Toxicity Testing with California Blackworms: Alcohol

This activity is being used with permission from the Center for Chemical Education at Miami University and has been modified from the "Toxicants and California Blackworms" activity developed by the Center for Chemical Education at Miami University (original activity is included with these materials). Contact at cce@muohio.edu.

Modifications were developed at the University of Arizona Southwest Environmental Health Sciences Center Community Outreach and Education Program.

TEACHER INFORMATION

Materials – for a class size of 30 working in 6 groups of 5-6 people each

½ ounce - Blackworms (can be purchased for ~ $1 at a tropical fish store)
42 large weigh boats, petri dishes, or similar container that will hold ~ 50 ml of liquid
1 gallon distilled water
7 - 500 ml beakers
6 – 50 ml graduated cylinders
24 – plastic transfer pipettes
100 ml Vodka (1 bottle can be used for 7 classes)
60 labels
6 timers
6 Student data sheets
24 plastic spoons

Teacher Preparation

1. Stock Preparation – 100 ml vodka (40%) + 300 ml water = 400 ml (80 mg/ml) (10% alcohol)

2. Dilutions – will be prepared by the students. You may prepare the dilutions if you want to save time. There are two dilutions made from the stock solution – 1:3 and 1:39

Overview

In this investigation, participants will work in groups to determine how various concentrations of alcohol (ethanol) 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 present their findings to the class.

Learning Objectives

Upon completion of the activity participants will be able to:

1. Explain the concepts "the dose makes the poison" and dose/response
2. Differentiate between exposure and dose and explain factors that can affect dose
3. Identify the routes of exposure

4. Differentiate between acute and chronic toxicity
5. Identify factors that can influence the effect of a chemical on living things

Narrative

In this investigation, participants will work in collaborative groups of 5 or 6 to first observe California Blackworms and establish what they consider normal behavior. This becomes the baseline measurement used for comparison when toxicants, such as alcohol, are tested. Several concentrations of the toxicant will be used and the participants will mark 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, 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.

Alcohol (ethanol) is the toxicant selected for use in this activity because the worms' responses are clearly different between the doses. However, if the maturity level of the class is such that the alcohol may be abused or mishandled, you may select a different toxicant. Please note that ethanol is drinking alcohol, not rubbing alcohol.

Procedures and Results

<u>Student Preparation</u>

The students will need to label 8 weigh boat dishes with "Control", Low, Medium, High, "Wcontrol", Water1, Water2, and Water3. They will also need to get a beaker full of distilled water and another beaker with the stock alcohol solution. The students will fill the "Control", "WControl", Water1, Water2, and Water3 dishes with distilled water. They will also make the dilutions and place them in the appropriate dish, low, medium, or high concentration. Have the students get at least 40 - 50 worms per working group and place them in the beaker containing distilled water.

<u>Student Observations</u>

Have the students place approximately 10 worms in the "water" weigh boats containing distilled water ("WControl," Water1, Water2, and Water3). Allow the participants approximately a few minutes to observe the worms' normal swimming behavior, with and without probing, and to familiarize themselves with the behavior categories listed on the Student Data Sheets. After the students become familiar with the normal swimming behavior, they will decide on individual roles (timer, data collector, worm mover, or one of the observers). They will then move the worms into the alcohol solutions. Please note that the worms will be moved from the

"WControl" weigh boat to the "Control" weigh boat to account for the changes in behavior from simply moving the worms.

The students will observe and mark the swimming behaviors of the worms exposed to the alcohol at 0, 3, 6, and 10 minutes. The students will then remove the worms from the alcohol solutions and the Control and place the worms in the corresponding dishes containing only distilled water (WControl, Water1, Water2, and Water3) for recovery. Again, they will observe the swimming behaviors of the worms at 0, 3, 6, and 10 minutes. Another recovery observation will occur in 24 hours. The students will discuss their individual group results with the class, and then the class should compile all of their results.

Worm Swimming Behavior

Swimming behavior/reaction categories:

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

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.

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

Data Compilation & Discussion

After the students have collected their data, it can be compiled on the page titled "Compiled Data Sheet." You may consider making an overhead of this sheet, that way all of the students can see the results. The average activity level for each time period and alcohol concentration will need to be calculated. Determining the average may be a whole-class exercise or assigned to the groups.

Once the averages are calculated, you can chart the results on the page titled "Compiled Data Chart." Again, you may want to make an overhead of this sheet and chart the results in different colors of ink.

