Toxicity Testing with California Blackworms: Chlorine

This activity is based on "Toxicants and California Blackworms" originally developed by the Center for Chemical Education at Miami University. Contact at cce@muohio.edu. Modifications were developed at the University of Arizona Southwest Environmental Health Sciences Center Community Outreach and Education Program. Contact at coep-info@pharmacy.arizona.edu.

TEACHER INFORMATION

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

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

Teacher Preparation

1. Stock Preparation – Bleach

Try to avoid bleaches containing other additives. The ingredients should be water and sodium hypochlorite (~5.25%).

Stock solution: 1:100 dilution (put 4 ml bleach in beaker and fill to 400 ml with distilled water)

2. Serial dilutions – Can be prepared by the students. You may prepare the dilutions if you want to save time (it is recommended the students at least make the final "drinking water" dilution). The dilutions are 1:100, 1:1000, 1:10,000, and 1:1,000,000.

If you prepare the dilutions ahead of time (there is enough to give each team's weigh boat ~ 50 ml of solution)

Solution High (1:100): stock solution = 5% bleach = 0.5 parts per thousand (ppt)
Solution Medium (1:1000): 30 ml stock solution + 270 ml distilled water = 0.05 ppt
Solution Low (1:10,000): 3 ml stock solution + 297 ml distilled water = 5 parts per million (ppm)
Drinking water levels: 30 ml Low Solution + 270 ml distilled water = 0.5 ppm

DO NOT USE TAP WATER

Overview

In this investigation, participants will work in groups to determine how various concentrations of chlorine bleach affect California Blackworms (*Lumbriculus variegatus*). This project represents an introduction to toxicology, which is an important component in environmental health science. The participants will discuss and analyze their data, and attempt to reach a conclusion about the relative safety of Chlorine levels in drinking water supply.

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 acute and chronic toxicity
3. Make educated decisions about relative risk

Procedures and Results

Background Information

It is recommended that some background information be provided about water borne diseases prior to this activity. This will give students a framework within which to assess risk. SEPUP's "Understanding Environmental Health Risks" activities on Drinking Water Safety complement this activity very well. Lab-Aids, Inc. ISBN 1-887725-05-9.

Student Preparation

1. The students will need to label 9 weigh boat dishes with: Control, Low, Medium, High, Wcontrol, Water1, Water2, Water3, and drinking water.

2. They will need to get a beaker full of distilled water and another beaker with the stock bleach solution and fill the "Control", "WControl", Water1, Water2, and Water3 dishes with distilled water.

3. They will also make the dilutions and place them in the appropriate dish: low, medium, high or drinking water concentration.

4. Students will calculate the concentration of chlorine in each of their samples (refer to student sheet "Chlorine Concentration Calculations"). Be sure to take this opportunity to emphasize the concentration levels of the drinking water dilution, how small of an amount it is.

5. Have the students get at least 40 - 50 worms per working group and place them in the beaker containing distilled water.

Student Observations

1. Have the students place approximately 10-20 worms in the "Water" weigh boats containing distilled water ("WControl," Water1, Water2, and Water3).

2. Allow the participants 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.

3. After the students become familiar with the normal swimming behavior, they will decide on individual roles (timer, data collector, worm movers, or one of the observers).

4. They will then move the worms into the bleach solutions. Please note that the worms will also be moved from the "WControl" weigh boat to the "Control" weigh boat to account for the changes in behavior from simply moving the worms.

5. The students will observe and mark the swimming behaviors of the worms exposed to the bleach at 0, 3, and 6 minutes.

6. The students will then remove the worms from the bleach solutions and the Control and place the worms in the corresponding dishes containing only distilled water (WControl, Water1, Water2, and Water3) for recovery. The "drinking water" worms will not be moved.

7. Again, they will observe the swimming behaviors of the worms at 0, 3, and 6 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.

<u>Worm Swimming Behavior</u>

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 should be "probed" gently with the plastic transfer pipette. 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 the high, medium, and low concentrations of bleach, they will initially become more active, won't clump, will eventually stop moving, and bleed. Bleeding is because bleach is corrosive. In the drinking water solution the worms will be more active but remain in the "normal" clumping state.

In the other blackworm experiments (e.g. alcohol), the worms go through a recovery period to demonstrate that the dose-response concept applies to recovery as well. Unfortunately, in this experiment, the worms in the low, medium, and high doses do not recover (in the drinking water level the worms are OK).

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 bleach 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. (Get concentration information from your "Chlorine Concentrations Calculations" worksheet.) Using this terminology, describe each for your investigation.

Exposure frequency: Once
Exposure duration: Six minutes
Exposure concentration: Drinking water levels = 0.5 ppm
Concentration Low = 5 ppm
Concentration Medium = 0.05 ppt
Concentration High = 0.5 parts per thousand (ppt)

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? *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 bleach 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. 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. 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.*

5. 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.*

6. 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.*

7. 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.

8. Chlorine (bleach) is corrosive. This means that it "eats away" or "dissolves" cells and tissues. Based on what you saw in your experiment, why might chlorine (bleach) be a good disinfectant?

