

TOXICANTS AND CALIFORNIA BLACKWORMS

Project overview

In this investigation, participants will work in groups to determine the normal behavior and pulse rate of California Blackworms (*Lumbriculus variegatus*). They will then test various concentrations of an assigned toxicant to determine how they affect the worms' behavior and pulse rate. This project represents an introduction to toxicology by introducing the worms as an excellent model for toxicology testing which is an important component in environmental health science.

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

Description of audience

This investigation is designed for use with middle school through high school participants.

National Standard Statement

This activity incorporates several standards from the National Science Education Standards. Through **science as inquiry**, participants will conduct a scientific investigation that will test various types and concentrations of toxicants on California Blackworms. This investigation also incorporates the content standard of **personal and community health**. By observing, understanding, and discussing biological consequences of products such as alcohol and tobacco, participants will be more informed to make better decisions about personal health practices. Finally, this investigation includes the **life science** standard of behavior by demonstrating how organisms respond to external stimuli through exposure to environmental changes.

Facilitator Notes

Narrative

In this investigation, participants will work in collaborative groups of 3 or 4 to first observe California Blackworms and establish what they consider normal behavior and pulse rate. This becomes the baseline measurement used for comparison when toxicants are tested. Participants will then be assigned a toxicant such as alcohol or caffeine to test their worms. Several concentrations will be used and the participants will be assigned observation criteria for the worms during the exposure. After analyzing data through a series of questions and group discussions, participants will present their data, contribute explanations, and suggest future experiments to the class.

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

Materials List

California Blackworms

California Blackworms may be ordered from Carolina Biological Supply (1 800-334-5551 #B3-L412). A better suggestion is to first check local tropical fish stores as they will be considerably cheaper. A good source in the Greater Cincinnati area is The House of Tropicals on River Road (471-2414).

Pipettes

The disposable pipettes need to be large bore pipettes. Eyedroppers may be used.

Chambers

Chambers can be small weigh boats or petri dishes placed over a white sheet of paper. Anything that will allow at least a 1cm depth for the solutions will work.

Probes

Special probes (Drewes 1997) are made by cutting a 1 inch piece of a narrow rubber band and then taping it to the end of an applicator stick or bamboo skewer stick. About 1/2 inch of the rubber band should extend from the end of the stick. Use electrical tape to attach the rubber band.

Magnifying glass or hand lens

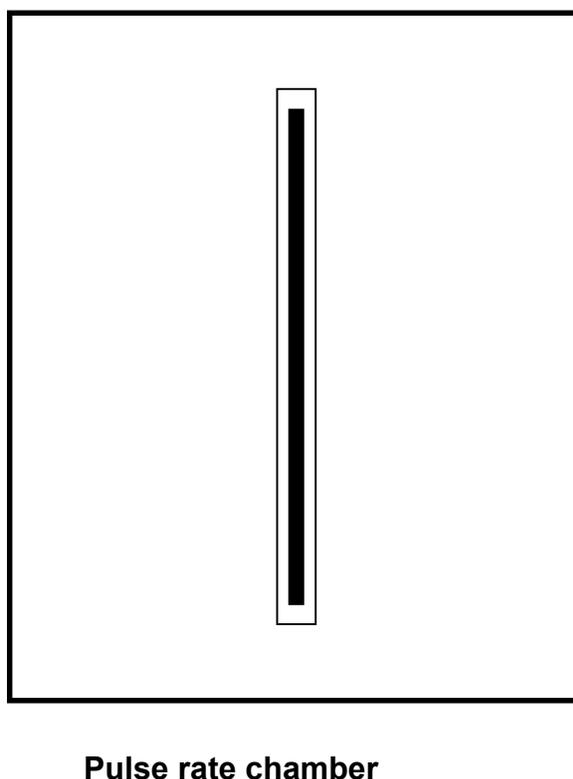
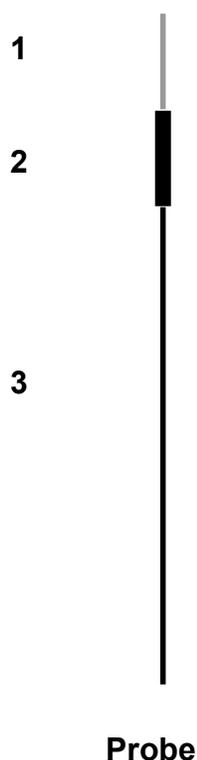
These are helpful but not required for the participants to initially distinguish the anterior from posterior end of the worms as well as get a closer look at segmentation and blood pulsations.