Below are some discussion questions and answer guidelines. The students have these questions (without the answers) at the end of the "Student Guidelines."

Discussion Questions

(You can refer to the section on Basic Toxicology for more information).

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.

Exposure frequency: Once
Exposure duration: Ten minutes
Exposure concentration: Concentration low = 2 mg alcohol/ml solution
Concentration medium = 20 mg/ml
Concentration high = 80 mg/ml

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? *Acute toxicity*
B. What would be the benefit of using an acute toxicity test? *It saves time, which makes it easier to conduct an experiment; You can see immediate responses to the toxicant; One can learn the single dose limit of tolerance to a toxin.*
C. What would be the benefit of using a chronic toxicity test? *One can learn the cumulative effects of the poison; It more closely approximates normal exposures to toxicants.*

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.

You 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 toxin, such as alcohol, entry would be through the mouth. Ingestion of a toxin by these worms would probably have to be associated with food. You can demonstrate this to the students by removing the head and the tail of the worms and show that the toxicant still has a dramatic systemic affect.

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 – *The old or the very young are more likely to be adversely affected*
   B. Genetic difference – *Genetic difference can increase or decrease the tolerance to a toxicant. Typically genetics governs physiological responses to the toxicant. For example, some people have a higher tolerance to alcohol because they can process and excrete the alcohol more quickly. Note that increased metabolism can either increase or decrease toxicity depending on the toxicant. If the metabolic processes break a chemical down into less toxic chemicals, then increased metabolism will likely decrease toxic effects. However, if metabolic processes bio-activate a chemical, then increased metabolism will likely increase toxic effects.*
   C. Body size – *Typically the larger the body size the less response to a toxicant. This is because the dose is smaller compared to someone who weighs less but ingested the same amount of toxicant. To calculate someone's dose, divide the amount of the hazard by the body weight. For example, the adult woman weighs 125 pounds and took 300 mg of aspirin. Her dose is 300 mg divided by 125 pounds or 2.4 mg/lb. It is important to express dose in terms of body weight because a small person who ingests the same amount of a chemical as a larger person actually receives a much higher dose. The concentration of the chemical in the small body is much higher than in the large body.*

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 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? *There are a number ways the worms can get rid of toxins in their bodies.*

   A. *Toxins that pass through the body wall and are in the worm's coelomic fluid could be dumped by some kidney-like organs (called paired nephridia) located in each segment.*
   B. *Some types of compounds could be broken down by enzymes in the worm's body (such as cytochrome P-450 – mixed function oxidases).*
   C. *Some compounds, such as heavy metals, may be bound up by special binding proteins in the body.*
   D. *Many compounds, like nicotine and caffeine, simply "wash out" of the body by diffusion once the external concentration is reduced.*
   E. *Some toxins may reside in the body for a long time or be eliminated slowly by the digestive track.*

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.
   Since the students only looked at the worms for a 24 hour period, they have to make an assumption that if complete recovery has not occurred within the given time period then the effects are irreversible. Given this assumption, the effects of the highest alcohol dose are

irreversible. At the two lower doses, the effects are reversible. You may want to point out that some damage from toxicants may be reversible over a long period of time.

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? *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.*

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

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? *Chlorine, ammonia, other forms of alcohol that are commonly released into the environment like antifreeze, etc.*

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

Lifestyles affect individual's health, metabolism, and limits of exposure to toxins. For example, risk increases if a person is in poor health. Limits of exposure to toxins can be affected by actions such as 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 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) then the dose of the toxicants could be insignificant.

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

Not directly. Effects can be extrapolated from an experimental system to another system if and only if the two types of systems can be shown to be sufficiently similar in relevant characteristics and behaviors.

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.

Basic summary should include terms like dose/response, exposure, acute toxicity, and chronic toxicity. It should also discuss concepts like "the dose makes the poison," or individual variation. They may also address factors that affect toxicity or risk.

Additional Toxicants

Students may want to observe the effects of other toxicants, such as nicotine and caffeine, on the worms. Such an extension can be used to demonstrate that different chemicals affect us in different ways and differ in their relative toxicity.

Below are instructions for how to prepare solutions containing nicotine and caffeine, as well as, brief descriptions of the effects of those toxicants on California Blackworms.

<u>Caffeine</u>: 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 Low: 16 ml stock solution + 184 ml water = 0.08 mg/ml
Solution Medium: 66 ml solution #1 + 134 ml water = 0.33 mg/ml
Solution High: 200 ml stock solution = 1 mg/ml

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. 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 but all should fully recover in 24 hours.

