy of American Association for Laboratory Animal Science

### 10. Help students recognize that they are not equipped to safely and ethically handle toxicology tests on animals. Tell them that there are many animal welfare laws, regulations, guidelines, and policies that govern the use of animals in research. Let students know that there are other living organisms that they can use for testing the mystery chemical in the classroom. Hold up a bag of seeds and ask students to tell you if they think the seeds are living organisms.

Seeds are living organisms that grow into plants. As living organisms, plants respond to chemicals in their environment, particularly in water. Plants are not the same as humans or other animals in structure, however, so results from tests that determine toxicity to plants might not be applicable to humans. Students will discuss this problem with the model system of plants when they analyze their data in Lesson 3.

### 11. Tell the students that they are going to perform toxicology tests on seeds, but first they need to formulate a question and design an investigation.

#### ACTIVITY 2: PLANNING THE SEED INVESTIGATION

##### 1. Put on your desk some of the chemicals from Lesson 1. Show the students the mystery chemical. Show the students the seeds in the bag. Ask the students what they want to know about the chemicals and the seeds.

Conduct this discussion as a brainstorming session, perhaps writing on the board all of the questions the students formulate. Help the students arrive at a general question, such as this one:

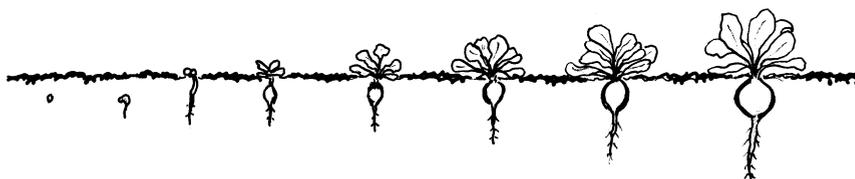
What effect does the chemical have on the seeds?

**2. Ask the students to refine their question by considering what they learned in the beaker demonstration about dose.**

The students' question should address both effect and dose. If students do not provide such a question on their own, suggest one like this:

What effect does the chemical have on seeds, and how is the effect different if the seeds receive different doses of the chemical?

**3. Refresh students' memories about the function of seeds in the life cycle of a plant. In doing so, discuss with students what they would expect a seed to do if it is exposed to moisture.**



Seeds are the most important part of a plant. In fact, the roots, leaves, and flowers of a plant all exist to produce seeds. Seeds have three parts: the protective outer seed coat, the embryo that becomes the new plant, and the endosperm that provides the food to nourish the embryo. Most students have looked at the germination of a bean seed in elementary school. Remind students that before a seed germinates, it absorbs moisture so that it swells and bursts its seed coat. At this time, the embryo starts to grow and the root tip, or radicle, pushes through the eye of the seed. This “sprouting” is the first evidence of growth that students can observe with the naked eye. Several days later, seed leaves emerge and the embryonic stem begins to extend upward. At this time, chlorophyll pigment is visible. If planted in favorable conditions with the right moisture, temperature, and light, the plant will develop leaves, lengthen its stem, flower, produce fruit, and develop mature seeds. Once the seeds dry, the cycle is complete and the seeds are ready to produce new plants.

**4. Ask students to further refine their question from Step 2 based on the discussion of what seeds do under normal conditions.**

Guide students to a question like this one:

How does the chemical affect the germination of the seeds, and how is the effect different if the seeds receive different doses of the chemical?

**5. Distribute copies of Master 2.2, *Making Solutions for Toxicity Testing*. Ask students to interpret the information presented in the handout by asking questions similar to these:**

- In which beaker is the *dose* of chemical going to be the highest? In which beaker is the *concentration* of chemical going to be the highest?

## Chemicals, the Environment, and You: Explorations in Science and Human Health

In both cases, the highest dose and highest concentration is in Beaker #6. Beaker #6 contains the greatest volume of chemical. Because the total amount of solution remains the same in each beaker, the highest concentration will be in the beaker that has the largest amount of chemical in it, Beaker #6.

