Instructor Notes

Toxicants and California Blackworms

In this investigation, participants work in groups to determine the normal behavior of California blackworms *(Lumbriculus variegatus)*. They then determine how various concentrations of assigned toxicants affect the worms' behavior. This investigation introduces testing of potential toxicants, an important component in environmental health science.

After testing different toxicants and concentrations, participants will investigate exposure pathways, nature of effects, acute and chronic exposure, and reversible and irreversible effects. The participants discuss and analyze their data, observe physiological effects, and present their findings to the class. Through extensions, participants can develop new investigations based on their findings.

➤ *The activity is written for workshop participants and may need modification for classroom use.*

Suggested Background Reading

- An Introduction to Toxicology

National Science Education Standards for Grades 5–12

Science as Inquiry

- Abilities Necessary to Do Scientific Inquiry

  Conduct scientific investigations. Students conduct an investigation that tests the behavioral effects of various concentrations of alcohol, caffeine, and nicotine on California blackworms.

  Formulate and revise scientific explanations using logic and evidence. After considering the data while answering a series of questions and participating in group discussions, students formulate explanations for the worms' behavior and suggest future experiments based on scientific knowledge, logic, and investigational evidence.

Life Science

- The Behavior of Organisms

  Organisms have behavioral responses to internal changes and external stimuli. Students recognize how organisms respond to external stimuli through exposure to environmental changes caused by the introduction of toxicants.

Science in Personal and Social Perspectives

- Personal and Community Health

  Personal choice concerning health involves multiple factors. By observing, understanding, and discussing biological consequences of products such as alcohol and tobacco, students will be able to make more informed decisions about personal health practices.

Safety

As the instructor, you are expected to provide participants with the necessary safety equipment (including personal protective equipment such as goggles, gloves, aprons, etc.) and appropriate safety instruction to allow them to work safely in the laboratory. Always follow local, state, and school policies. Read and follow all precautions on labels and MSDSs provided by the manufacturer for all chemicals used.

Materials

For Getting Ready

Per class

- materials to make a probe
  - narrow rubber bands
  - electrical tape
  - applicator stick or bamboo skewer
- containers for preparing, measuring, and storing stock solutions
- Bunsen burner or hot plate
- glass stirring rod
- distilled water or untreated spring water
- toxicants
  - 80-proof vodka (for ethanol)
  - Vivarin® (for caffeine)
  - cigarettes of regular length and strength (for nicotine)

For the Procedure

Per class

- California blackworms
  California blackworms may be ordered from Carolina Science & Math (800/334-5551; #B3-L412). The worms may also be available at local tropical fish stores.
- recovery container for blackworms

Per group

- disposable Beral pipets (with tips cut off) or eyedroppers
- 4 chambers
  Chambers can be small weighing dishes or petri dishes placed over a white sheet of paper. Any small, clear container that will allow at least a 1-cm depth for the solutions will work.
- distilled water
- filter paper
- toxicant solutions (prepared in Getting Ready)
- waste beaker

- probes (prepared in Getting Ready)
- stopwatch or other timer
- black permanent marker

Getting Ready

Solution Preparation

In each case, make the stock solution first and then use it for dilutions. Use **ONLY** distilled water or spring water for making all dilutions and holding the worms. Tap water may kill the worms because they are very sensitive to chlorine.

Ethanol

Do not use denatured ethanol, methanol, or isopropyl alcohol. Vodka is recommended because it is clear and nearly odorless. To prepare the stock solution of ethanol, to 100 mL 80-proof vodka (40% ethanol), add distilled water to bring to a final solution volume of 400 mL (10% ethanol). Use this stock solution to prepare the following solutions:

- Solution #1: To 5 mL stock solution, add distilled water to bring to a final solution volume of 200 mL (0.25% ethanol).
- Solution #2: To 50 mL stock solution, add distilled water to bring to a final solution volume of 200 mL (2.5% ethanol).
- Solution #3: Measure out 200 mL stock solution (10% ethanol).