<u>Nicotine</u>: 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 2 cigarettes (1.1 mg nicotine/cigarette) in 500 ml 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 (0.0044 mg/ml)
Solution Low: 10 ml stock solution + 190 ml water = 0.00023 mg/ml
Solution Medium: 50 ml stock solution + 150 ml water = 0.00147 mg/ml
Solution High: 200 ml stock solution = 0.0044 mg/ml

With nicotine, the worms may twitch in the solution. The tail may curl with loss of response. In the high concentration, paralysis or death 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 but most all of the worms should recover in 24 hours.

Basic Toxicology

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

<u>Exposure vs. Dose</u>

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 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 their lungs. If they are breathing quickly, they will breathe in more of the gas than if they are breathing slowly. So 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 toxin - 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)

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 LD_{50}. The LD_{50} 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 LD_{50} and the approximate human LD_{50} for some common toxicants.

TABLE 1. LD_{50} for various toxicants administered to rats

Chemical	Rat LD_{50} (milligrams/kilogram)	Approximate Human LD_{50} (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

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

Alcohol Toxicity

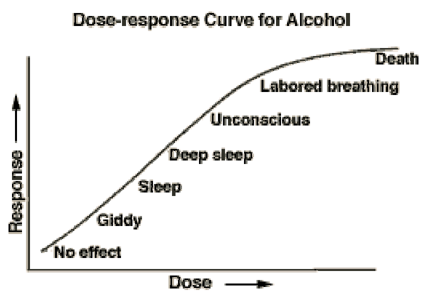
Acute Dose-Response for **ETHANOL** (the alcohol in beverages):

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, it would require approximately 3oz of pure alcohol to 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.



Special Note

This version has been modified from the original version developed by the Center for Chemical Education. The original version demonstrates how to measure the pulse rate of the worms and includes crawling as another means of measurement. Attached is the original activity for your reference.

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

"Chemicals & Human Health Website", Southwest Environmental Health Sciences Center & the Biology Project, the University of Arizona. http://www.biology.arizona.edu/chh/

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.

The Institute for Environmental Toxicology. "Toxicology for the Citizen" University Publications, 1991. Also available on the web at http://www.iet.msu.edu/toxconcepts/toxconcepts.htm

Snyder, Robert and Larry S. Andrews. 1996. "Toxic Affects of Solvents and Vapors." Casarett and Doull's Toxicology: The Basic Science of Poisons. 5th edition. Curtis Kassen, Ed. McGraw Hill.

STUDENT GUIDELINES: Alcohol & California Blackworms Activity

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 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 (per group of students)

40 blackworms
pipettes or eye droppers
8 weigh boats or petri dishes
1 - 500 ml beaker
1 – 100 ml beaker
distilled water
alcohol solution
magnifying lens (if available)
marking pen
10 labels
50 ml graduated cylinder
plastic spoons

Procedure (You will work in groups of 5 or 6)

<u>Preparation</u>

1. Obtain 8 weigh boats, 8 labels, and a marking pen. Label each container as follows: Control, W Control, low, medium, high, Water1, Water2, and Water3.

2. Fill the 500 ml beaker with distilled water and label "distilled". Fill the weigh boats labeled Control, W Control, Water1, Water2, and Water3 with approximately 50 ml distilled water. The distilled water will also be used to make your dilutions. (Do not use tap water because the chlorine will negatively affect the worms).

3. Fill the 100 ml beaker just over ½ full with the alcohol solution and label "alcohol".

4. Arrange your weigh boats as follows:

Distilled Only	Alcohol
Water 3	High
Water 2	Med
Water 1	Low
W Control	Control

5. Using the 50 ml graduated cylinder and pipettes make your alcohol dilutions as follows:

    6a. Solution #1 – Low concentration- Using a pipette to transfer the liquid, place the following amounts of alcohol and distilled water into a graduated cylinder. Suggestion: Fill the graduated cylinder with the water first (i.e. to the 39 ml mark), then add the alcohol (adding 1 ml will make the total solution 40 ml). Dilution ratio: **1 ml stock:39 ml distilled water**. Pour the mixture into the weigh boat labeled "Low". Rinse the graduated cylinder and pipette with distilled water.

    6b. Solution #2 – Medium concentration – Using a pipette to transfer the liquid, place the following amounts of alcohol and distilled water into a graduated cylinder . Suggestion: Fill the graduated cylinder with the water first (i.e. to the 30 ml mark), then add the alcohol (adding 10 ml will make the total solution 40 ml). Dilution ratio: **10 ml stock:30 ml distilled water**. Pour the mixture into the weigh boat labeled "medium". Rinse the graduated cylinder and pipette with distilled water.