- **How do you calculate the percent concentration of chemical in the solution?**

The percent concentration refers to the amount of chemical compared with the total volume in the beaker. For example, students put 2.50 mL of chemical and 17.50 mL of water in Beaker #3. The total amount of liquid in the beaker is 20 mL. Therefore, the amount of chemical divided by the total amount of liquid is

$$\frac{2.50 \text{ mL of chemical}}{20.00 \text{ mL of liquid}} = 0.125, \text{ or } 12.5\% \text{ concentration}$$

If you have chosen to do so, have students calculate the percent concentration for each beaker and fill in the chart on Master 2.2.

**Tip from the field test:** Some students may need a review of the conversion of decimals into percentages.

- **Why do you think you need a beaker that is just water (0% concentration)?**

Remind students that they need to have a control for their experiment. In this way, students can see what happens to seeds under normal conditions and compare that with what they observe in the seeds treated with various concentrations of chemicals.

- **Suppose you expose seeds to the different concentrations of chemical suggested in the chart on this worksheet. What predictions can you make about how they will respond?**

Give students time to make some general predictions about what they think could happen to seeds exposed to high, medium, and low concentrations of different chemicals. Students will have a chance to write specific predictions about their own chemical once it is assigned or chosen, so make this discussion brief.

- 6. Instruct teams to put their copies of Master 2.2 in their science notebooks so that they can use them during the next class period.**
- 7. Make the CD-ROM available at a computer center for students who want more practice determining concentrations of chemicals.**



Insert the CD-ROM into the computer and go to the main menu. Click on *The Dose Makes the Poison: Dose-Response Relationships*. This brings up the page on which students can manipulate chemical concentrations. Instruct the students not to click *Do Experiment* at this time.



**Content Standard A:** Students use mathematics in all aspects of scientific inquiry.

**ACTIVITY 3: SETTING UP THE SEED INVESTIGATION**

The following procedures describe how to conduct a hands-on laboratory investigation, which is the preferred method of instruction for this activity.

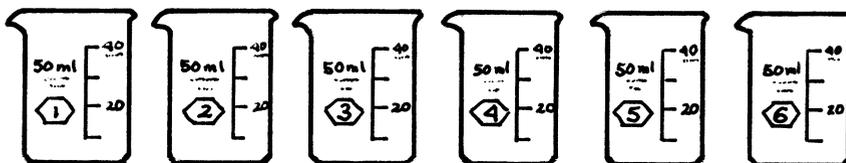
**Important note to teachers:** Activity 3 requires at least three consecutive school days (without interruption by a weekend or holiday). Usually, that means you need to start no later than the Wednesday of a full week of school.

1. **Direct students' attention to the beakers of chemicals you prepared. Distribute chemicals on the trays, one chemical to each team.**

Prepare at least one chemical per team and perhaps more if you want to give teams a variety of choices for testing. Alternately, you can assign chemicals to teams. Ask one team to test the mystery chemical or you can test it for the class.

2. **Ask teams to get out their copy of Master 2.2, *Making Solutions for Toxicity Testing*, from the previous class period. Ask students, How are you going to label your beakers of solutions so that you know what is in each one?**

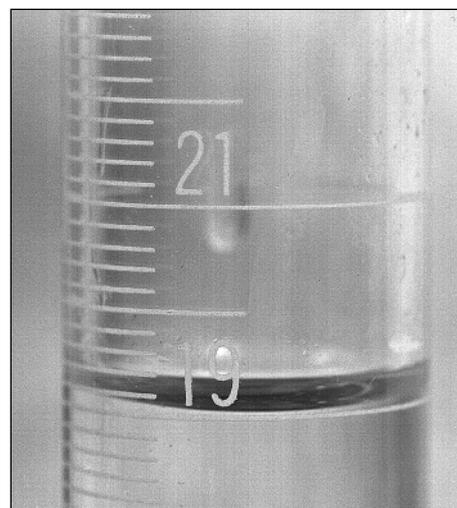
Answers will vary, but impress upon students that it is important to keep accurate records in toxicology testing. At the minimum, students should number the beakers 1 to 6 to correspond with the beaker numbers on the chart on Master 2.2.



3. **Direct students to gather their materials, put on their gloves and safety glasses, and follow the chart on Master 2.2 to make their solutions.**

If necessary, review proper measuring techniques for fluids in a graduated cylinder before the students attempt to make their solutions. Make sure they know how to measure at eye level so that they can see when the meniscus is right on the line that demarcates the target volume. Demonstrate to students how they can use the eyedropper to add or subtract small amounts of volume.