Caffeine

Vivarin is recommended as the source for caffeine rather than NoDoz® tablets, which contain a mint flavoring. To prepare the stock solution of caffeine, place two Vivarin tablets (total 400 mg caffeine) in an Erlenmeyer flask and add distilled water to bring to a final solution volume of 400 mL. Heat while frequently stirring to dissolve the tablets. It helps to gently break the tablets by tapping them with a glass rod while the solution is being heated. Do not let the solution boil. Use this stock solution to prepare the following solutions:

- Solution #1: To 16 mL stock solution, add distilled water to bring to a final solution volume of 200 mL.
- Solution #2: To 66 mL stock solution, add distilled water to bring to a final solution volume of 200 mL.
- Solution #3: Measure out 200 mL stock solution.

Nicotine

Use any generic or name-brand cigarette that is regular length and strength (do not use menthol, 100's, or ultralights). To prepare the stock solution of nicotine, stir the tobacco from two cigarettes (total 2.2 mg nicotine) in 500 mL very warm distilled water for 15–20 minutes. Strain or filter the solution after soaking. (You will lose about 50 mL of solution through straining). This process makes about 450 mL (0.011 mg/mL). Use this stock solution to prepare the following solutions:

- Solution #1: To 10 mL stock solution, add distilled water to bring to a final solution volume of 200 mL.
- Solution #2: To 50 mL stock solution, add distilled water to bring to a final solution volume of 200 mL.
- Solution #3: Measure out 200 mL stock solution.

The intent of this investigation is to determine what behavior changes occur at different sublethal concentrations. However, as a demonstration you may wish to expose one worm to a double-strength alcohol solution and one to a double-strength nicotine solution. Both of these are usually fatal.

Probes

Make probes by cutting a 2.5-cm piece of a narrow rubber band and then tape it to the end of an applicator stick or bamboo skewer. Use electrical tape to attach the rubber band. About half of the rubber band should hang from the end of the stick (Drewes, 1997).

Procedure Notes and Outcomes

In this investigation, participants work in collaborative groups of three or four to observe California blackworms and establish normal behavior. This baseline observation is used for comparison when toxicants are tested. Participants then test their worms with their assigned toxicant: alcohol, caffeine, or nicotine. Three concentrations are used and the participants are assigned observation criteria for the worms during the exposure. After answering a series of questions and participating in a group discussion, participants present their data, proper explanations, and suggest future experiments to the class.

This investigation can range from structured to open-ended, depending on the intended grade and ability level as well as on the length of the class period and the time devoted to the study. For instance, participants can mix their own dilutions and calculate the concentrations, or the solutions can already be prepared. Participants can use more concentrated stock solutions to test concentrations that determine the range for lethal, sublethal, or no effect. Participants can also investigate the physiological effects of these concentrated solutions on organisms. The time of exposure can be changed.

First, participants must to learn to manipulate the worms and to observe normal behaviors. They will soon develop their own "system" for handling the worms. They could be assigned to read the article in *Carolina Tips* (see References) to complete their introduction to the worms.

For all experiments, use full-length worms that are uniform in color. Worms that are dark with lighter sections have recently undergone regeneration and should not be used. Use care in handling the worms with the pipet so as not to fragment them. The worms should be "probed" only with the special probes made for this activity, never the tip of the pipet or forceps.

Participants should determine the anterior end from the posterior end. The anterior end is blunter and more darkly pigmented than the posterior end. It is also the end that will move first. If several worms are in the chamber, they will clump together in a ball, as they like to "cling" to things. This in itself is a normal behavior that the participants will want to note. Participants can separate the group by gently probing the worms or pipetting with water.

Once the anterior and posterior ends are established, participants should begin observing the swimming behavior. Touch the probe to the posterior end. The worm will swim forward in a corkscrew fashion, alternating clockwise and counterclockwise. When the worm is probed on the anterior end, it will coil and reverse its position. Both these movements are quite rapid, and it may take some time to note the differences.