Water

All water that the worms come in contact with must be nonchlorinated as they are highly sensitive to chlorine. Distilled water, spring water, or purchased drinking water is fine. Be cautious in using aged tap water that might have traces of chlorine.

Pulse rate chamber (Paraffin block with trough)

To measure pulse rate, it is helpful to have a device to hold the worm in place under a dissecting scope. An accepted device (Drewes 1997) can be made from paraffin blocks that are used in canning. There are 4 blocks in each box that can be cut in half. Place a ruler on the paraffin block and using the edge of a small paper clip, make a trough 4-5cm long and about 1mm wide and deep. Cut a piece of dark thread slightly shorter than the length of the trough and with the edge of the paper clip, gently embed it into the trough. If dissecting scopes are not available, then (although more difficult) participants can use a regular microscope with the lowest objective 4X. Cut a piece of filter paper just a little smaller than a microscope slide and place it on the slide. Moisten it with distilled water and then place the worm on it. Be sure to remove excess water. Although the worm will crawl on the filter paper, with a little skill it is possible to keep up with the worm and get the pulse rate.



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|-------------------------------|--------------------------------|
| 1) Rubber band tip (1/2 inch) | paraffin block |
| 2) Electrical Tape | trough 4cmX1mmX1mm |
| 3) Stick (6-8 inches) | dark thread embedded in trough |

Solution preparation

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

Alcohol

You cannot use ethanol or rubbing alcohol because it is denatured and toxic. Therefore vodka is recommended because it is clear and odorless. Just refer to it as alcohol and participants will assume it to be ethanol.

Stock solution: 100 ml vodka (40%) + 300 ml water = 400 ml (80 mg/ml) (10% alcohol)

Solution #1: 5 ml stock solution + 195 ml water = 2 mg/ml (0.25% alcohol)

Solution #2: 50 ml stock solution + 150 ml water = 20 mg/ml (2.5% alcohol)

Solution #3: 200 ml stock solution = 80 mg/ml (10% alcohol)

Caffeine

Vivarin is recommended for the caffeine tablets as NoDoz contained a mint flavoring.

Stock solution: Crush 2 caffeine tablets (200 mg caffeine/tablet) and add to 400 ml water (heat if necessary to dissolve tablets) = 400 ml (1 mg/ml)

Solution #1: 16 ml stock solution + 184 ml water = 0.08 mg/ml

Solution #2: 66 ml solution #1 + 134 ml water = 0.33 mg/ml

Solution #3: 200 ml stock solution = 1 mg/ml

Nicotine

Use any generic or namebrand cigarette that is regular length and strength (do not use menthol, 100's, or ultralights).

Stock solution: Stir the tobacco from 5 cigarettes (1.1 mg nicotine/cigarette) in 500 ml water of very warm water for 15-20 minutes. Strain or filter the solution after soaking. (You will lose about 50 ml through straining) = 450 ml (0.011 mg/ml)

Solution #1: 10 ml stock solution + 190 ml water = 0.00055 mg/ml

Solution #2: 50 ml stock solution + 150 ml water = 0.00275 mg/ml

Solution #3: 200 ml stock solution = 0.011 mg/ml

The solution concentrations are designed to give little or no change in the first solution. The second solution should produce a more dramatic response. The third solution should demonstrate that exposure concentrations above the second have reached their maximum. Actual responses will depend on such things as the worms' health, size, etc. Stock solutions can be made double strength which in the case of

alcohol and nicotine are usually fatal to the worms. A demonstration could be to sacrifice a worm to each. However, the intent of this investigation is not to kill the worms but rather to determine what behavior and pulse rate changes occur at different sublethal concentrations.

