Updated June, 2005

Toxicity Testing with California Blackworms Using Alcohol

This activity has been modified from "Toxicants and California Blackworms" and is being used with permission from the Center for Chemical Education at Miami University. Modifications were developed at the University of Arizona Southwest Environmental Health Sciences Center Community Outreach and Education Program. This activity as well as the original can be downloaded at http://coep.pharmacy.arizona.edu/curriculum/blackworms/index.html.

Upon completion of the activity participants will be able to:

- Explain the concepts "the dose makes the poison" and dose/response.
- Differentiate between exposure and dose and explain factors that can affect dose.
- Identify the routes of exposure.
- Differentiate between acute and chronic toxicity.
- Identify factors that can influence the effect of a chemical on living things.

Overview

In this investigation, participants will work in groups to determine how various concentrations of alcohol (ethanol), caffeine, and nicotine affect the swimming behavior of California Blackworms (*Lumbriculus variegatus*). This project represents an introduction to toxicology, which is an important component in environmental health science. By exposing the worms to a toxicant, such as alcohol, in varying concentrations, participants will witness and discuss exposure pathways, nature of effects, acute and chronic exposure, as well as reversible and irreversible effects. The participants will discuss and analyze their data and share their findings with 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, sub-lethal, or no effect. Also, depending on the level of participant and time frame, participants can investigate the physiological effects (such as effects on pulse rate) 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. In addition, different toxicants (e.g. caffeine or nicotine) can be used.

References

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Updated June, 2005

Student Directions: Toxicity Testing with California Blackworms

Learning Objectives

Upon completion of the activity participants will be able to:

- Explain the concepts "the dose makes the poison" and dose/response.
- Differentiate between exposure and dose and explain factors that can affect dose.
- Identify the routes of exposure.
- Differentiate between acute and chronic toxicity.
- Identify factors that can influence the effect of a chemical on living things.

Introduction

This investigation represents a model for toxicology testing in organisms. You will determine the behavioral changes that occur when blackworms are exposed to different concentrations of alcohol, caffeine, and nicotine through a controlled experiment. In this investigation, participants will work in groups to determine how various concentrations of alcohol (ethanol), caffeine, and nicotine affect the swimming behavior of California Blackworms (*Lumbriculus variegatus*). This project represents an introduction to toxicology, which is an important component in environmental health science. By exposing the worms to a toxicant, such as alcohol, in varying concentrations, participants will witness and discuss exposure pathways, nature of effects, acute and chronic exposure, as well as reversible and irreversible effects. The participants will discuss and analyze their data and share their findings with the class.

At the end of the investigation, you will analyze your data, share your findings with the class, and suggest other possible experiments.

Materials (per group of 5-6 students)

8 weigh boats or petri dishes or cups	marking pencil/ sharpie
1 – 500 mL beaker	1 – 100 mL beakers
1 – 50 or 100 mL graduated cylinder	distilled water
alcohol /nicotine/ caffeine stock solns.	4 pipettes
4 plastic spoons	40-50 California Blackworms
magnifying lens (optional)	timer (optional)

Background Information: Basic Toxicology

This activity demonstrates several very important, fundamental concepts in toxicology including exposure, dose, dose/response, and acute toxicity.

Exposure vs. Dose

To cause harm to a person (or animal), a hazard must enter the body. Merely being exposed will not cause harm if the hazard does not actually enter the body. For example, a pack of cigarettes in a man's shirt pocket does not cause harm to him because nothing from the cigarettes has entered his body. If, however, he smokes one of the cigarettes, the smoke has entered his body through his lungs and can cause harm. There are three primary ways that a hazard can enter the body:

- **Ingestion** - Chemicals that are ingested enter the body by being eaten. From the digestive track, they can go to the liver or the lymphatic system and then on to the bloodstream. Some chemicals are not absorbed by the digestive track, so they pass through the body and are excreted in the feces. The alcohol enters the worms' bodies through ingestion.

- **Inhalation** – Chemicals can be breathed into the lungs, called inhalation. The inside surface of the lungs is very large and is a poor chemical barrier. Many chemicals that are inhaled can easily and quickly enter the bloodstream from the lung tissue.

- **Absorption** - Chemicals can enter the body by moving through the skin, called absorption. The skin is a very good barrier and provides protection from many hazards, but some substances can penetrate the skin, then enter the blood stream and be carried to all parts of the body.