Next, participants can observe crawling behavior. The worm can be placed in a petri dish or weighing dish on moistened filter paper (remove all excess water). Again they should probe both the posterior and anterior ends of the worm. In each case the worm will move by peristaltic crawling (successive waves of muscle contractions) in the opposite direction.

Results that might be noted for the suggested toxicants are listed below. These sample results represent some of the behavior changes that participants have observed using the given concentrations. You may note different observations on different days, depending on how participants observe and perceive the changes as well as on the size and health of the worms used.

Sample Data

In ethanol, the worms will be less likely to clump and will become rather inactive as the concentrations increase. In the highest concentration, they may straighten out in the middle but have their ends curled. The anterior area will be more affected. The worms may need to be probed several times to stimulate a response. They will have less skill in swimming, although they will still be able to crawl. They should begin to recover in 15 minutes after exposure to the first two solutions. Although the worms in the first two solutions will recover completely within 24 hours, recovery may not be complete after 24 hours with the third solution.

In caffeine, the worms become very active as the concentration increases, but they may try to clump at the lower concentration. They will show a greater sensitivity to probing both in swimming and crawling. At the higher concentration they may first curl in a ball and then stretch out. Some recovery should be seen after 15 minutes in the lower concentrations, and all should fully recover within 24 hours.

With nicotine, the worms may twitch in the solution. The tail may curl with loss of response. In the highest concentration, paralysis will occur. With paralysis, the worm will stretch and just seem to float in the water. There should be some recovery at the lower concentration in 15 minutes, and all worms should recover within 24 hours.

Plausible Answers to Questions

1. Exposure occurs when an organism comes in contact with a toxicant. Exposure frequency refers to how often, exposure duration refers to how long, and exposure concentration refers to how much. Using this terminology, describe each for your investigation.
   Answers will vary.

2. Two types of toxicity tests can be performed. Acute toxicity tests are a high single exposure for a brief duration. Chronic toxicity tests are usually a persistent and longer exposure (depending on the organism's lifespan) at a lower concentration than the acute test.

   a. Based on this information, which type of test was done in this investigation?
      This was an acute toxicity test.

   b. What are the benefits of using an acute toxicity test?
      It saves time, one can see immediate responses to the toxicant; and one can learn the per dose limit of tolerance to a toxicant.

   c. What are the benefits of using a chronic toxicity test?
      One can learn the cumulative effects of the toxicant; it more closely approximates normal exposure to toxicants.

3. Using the data from your assigned toxicant, design a chronic toxicity test that you might perform on the blackworms. Predict what your results might be.
   One might begin by exposing the worms to the lowest alcohol concentration used in this experiment, as well as exposing them to even lower concentrations. Since the exposure needs to be chronic, the experiment would last over a much longer period of time (months or even years). The exposure could be constant or periodic. Periodic exposures, meaning the worms are given some recovery time in between exposures, would be more representative of "real life" exposures.

4. The exposure pathway is how a toxicant enters the body. What was the exposure pathway for your toxicant?
   The exposure pathway is through the surface membrane of the worm. It is unlikely that for a water-soluble toxicant, such as alcohol, entry would be through the mouth. Ingestion of a toxicant by these worms would probably have to be associated with food. (You can demonstrate this to the participants by removing the head and the tail of a worm and showing that the toxicant still has a dramatic systemic effect.)

5. Toxicity is affected by both intrinsic and extrinsic factors. Extrinsic factors, such as temperature or barometric pressure, occur outside the body. Intrinsic factors, such as age, metabolism, and genetic differences, are inherent to an individual organism. Using the following factors, predict how you think each could affect the results with your toxicant. Be specific!

a. temperature
b. age
c. metabolism
d. genetic difference

Answers will vary.

6. Your concentrations represent sublethal concentrations of the toxicant. Explain what you think this means.
The concentration of the toxicant is not high enough to kill the worms during the period of time they were exposed.

7. The potency of a toxicant is the measure of its strength. Paracelsus (1493–1541) is quoted as saying "The dose makes the poison." The more potent the toxicant, the less it takes to evoke a response. Based on the concentrations listed above and your observations, which toxicant do you think is the most potent and why?
Answers will vary.