    6c. Solution #3 – High concentration – Pour about 40 ml of the stock alcohol mixture into the weigh boat labeled "High".

6. Take the 500 ml beaker and partially fill ¼ – ½ full with distilled water. You may use any left over distilled water already in the beaker. Using a pipette, spoon, or your probe, transfer at least 50 worms to your beaker.

<u>Observation</u>

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

8. Observe their behavior for a few minutes. Familiarize yourself with the behaviors listed on your data sheets, including the activity level ratings. You may gently probe the worms and observe their response.

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

<u>Timer</u> (1 person)– Will let the group know when 0, 3, 6, and 10 minutes has passed and will record the start time for each concentration on the Time Log Sheet.

<u>Worm Movers</u> (4 people) – There will be one worm mover per set of containers and you will move the worms to and from the containers with and without alcohol (i.e. WControl to Control; Water1 to Low; Water2 to Medium; and Water3 to High). **Worm Moving Tip: Allow the worm 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 container (i.e. you don't want to introduce alcohol to the distilled water-only containers or dilute the alcohol solutions).

<u>Data Collector</u> – Records the observations made by each observer on the Student Data Sheet.

<u>Observers</u> (4 people – one for each concentration level) - Will observe the behavior of the worms once they are moved from one container 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).

10. Transfer the worms and start the clock immediately.

11. Observe and record behavior at time 0, 3, 6, and 10 minutes.

12. After 10 minutes, gently remove your worms from each dish and place in back into the dishes labeled W Control, Water1, Water 2, and Water 3. This begins the recovery time for the worms.

13. Observe the worms at 3, 6, and 10 minutes on all four dishes**,** including the control. Record the recovery levels of the worms.

14. Leave the worms in their chambers overnight and observe again the next day. Be sure to record the number of deaths that might have occurred.

Data Evaluation

1. When you are finished, the groups will share their data as a class.
2. Provide your data to the teacher, who may assign groups to calculate the averages.
3. Once the averages have been calculated, the class may chart the results as a whole.
4. Based on the class results, discuss and answer the following questions.

Alcohol & California Blackworms Questions, pg. 1

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 lifespan) 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. Your concentrations represent sublethal concentrations of the toxicant. Explain what you think this means.

Alcohol & California Blackworms Questions, pg. 2

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.

Student Data Sheet

Timer: ________________________
Data Collector: ____________________
Worm Movers: ____
Observers: ____

Directions: Give an activity rating at each time interval (see rating scale below). Place a check mark next to "clumped" or "not clumped" to indicate the behavior of the majority of worms. Record any additional observations under "other."

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

EXPOSURE

Swimming Behavior	Control	Alcohol Low	Alcohol Medium	Alcohol High
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
	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
	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
	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 – clumped, not clumped, bleeding, curled, straight, etc.	0 min	0 min	0 min	0 min
	3 min	3 min	3 min	3 min
	6 min	6 min	6 min	6 min
	10 min	10 min	10 min	10 min

Student Data Sheet, pg. 2

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

RECOVERY

Swimming Behavior	Control		Alcohol Low		Alcohol Medium		Alcohol High	
R = Completely Recovered P = Partially Recovered N = Not Recovered D = Dead (mark only after 24 hrs)	3 min		3 min		3 min		3 min	
	6 min		6 min		6 min		6 min	
	10 min		10 min		10 min		10 min	
	24 hrs		24 hrs		24 hrs		24 hrs	

TIME LOG SHEET

Timer: ______________________________

Directions: The timer needs to record the start times for the worm transfers, then calculate what time observations will need to be recorded on the data sheet. (Times should be the same for all levels if the worm transfers occurred simultaneously).

EXPOSURE TIME SHEET

From Container ⇒ To Container	Time at 0 min (start time)	Time at 3 minutes	Time at 6 minutes	Time at 10 minutes
From Container ⇒ To Container				

RECOVERY TIME SHEET

From Container ⇒ To Container	Time at 0 min	Time at 3 minutes	Time at 6 minutes	Time at 10 minutes
From Container ⇒ To Container				

COMPILED DATA SHEET

Directions: Record the activity level observed by each group at each time interval, then average and graph.

EXPOSURE

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

COMPILED DATA CHART

Directions: Color in the squares up to the average activity level.

Exposure Chart

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

TIME (minutes)