(Source: Chemicals & Human Health Website: http://www.biology.arizona.edu/chh/default.html)

Dose/response

The **dose** is the specific amount of a chemical that enters the body. When a person is exposed to a hazard, such as alcohol, there are several things that determine the amount that actually enters the body. One way to determine a person's dose is to do a blood test to measure the amount of chemical in their body. For many chemicals, there is no easy way to measure them in the blood. Scientists must measure other factors to estimate dose. Some measurements that can be used are:

- **respiration rate** - A hazardous gas usually enters a person's body through inhalation into the lungs. If they are breathing quickly, they will breathe in more of the gas than if they are breathing slowly. Thus, their dose is higher if they are breathing heavily.

- **hazard concentration** - A higher concentration of a hazard generally means a higher dose because there is more of the hazard to enter the body. For example, a person who drinks a beer with a shot of tequila in it will receive a higher dose of alcohol compared to someone who drinks only the beer.

- **frequency of exposure** - A person exposed only once is likely to have a smaller dose than a person exposed many times.

- **length of exposure** - A person exposed for a short time will have a lower dose than a person exposed for a long length of time.

- **properties of the toxicant** - Some toxicants are not easily absorbed by the human body and exposure does not lead to as high a dose as exposure to a toxicant that is easily absorbed. In addition, different toxicants affect different bodily functions and are processed by the body differently. The severity of the response to a toxicant will depend on how the body processes the toxicant and the physiological functions it affects.

The amount of damage (**response**) caused by a chemical that has entered the body depends on the dose, or amount entering the body. This relationship, called **dose/response**, follows a predictable pattern. At very low amounts, there will be no detectable effect of the chemical. In the midrange of doses, the amount of damage will increase as the dose increases. At very high doses, a maximum level of damage is reached. Thus, **it is the dose of a chemical that makes the poison**.

(Source: Chemicals & Human Health Website: http://www.biology.arizona.edu/chh/default.html)

Updated June, 2005

Acute vs. Chronic Toxicity

Acute toxicity refers to a high toxicant dose over a short period of time, whereas, **chronic toxicity** refers to small doses over a long period of time. Acute toxicity is commonly measured as the Lethal Dose 50 or LD50. The LD50 is the dose of a substance that is lethal to 50% of the animals being tested (most commonly mice or rats). Below is a table illustrating the Rat LD50 and the approximate human LD50 for some common toxicants.

LD50 for Various Toxicants Administered to Rats

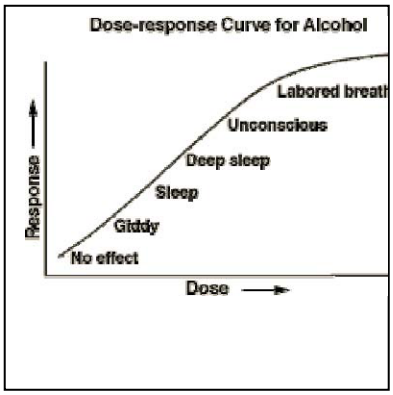
Chemical	Rat LD50 (milligrams/kilogram)	Approximate Human LD50 (for a 160 lb. human)
Sugar (sucrose)	29,700	3 quarts
Alcohol (ethanol)	14,000	3 quarts
Salt (sodium chloride)	3,000	1 quart
Arsenic (arsenic acid)	48	1-2 teaspoons
Nicotine	1	½ teaspoon
Dioxin (TCDD)	0.001	speck
Botulinum toxin	0.00001	too small to be seen

(Source: Toxicology for the Citizen, Institute for Environmental Toxicology, Michigan State University, 1991. http://www.iet.msu.edu/toxconcepts/toxconcepts.htm.)

Alcohol Toxicity

Acute Dose-Response for ETHANOL (the alcohol in beverages):

Blood ethanol level (mg/100mL)*	Effect
50-150	Incoordination, slow reaction time, blurred vision
150-300	Visual impairment, staggering, slurred speech; hypoglycemia, especially in children
300-500	Marked incoordination, stupor, hypoglycemia, convulsion
+500	Coma and death (different in tolerant individuals)



* mg/100mL = mg% = mg/deciliter—concentration of alcohol in blood

In a 70kg human, ingestion of approximately 3oz of pure alcohol would achieve a blood alcohol level of 90-150 mg/100mL (legally drunk)—this is equivalent of 6oz of 100 proof whiskey, 12oz of fortified wine (sherry) or 8 12-ounce bottles of beer. The amount of alcohol necessary to result in a certain blood level or certain effects varies with the individual (genetics, tolerance, size), fasting state, and the period of time over which the drinks are consumed. Absorption from the digestive tract is very rapid with peak blood levels attained 30-60min after ingestion. In general, the liver metabolizes ethanol at a rate that reduces the blood alcohol level approximately 15-20mg/100mL per hour, so that it would require 6-8hrs for a 120mg/100mL level to reach negligible levels.