8. Based on your toxicant, what body systems do you think were affected and why?
Answers will vary.

9. At the end of the 24-hour recovery, you can generally determine whether the effects of your toxicant are reversible or irreversible. Based on the toxicant that you used, tell whether the effects were reversible or irreversible at each concentration.
All of the worms recovered within 24 hours, so the effects of these toxicants are reversible. The effects of the strongest ethanol solution is a possible exception.

10. Did all of your worms (at each concentration) demonstrate the same behavior? Assume that one worm demonstrated normal behavior and the other four demonstrated abnormal behavior. How would you explain this?
No, all of the worms at each concentration did not demonstrate the same behavior. This can be explained by variability, or differences, between the worms. For example, there may be genetic, age, health, or size differences between the worms.

11. The investigation that you did was a controlled experiment.
    a. What was the control?
    A set of blackworms whose behavior was observed but to which no toxicant was applied; the blackworms in only distilled water.
    b. Why is a control necessary in a scientific experiment?
    To be able to determine what is "normal" so we can compare and determine deviations from normality.

12. Risk assessment of a toxicant is the estimate of severity and the likelihood of harm to human health or the environment that occurs from exposure to a risk agent (toxicant). The toxicants that you tested are relevant to human health. Name some toxicants you might test that would harm the environment and thus pose a threat to the worms.
Ammonia, chlorine, or other forms of alcohol that are commonly released into the environment, such as antifreeze.

13. How do lifestyles play a part in risk assessment of human health toxicants?
Lifestyles affect an individual's health, metabolism, and limits of exposure to toxicants. For example, risk increases if a person is in poor health. Limits of exposure to toxicants can be affected by actions such a frequency of exposure to alcohol or career choice. Risk increases as the frequency of alcohol (ethanol) exposure increases. Also, a mechanic will probably be exposed to more toxicants than a secretary; thus a mechanic's risk for exposure to certain chemicals would be higher. Remember, exposure does not necessarily mean the toxicant has entered the person's body. If the mechanic is very careful (which is also a lifestyle choice), the dose of the toxicants would be zero.

14. Can the results of your tests be applied to humans or other vertebrates? Why or why not?
Not directly. Effects can only be extrapolated from an experimental system to another system if the two types of systems can be shown to be sufficiently similar in relevant characteristics and behavior.

15. Based on what you have learned from your investigation and your answers to the questions above, analyze your data and summarize any conclusions that can be drawn from the results.
Answers will vary.

16. As a group, discuss your findings. Using reference material, look up any information about your toxicant that will help in further analyzing your data. Suggest additional investigations using your toxicant and make a group presentation of your findings to the class.
Answers will vary.

Extensions

Participants may test other toxicants. They will need to determine the initial concentration for their stock solution by trial and error. The goal of the experiment is to determine three sublethal concentrations that produce the following results:

1. low concentration—evokes little or no response
2. middle concentration—evokes a near maximum response
3. high concentration—is sublethal and evokes a maximum response

The following are just some examples of toxicants that might be tested:

- Aleve®, Nuprin®, aspirin, Tylenol®, Excedrin® PM
- melatonin, Nytol®, Dexatrim®, vitamins
- Benadryl®, Sudafed®
- saccharin, Nutrasweet®
- chlorinated water
- pesticides, antifreeze, detergents
- UV radiation

References

Drewes, C.D. "Those Wonderful Worms," *Carolina Tips. 59* (3). Carolina Biological Supply: Burlington, NC, 1996.

Drewes, C.D. "Screening for Sublethal Behavioral Effects in a Freshwater Oligochaete, *Lumbriculus variegatus*." Lab for Dr. Drewes' toxicology class, 1995.

Lesiuk, N.M.; Drewes, C.D. "Blackworms, Blood Vessel Pulsations, and Drug Effects," *The American Biology Teacher.* 1999, *61* (1), 48–53.