Procedures and Results

It is best to allow participants one class period to learn how to manipulate the worms and discover for themselves behaviors that they will be observing as well as determining accurate pulse rates. Participants soon develop their own “system” for handling the worms. At the end of the period, they could then be assigned the article in “Carolina Tips” as a reading assignment to complete their introduction to the worms and lab.

For all experiments, participants should 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. Participants need to use care in handling the worms with the pipette so as not to fragment the worms. The worms should be “probed” only with the special probes made, never the tip of the pipette or forceps.

In lab, participants should first 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 be moving first. If several worms are in the chamber, they will clump into a ball as they like to “cling” to things. This in itself is a normal behavior that the participants will want to note. By gently probing the worms or pipetting water, the group will separate.

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

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

Pulse rate takes a little more skill. Using the pulse rate chamber, the participant should carefully place the worm using the pipette on top of the trough. The excess water then needs to be drawn back up in the pipette. Once the water is removed, the worm will sink into the trough or can be coaxed in with the aid of the probe. After a couple of minutes they can use the dissecting scope to watch the pulsations and begin timing. Pulsations are usually higher in the tail than the head so it might be advisable for the participants to count the pulsations in the middle of the worm. Depending on the activity level of the worm, it is acceptable to take a count for 15 seconds and multiply by four for pulsations/minute. If the worm less active, then 30 seconds is better. The normal pulse rate is around 12 pulsations/minute.

Results that might be noted for the suggested toxicants are as follows:

Alcohol

The worms will be less likely to clump and become rather inactive as the concentrations increase. In the high concentration, they may straighten out in the middle but have their ends curled. The head area is more affected. The worms may need to be probed several times to stimulate a response. They will have less skill in swimming although they are still able to crawl. Their pulse rate will decrease. 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 in 24 hours, recovery may not be complete after 24 hours with the third solution.

Caffeine

The worms become very active as the concentration increases but may try to clump at the lower concentration. They 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. The pulse rate will also increase but may be difficult to measure due to their activity level. Some recovery should be seen after 15 minutes in the lower concentrations but all should fully recover in 24 hours.

Nicotine

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

These sample results represent some of the behavior and pulse rate changes that participants have observed using the given concentrations as well as other concentrations. You may note different observations on different days depending on how participants observe and perceive the changes as well as the size and health of the worms used.

Special Note

The idea of using *Lumbriculus variegatus* came from Dr. Charles Drewes, who is a professor of Zoology at Iowa State University (Ames, Iowa). After attending two different workshops presented by Dr. Drewes at NABT, it was apparent that these worms offered a wealth of experimental opportunities for participants of all levels. The possibilities of labs that can be designed by participants and facilitators are endless. In fact, three projects at the Intel International Science Fair (spring 1997) were based on research with these worms. Dr. Drewes can be reached at (515) 294-8061 or by email at cdrewes@iastate.edu.

References

Drewes, Charles D. 1996. "Those Wonderful Worms." Carolina Tips 59(3). Carolina Biological Supply Company, Burlington, NC.

Drewes, Charles. 1995. "Screening for Sublethal Behavioral Effects in a Freshwater Oligochaete, *Lumbriculus variegatus*. Lab for Dr. Drewes Toxicology class.

Lesink, Nalena M. and Charles Drewes. (fall) 1997. "Blackworms, Blood Vessel Pulsations, and Drug Effects". The American Biology Teacher.