Rule of thumb: 1mL of absolute ethanol per kilogram of body weight results in a level of 100mg/100mL in 1 hour. Absorption from the digestive tract is very rapid with peak blood levels attained 30-60min after ingestion.

Updated June, 2005

Preparation Your group will test either alcohol or caffeine or nicotine

1. Obtain **8 weigh boats (plastic cups or petri dishes can also be used**) and a marking pencil/tape. Label each container as follows: Control, WControl, Low, Medium, High, Water1, Water2, and Water3. If you are using weigh boats, petri dishes or plastic cups, be sure to label them on the lip so that you can easily read the label. Make sure to rinse all glassware with distilled water to remove any residue as the worms are sensitive to chorine compound, and detergents/soaps.

2. Using a graduated cylinder, fill the weigh boats labeled Control, WControl, Water1, Water2, and Water3 with 40-50 mL distilled water. The remaining distilled water in the beaker will also be used to make your dilutions. (**Do NOT** use tap water because the chlorine will negatively affect the worms). **Once the solutions have been prepared pour the solutions into the low, medium and high weigh boat, petri dish, or plastic cup with labels.**

Solution preparation

In each case, **use the provided stock solutions to make the dilutions (low, medium, and high) required**. 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 think of it as slightly diluted 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

3. Arrange your weigh boats as follows:

Distilled Water Alcohol / Caffeine / or Nicotine solutions

Water3	Water2	Water1	Wcontrol Distilled water
High	Medium	Low	Control Distilled water

4. Using a pipette or spoon, transfer ~40-50 worms into a plastic cup and fill the cup or beaker with distilled water.

Observations

5. Using a pipette or spoon, **transfer about 10 worms from the beaker to the WControl dish, ~ 10 worms to Water1, ~ 10 worms to Water2, and ~ 10 worms to Water3**. The numbers do not have to be exact.

6. **Observe their behavior for at least 5 minutes.** Familiarize yourself with the behaviors listed on your data sheets and further descriptions in step #8 of procedure, including the activity level ratings. You may gently probe the worms and observe their response.

7. Decide who in your group will have the following assignments (some of you may have multiple assignments):

   Timer – Will let the group know when 0, 3, 6, and 10 minutes has passed and will record the start time for each concentration.

   Worm Movers – There will be one worm mover per set of weigh boats to move the worms to and from the weigh boats with and without alcohol (i.e. WControl to Control; Water1 to Low; Water2 to Medium; and Water3 to High). **Worm Moving Tips**: Allow the worms to clump together**,** then scoop up as many worms as possible with a spoon or pipette. **Try not to spill the liquid into the new weigh boat (i.e. you don't want to introduce alcohol to the distilled water-only weigh boats or dilute the alcohol solutions).**

   Observers – one for each concentration level) - Will observe the behavior of the worms once they are moved from one weigh boat to another. You will report the activity rating, clumping behavior, and any other observations to the data collector. Be sure to observe the worms' behavior immediately upon entering the solution (Time 0).

   Data Collector – Records the observations made by each observer on *Student Data Sheet #1: Blackworm Observations*.

8. Transfer the worms and start timing immediately.

Observe and record behavior at time 0, 3, 6, and 10 minutes. wimming behavior/reaction categories:

Activity Rating: 0 – 4 (where 0 = no activity, 2 = normal activity level, and 4 = high activity level)

Description: Clumped, Not Clumped, Other (e.g. bleeding, bulging, breaking into segments, etc.)

The worms can be "probed" with the plastic transfer pipette or spoon. 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, the group will separate. When the worms are exposed to alcohol, they will be less likely to clump and become rather inactive as the concentrations increase. In the second highest

concentration, they may straighten out in the middle but have their ends curled. The worms may need to be probed several times to stimulate a response. In the highest concentration, the worms may actually begin to bleed.

When the worms are reintroduced to clean, distilled water, they should begin to recover within 10 minutes after exposure to the two lowest concentration 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. Death cannot be confirmed until the 24-hour observation.

(Note→ 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" carefully with the probes made, never use a sharp probe or use too much force.

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.

9. After 10 minutes, gently remove your worms from each weigh boat and place in back into the dishes labeled WControl, Water1, Water 2, and Water 3. This begins the recovery time for the worms.

10. Observe the worms at 3, 6, and 10 minutes on all four weigh boats**,** including the control. **Record the recovery levels of the worms on *Student Data Sheet #2**: Recovery Observations.***

12. **Leave the worms in their weigh boats/containers overnight** and observe again the next day. Be sure to record the number of deaths that might have occurred.