**Tip from the field test:** If students have not measured liquid volume before, they might need to practice before completing this step of the activity. Refer to measurements from Master 2.2, *Making Solutions for Toxicity Testing*, and, using clear and colored water, set up a practice lab for inexperienced students. For further tips from the field test, see Step 5.





**Content Standard A:**  
Students design and  
conduct a scientific  
investigation.

**4. Once teams have completed their solutions and have six small beakers of chemical solutions on their work tables, ask students to discuss how they might test the toxicity of these solutions on seeds.**

This discussion can be brief, but it helps students design their own investigation rather than simply following directions without giving a thought to their purpose. Students will recognize that they want to put the seeds in contact with the chemical solutions in some way. Many will remember seed germination activities from previous years and will suggest moist paper towels in petri dishes or plastic bags. Show students the assembly they will use in this investigation.

**Ask students how they will know if a chemical was toxic to seeds.**

Ask students what they predict should happen to healthy seeds in a nontoxic environment (the seeds will germinate). Help students recognize that they will want to look at nongermination as an indicator of toxicity in their investigation.

**Ask students how they will know it was the chemical or a particular dose of the chemical that caused the observed effect and not some other factor.**

Take this opportunity to discuss experimental design:

- Emphasize careful measuring and cleanliness (making sure not to contaminate the beakers or graduated cylinders with solutions of chemicals that are different from the intended solution).
- Discuss the experimental control. Remind students that a control is a group of experimental subjects that are not exposed to the chemical being investigated so they can be used as a comparison against tested subjects. The control will be the seeds they do not expose to any chemical other than water (the 0% chemical solution in Bag #1). They will compare the germination of seeds in this control with the germination of the seeds in Bags #2–6. Then they will determine whether the addition of the chemical affects the germination of seeds.
- Discuss the importance of controlling variables. Ask students to think of some variables they will want to control. Some possibilities might be
  - \* number of seeds tested for each solution
  - \* volume of chemical solution put on each group of seeds
  - \* amount of light
  - \* temperature
  - \* kinds of containers in which the seeds are placed
  - \* number of paper napkins used for each group of seeds
  - \* type of water source

**5. Distribute copies of Master 2.3, *Toxicity Testing on Seeds*, one to each student. Direct teams to work together to set up the investigation.**

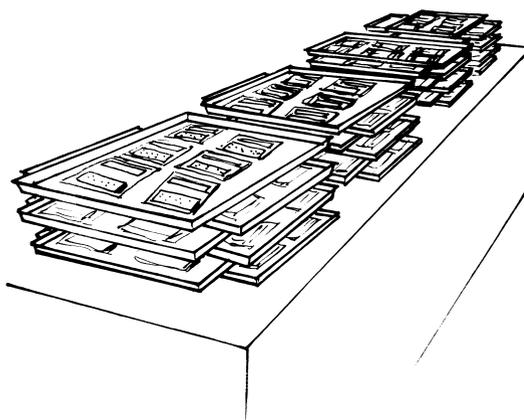
**Tip from the field test:** During the field test, some students and teachers tried to short-cut the solution preparation and set-up steps by adding water and then chemical directly to the bag in which they already had

placed the paper napkins and seeds. This resulted in an unequal distribution of chemical; some areas on the napkins had a high concentration of chemical and others had a low concentration. In addition, the seeds flowed to the bottom of the bag and got lost under the layers of napkin. The seeds should be added to the bag only after the napkins are saturated with completely mixed solutions of chemicals. Encourage your students to work carefully and methodically through the preparation of the chemical solutions (see Step 3 above) and then the preparation of the seed bags as outlined on Master 2.3, *Toxicity Testing on Seeds*.



- 6. Provide space for the trays of seeds from each team. Tell the students that they will check on their seeds during the next class.**

**Tip from the field test:** Storing multiple sets of seeds for multiple science classes can be a challenge. Remember that germinating seeds do not need light, so the seed bags for each team can be stacked. One field-test teacher gave each team a dissecting tray and told the teams to stack their seed bags in the tray. She then stacked the trays for each class. In this way, she was able to fit the ongoing investigations for four science classes on one shelf in her classroom.