TOXICANTS AND CALIFORNIA BLACKWORMS

Introduction

This investigation represents a model for toxicology testing in organisms. You will determine the behavioral and/or pulse rate changes that occur when blackworms are exposed to different concentrations of an assigned toxicant through a controlled experiment. At the end of the investigation, you will analyze your data, present your findings to the class, and suggest other possible experiments.

Materials

20 blackworms

4 disposable pipettes

4-5 chambers (weigh boats or petri dishes)

4 probes

beaker of distilled water

waste beaker

magnifying lens

marking pen

flasks with different concentrations of toxicant

For pulse rate

pulse rate chamber

dissecting scope

For crawling behavior

chamber with moistened filter

paper

Procedure

1. You will work in groups of 3-4 and be assigned a toxicant to test. You will also be assigned criteria to observe from the list below.

A) clumping behavior - Do the worms clump in a ball?

B) swimming behavior - Do the swim forward and backward in a normal fashion?

C) crawling behavior - When probed, do they crawl forward and backward normally?

D) pulse rate - How many pulsations/ minute occur?

***Also make note of activity level (faster or slower than normal) and individual position in the water such as stretched out, curled in a ball, ends curled as well as anything else that might be considered an unusual response.

2. Obtain 4 chambers and a marking pen. Label each as follows: Control, #1, #2, and #3 along with the name of the toxicant you've been assigned. Add enough distilled water to each chamber to produce a depth of 1 cm.

3. Take the chamber labeled "Control" to the large container of worms. Using a pipette, transfer 20 worms to your chamber. Be sure to choose worms that are full length and uniform in color. Do not select worms that have areas distinctly lighter than the rest of the worm.

4. Transfer 5 worms from the control chamber to chamber #1, then transfer 5 worms to chamber #2, and transfer 5 worms to chamber #3. Observe the behavior of the worms in all 4 chambers based on your assignment. Note whether the worms tend to clump together in a ball. If you have been assigned swimming behavior, use the probe to touch the anterior end (blunter and more darkly pigmented) of the worm and observe the movement. Then touch the posterior end and again observe the movement.

If you have been assigned crawling behavior, transfer the worm with the pipette onto moistened filter paper in another chamber and remove all excess water. Probe the worm first on the anterior end and then the posterior end to make your observations.

If you have been assigned to pulse rate, transfer the worm with the pipette onto the top of the trough of the pulse rate chamber. Using the pipette, draw all the excess water off. If the worm does not sink into the chamber, gently use the probe to move it. Using the dissecting microscope, count the number of pulsations in the middle of the worm for 15 seconds. Multiply your answer by 4 to give you the number of pulsations/minute. Record all your assigned observations on the data sheet.

5. Gently draw your worms from each chamber (except the control) back into a pipette. (If this is too difficult, then get another chamber with some distilled water and put your worms temporarily there.) Pour the distilled water back into the beaker and then add your assigned toxicant solution in chamber #1, #2, and #3 to a depth of 1 cm. Transfer your worms back into the chamber. Expose the worms in the toxicant for at least 15 minutes. During the exposure period, make your assigned observations every three minutes on **all four chambers** of your assigned toxicant and record them by three minute intervals on your data sheet.

If you are observing behavior, make note if all worms exhibit the same behavior or if some remain normal while others exhibit a change. If you are observing pulse rate or crawling behavior, use a different worm at each 3 minute interval. Also look for physical changes such as bulging in the center, bleeding, or fragments breaking off. If any worms die, record the mortality.

6. After the exposure period has ended you will begin a recovery period for the worms. Draw your worms into a pipette. Empty the toxicant into the waste beaker and fill the chamber to a depth of 1cm with distilled water. Add the worms. (Try to expel as much toxicant out of the pipette first if they have been held in the pipette). Again observe the worms at three minute intervals on **all four chambers** for at least 15 minutes during the recovery period and record your observations just as you did before. Be sure to make notes about any recovery (a return to normal behavior or pulse rate) that takes place.