Data Compilation and Discussion

11. When you are finished, groups may share their data and calculate class averages using *Student Data Sheet #4: Compiled Data*. Collect group data only from groups that tested the same variable treatment- alcohol, caffeine, or nicotine.

12. You may then create a bar graph using *Student Data Sheet #5: Bar Graph*.

13. Based on the class results, discuss and answer the questions *on Student Questions: Toxicity Testing with California Blackworms*.

15. Clean up your lab materials.

Updated June, 2005

Toxicity Testing with California Blackworms and Alcohol

Student Data Sheet #1: Blackworm Observations

Timer: _____Data collector: _____
Worm movers:_____Observers: _____
Directions: Record an activity rating at each time interval (see rating scale below). Record your observations (i.e. clumped, not clumped, bleeding, etc.).

Activity Rating: **No Activity** **Normal** **Very Active**
0 1 2 3 4

Exposure variable_____ Fill this in!!!

Swimming Behavior	**Control**	**Low Concentration**	**Medium Concentration**	**High Concentration**
Activity Rating (circle a number)	0 min 0 1 2 3 4	0 min 0 1 2 3 4	0 min 0 1 2 3 4	0 min 0 1 2 3 4
	Notes:	Notes:	Notes:	Notes:
	3 min 0 1 2 3 4	3 min 0 1 2 3 4	3 min 0 1 2 3 4	3 min 0 1 2 3 4
	Notes:	Notes:	Notes:	Notes:
	6 min 0 1 2 3 4	6 min 0 1 2 3 4	6 min 0 1 2 3 4	6 min 0 1 2 3 4
	Notes:	Notes:	Notes:	Notes:
	10 min 0 1 2 3 4	10 min 0 1 2 3 4	10 min 0 1 2 3 4	10 min 0 1 2 3 4
	Notes:	Notes:	Notes:	Notes:

Updated June, 2005

Toxicity Testing with California Blackworms→Student Data Sheet #2: Recovery Observations

Directions: Place the appropriate letter that applies to the majority of the worms at each time period.

Recovery Exposure variable_____

Swimming Behavior	**Control**		**Low Concentration**		**Medium Concentration**		**High Concentration**	
R= completely recovered	3 min		3 min		3 min		3 min	
P= partially recovered	6 min		6 min		6 min		6 min	
N= not recovered	10 min		10 min		10 min		10 min	
D= dead (mark only after 24 hrs)	24 hrs		24 hrs		24 hrs		24 hrs	

Toxicity Testing with California Blackworms

Student Data Sheet #5: Bar Graph *Directions*: Color in the squares up to the average activity rating.

EXPOSURE BAR GRAPH

ACTIVITY RATING

4																
3																
2																
1																
0																
	0 3 6 10 Control				0 3 6 10 Low				0 3 6 10 Medium				0 3 6 10 High			

TIME (minutes)

Updated June, 2005

Toxicity Testing with California Blackworms and Alcohol/Student Data Sheet #4: Compiled Data

Directions: Record the activity rating observed by each group at each time interval, then calculate the averages.

EXPOSURE **Collect group data from the other groups that test the same variable as your group!**

Exposure (variable) treatment_____ **Use activity values 0 to 4 from above!**

	Time	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Average
Control	0 min							
	3 min							
	6 min							
	10 min							
Low	0 min							
	3 min							
	6 min							
	10 min							
Medium	0 min							
	3 min							
	6 min							
	10 min							
High	0 min							
	3 min							
	6 min							
	10 min							

Updated June, 2005

Name(s)_____

Date_____

Student Questions: Toxicity Testing with California Blackworms using Alcohol

1. 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.

2. 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 life span) exposure with a lower concentration than the acute test.

A. Based on this information, which type of test was done in this investigation?

B. What would be the benefit of using an acute toxicity test?

C. What would be the benefit of using a chronic toxicity test?

3. 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.

4. The exposure pathway is how a toxicant enters the body. What was the exposure pathway for your toxicant?

5. 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.

A. Age

B. Genetic difference

C. Body size

6. Explain what is meant by sublethal and lethal concentrations of the toxicant.

7. 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?

8. 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.

9. 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?

10. 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?

11. 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 toxicant you tested applies to human health. Name some toxicants you might test that would harm the environment and thus pose a threat to the worms?

12. How do lifestyles play a part in risk assessment of human health toxicants?

13. Can the results of your tests be applied to humans or other vertebrates? Why or why not?

14. 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 in the space below.