- 7. Students might need to complete the rest of the work on Master 2.3 at home. Remind students to construct a data table in their science notebooks (or to put the data table from Master 2.3 in their notebooks). Ask them to fill out Day 1 on the data table with observations of their seeds at the start. Instruct them to answer the questions at the end of the worksheet.**



When students return to class with their worksheets complete, review their answers to assess their understanding of the investigation and underlying toxicological principles. Do they know the primary route of exposure of a human to their chemical? Can they describe how concentration is determined in a beaker? Are they able to make a reasonable prediction about the outcome of the investigation based on an understanding of dose, concentration, and response?

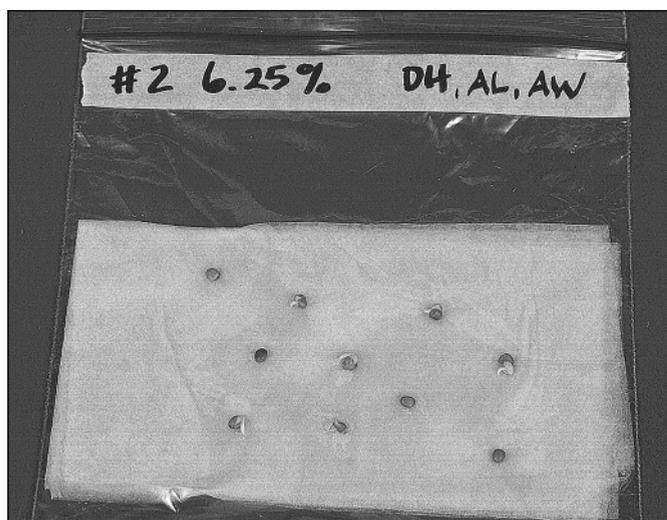


**Content Standard A:**  
Students use appropriate tools and techniques to gather, analyze, and interpret data.

#### ACTIVITY 4: GATHERING DATA

1. As students come into the room, ask them to get their team's seed investigation from Activity 3 and bring it to their team's table.
2. Ask students to observe their seeds and share with the class what is happening with their seeds.

Ask students to make observations that are *qualitative* (descriptive) and *quantitative* (measurable). An example of a qualitative observation might be that the seeds seem to be "puffed up" and are turning the paper napkin yellow underneath them. An example of a quantitative observation might be that six out of the 10 seeds in Bag #2 have germinated.



For those classes not equipped to conduct a laboratory investigation, the CD-ROM includes a simulation of the results students can expect from the Dose-Response Seed Germination Experiment using three different chemicals.



Insert the CD-ROM into the computer and go to the main menu. Click on *The Dose Makes the Poison: Dose-Response Relationships*. This brings up the page on which students can manipulate chemical concentrations. Click on *Do Experiment* to access the simulation of the experiment.

3. Direct the students to the question that guided the seed investigation:

**How does the chemical affect the germination of the seeds, and how is the effect different if the seeds receive different doses of the chemical?**

**Note to teachers:** The term dose is used rather loosely here because it is difficult to know how much chemical penetrates each seed. It is reasonable to assume, however, that seeds exposed to a higher concentration of chemical receives a higher dose. Getting students accustomed to thinking about the term dose will prepare them for activities later in the unit.

Help students see that they can begin to answer this question based on their observations by asking them the following questions:

- **How do you know that a chemical is having an effect?**

You can look at the control and see what a population of seeds does under normal conditions and compare control seeds with seeds exposed to the chemical.

**Tip from the field test:** Begin to talk about the seeds in each bag as a “population” of individuals. This reference will help students understand how to translate their data into a dose-response curve in Lesson 3.

- **What is happening to the population of seeds in the control?**

Seeds are germinating.

- **What is happening to the populations of seeds in the other five bags? Is the same thing happening in all the bags to which chemicals were added?**

Answers will vary: Some seeds might be germinating, some might not. Students might notice differences in seed germination between the seeds in the bag with the lowest dose of chemical and those in the bag with the highest dose of chemical.

- **What is the reaction that your populations of seeds have to your chemical? This reaction is called the response.**

Answers will vary, but students should begin to describe what is happening with their populations of seeds in terms of a **response**. The response to the chemical might be that seeds do not germinate as fast as they do in the control bag. Students might be able to see that more seeds are responding to the chemical (not germinating) in the bags with the highest dose of chemical.