7. Leave the worms in their chambers overnight and observe again the next day. Be sure to record the number of any new mortalities that might have occurred overnight. Fully recovered worms will return to the original large container after 48 hours. Any worms that don't appear to be in good health or fully recovered after 48 hours should go in a separate container marked "Recovery".

Questions and Conclusions

Directions: Collaborate with your group to obtain the data for all concentrations of your toxicant. Complete your data table with the following information on toxicant concentrations and then answer the questions.

	Alcohol	Caffeine	Nicotine
Solution #1	2 mg/ml	0.08 mg/ml	0.00055 mg/ml
Solution #2	20 mg/ml	0.33 mg/ml	0.00275 mg/ml
Solution #3	80 mg/ml	1.0 mg/ml	0.011 mg/ml

Part A

- Based on your assigned observations, graph your results.
- Exposure occurs when the 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.
- There are two types of toxicity tests that can be performed. Acute toxicity tests are a high single exposure for a brief duration. Chronic toxicity tests are usually a persistent and longer (depending on the organism's lifespan) exposure with a lower concentration than the acute test.
 - Based on this information, which type of test was done in this investigation?
 - What would be the benefit of using an acute toxicity test?
 - What would be the benefit of using a chronic toxicity test?
- Using the data from your assigned toxicant, design a chronic toxicity test that you might perform on the blackworms. Predict (hypothesize) what your results might be.
- The exposure pathway is how a toxicant enters the body. What was the exposure pathway for your toxicant?
- Extrinsic factors that affect toxicity occur outside the body such as temperature or barometric pressure. Intrinsic factors are within an individual organism such as age, metabolism, and genetic difference. Using the following factors, predict how you think each could affect the results of your toxicant. Be specific!
 - temperature
 - age
 - metabolism
 - genetic difference
- Your concentrations represent sublethal concentrations of the toxicant. Explain what you think this means.

8. The potency of a toxin is the measure of its strength. Paracelsus (1493-1541) is quoted as saying “the dose makes the poison”. The more potent the toxin, 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?
9. Based on your toxicant, tell what body systems you think were affected and why?
10. The recovery period for the worms represents detoxification. What body systems in your worms were involved in this process and how do you think they functioned?
11. At the end of the 24 hour recovery, it is important to determine the nature of the effects of your toxicant. The effects can be reversible or irreversible. Based on the toxicant that you used, tell whether the effects were reversible or irreversible at each concentration.
12. 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?
13. The investigation that you did was a controlled experiment.
 - A. What was the control?
 - B. Why is a control necessary in an actual scientific experiment?
14. 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 apply to human health. Name some toxicants you might test that would harm the environment and thus pose a threat to the worms?
15. How do lifestyles play a part in risk assessment of human health toxicants?
16. Can the results of your tests be applied to humans or other vertebrates? Why or why not?
17. Based on what you have learned from your investigation and what you have learned from the above questions, analyze your data and write your own conclusion using the proper terminology and concepts.

Part B

1. 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 further investigations using your toxicant and make a group presentation of your findings to the class.

Extensions

Participants may wish to 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 yet evokes no increase in 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

Sample Data Sheet

Toxicant: _____	Observed behavior or pulse rate before exposure	Observed behavior or pulse rate during exposure (Exposure time _____)	Observed behavior or pulse rate during recovery (Recovery time _____)	Observed behavior or pulse rate after 24 hrs. recovery
Control		3 min 6 min 9 min 12 min 15 min	3 min 6 min 9 min 12 min 15 min	
Solution #1 Conc: _____		3 min 6 min 9 min 12 min 15 min	3 min 6 min 9 min 12 min 15 min	
Solution #2 Conc: _____		3 min 6 min 9 min 12 min 15 min	3 min 6 min 9 min 12 min 15 min	
Solution #3 Conc: _____		3 min 6 min 9 min 12 min 15 min	3 min 6 min 9 min 12 min 15 min	