- **Why might the whole population in each bag (all 10 seeds) not respond in the same way?**

By asking this question, you will help students begin to think about **individual susceptibility**, a concept students will explore further in Lesson 3. Just as there are individual differences among people, there are individual differences among seeds that affect how each seed responds to the chemical. In addition, students can begin to critique their experimental procedure and decide if they think they had enough liquid in each bag, spaced the seeds evenly, or in some way influenced the results through experimental error.

- **How do you measure the response your populations of seeds have to the chemical?**

Since the seeds’ response to the chemical is a variation in germination, the measurement would be to count the number of seeds in each bag that germinated and those that did not germinate. By focusing students on measurable observations rather than only qualitative ones, you will make it easier for students to graph the results of their investigation in Lesson 3.



**Content Standard A:** Students develop descriptions, explanations, predictions, and models using evidence.

- 4. Instruct students to fill in Day 2 of their data table on Master 2.3, which they placed in their science notebooks (see Activity 3, Step 7). Remind students that they will observe the seeds again on the next day to see if there are further changes.**
- 5. Direct students to place their trays of seeds in the same spot as the day before.**

---

### Extension Activity

---

The hazard of chemicals in the environment is one that often is exaggerated. Because we are exposed to many chemicals, both naturally occurring and synthetically produced, it makes sense to understand when to worry and when not to worry. The problem arises when people do not know the dose of a chemical required to cause an adverse effect. In many cases, people assume that any exposure to a chemical that can cause harm is harmful.

To illustrate the flaw in this reasoning, the American Council on Science and Health publishes a Holiday Dinner Menu each year. In this menu, scientists from the council analyze the foods we eat at Thanksgiving to determine our exposure to natural chemicals known to cause adverse effects in rats. For example, people consume natural chemicals such as ethyl benzene in their coffee, hydrogen peroxide in their tomatoes, and furan in their sweet potatoes. How are humans not being poisoned by their own food supply?

- 1. Invite students to log on to the American Council on Science and Health Web site to learn more about the importance of dose with respect to the dangers of chemicals in our food source:**  
*[www.acsh.org/publications/booklets/menu98.html](http://www.acsh.org/publications/booklets/menu98.html)*
- 2. Ask those students who choose to explore this extension to share their findings with the class.**
- 3. Encourage students to design a bulletin board that illustrates how much of a food containing a harmful chemical a human would have to consume to get a toxic dose of the chemical. Such calculations are presented on the council's Web site. Past years' examples have included the amount of turkey a person would have to eat to get a toxic dose of malonaldehyde and the amount of bread a person would have to eat to get a toxic dose of furfural. The calculations for the bread example are shown in the box on page 59.**

The bread in Thanksgiving stuffing contains furfural, a chemical that can cause cancer in rats when they are fed high doses of it. But, before you start to worry about your Thanksgiving dinner, take into account the difference in body weight between a human and a rodent. How much bread would *you* have to eat to consume an amount of furfural equal to the amount that increased the risk of cancer in rodents?

Here are the facts:

- One slice of white bread contains 167 micrograms of furfural.
- The carcinogenic dose of furfural for a rodent is 197 milligrams per kilogram of body weight per day, fed every day of its life. (This is the same as 197,000 micrograms/kilogram/day because there are 1,000 micrograms in a milligram.)
- The weight in kilograms of a middle school student who weighs 110 pounds is 50 kilograms because there are 2.2 pounds in every kilogram.

Here is the solution:

$$\frac{197,000 \text{ micrograms}}{1 \text{ kilogram}} \times \frac{50 \text{ kilograms}}{\text{person}} = 9,850,000 \text{ micrograms per day, a carcinogenic dose for an average middle school student.}$$

$$\frac{9,850,000 \text{ micrograms}}{167 \text{ micrograms/slice}} = 58,982 \text{ slices of bread}$$

What does this mean?

The average middle school student would have to eat 58,982 slices of bread a day to get a carcinogenic dose of furfural, assuming he or she responded the same way experimental animals do. When looking at this example, remember the conditions of animal studies: Doses are fed every day of the rodent's life (usually two years). To get an equivalent carcinogenic dose, a human would have to consume those 58,982 slices every day for years.