   Pathogens are very small and it would not take much chlorine to corrode their cells or structure (in the case of viruses) to the point where they die or become non-functional. Chlorine works via a physical reaction, compared with antibacterial soaps which work via a biological reaction. Biological reactions allow pathogens to mutate and become resistant more easily.

9. 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). Based on your results of the different exposure levels of chlorine to the worms, what would your recommendations be about levels of chlorine to use in drinking water?

Answers will vary. Make sure answers are logical and statements are backed up by data from this experiment.

10. How would you design an experiment to determine safe drinking water levels of chlorine? What things might you consider?

Answers will vary. Make sure answers are logical and the experimental design will address the question they want to answer.

Additional Toxicants

Students may want to observe the effects of other toxicants, such as nicotine, caffeine, and alcohol 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, caffeine, and alcohol as well as, brief descriptions of the effects of those toxicants on California Blackworms.

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

Stock solution: Stir the tobacco from 6 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 (0.0132 mg/ml)
Solution Low: 30 ml stock solution + 270 ml water = 0.00132 mg/ml
Solution Medium: 100 ml stock solution + 200 ml water = 0.0044 mg/ml
Solution High: stock solution = 0.0132 mg/ml

With nicotine, the worms may twitch in the solution and not clump. 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.

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 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.

Alcohol:
Stock solution: 100 ml vodka (40%) + 300 ml water = 400 ml (80 mg/ml) (10% alcohol)
There are two dilutions made from the stock solution – 1:3 and 1:39

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. In the highest alcohol concentration at Time 0 (upon immediate immersion) they will become extremely hyper then quickly reduce their movements to a level 1 or 0. The worms may need to be probed several times to stimulate a response.

BASIC TOXOCOLOGY

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.

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)

<u>Acute vs. Chronic Toxicity</u>

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
Bleach (sodium hypochlorite 12.5%)	1200 oral	
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. & Chemical Desk MSDS http://watertreatment.chemicaldesk.com/msds/74_2msds.html ; http://chem-courses.ucsd.edu/CoursePages/Uglabs/MSDS/sodium.hypochlorite.-info.html

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.

The idea of using *Lumbriculus variegatus* came from Dr. Charles Drewes, who is a professor of Zoology at Iowa State University (Ames, Iowa). 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

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STUDENT GUIDELINES: Chlorine & California Blackworms

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 chlorine through a controlled experiment. At the end of the investigation, you will analyze your data, present your findings to the class, and present a conclusion on the relative risk of water chlorination.

Materials (per group of students)

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

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

Preparation

1. Obtain 9 weigh boats, 9 labels, and a marking pen. Label each container as follows: Control, W Control, low, medium, high, Water1, Water2, Water3, Drinking Water.

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 it will add chlorine to the experiment).

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

4. Arrange your weigh boats as follows:

Distilled Only	Chlorine
Water3	High
Water2	Med
Water1	Low
W Control	Control
	Drinking Water

<u>Dilutions</u>

5. Using the 100 ml graduated cylinder and pipettes make your bleach dilutions as follows:

Medium concentration – Using a pipette to transfer the liquid, place the following amounts of distilled water and bleach stock solution into a graduated cylinder .

<u>Suggestion</u>: Fill the graduated cylinder with the water first then add the bleach stock

<u>Dilution</u>: **10 ml stock:90 ml distilled water (total of 100 parts)**. Pour some of the mixture into the weigh boat labeled "medium". Rinse the graduated cylinder and pipette with distilled water.

Low concentration- Using a pipette to transfer the liquid, place the following amounts of distilled water and bleach stock solution into a graduated cylinder.

<u>Suggestion</u>: Fill the graduated cylinder with the water first, then add the bleach stock solution

<u>Dilution:</u> **1 ml stock:99 ml distilled water (total of 100 parts)**. Pour some of the mixture into the weigh boat labeled "Low". SAVE 10 ML OF THIS SOLUTION FOR YOUR NEXT DILUTION!

Drinking Water concentration – Fill the graduated cylinder with the distilled water, then add the bleach stock solution. DO NOT USE TAP WATER.

Dilution: **10 ml Low Solution: 90 ml distilled water**

6. Fill out the "Dilution Worksheet."

Observation

7. 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 or spoon transfer at least 50 worms to your beaker (a medium sized clump).

8. Using a pipette, spoon, or your probe, 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.

9. Observe their behavior for a few minutes. Familiarize yourself with the behaviors listed on your "Data Sheets", including the activity level ratings.

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

Timer (1 person)– Will let the group know when 0, 3, 6, and 10 minutes has passed .

Worm Movers (1-4 people) – There can be one worm mover per set of containers and you will move the worms to and from the containers with and without bleach (i.e. WControl to Control; Water1 to Low; Water2 to Medium; and Water3 to High). **Worm Moving Tip: Let the worms clump first,** then scoop up as many worms as possible with a spoon. Try not to transfer the liquid into the new container (i.e. you don't want to introduce nicotine to the distilled water-only containers or dilute the nicotine solutions).

Data Collector – Records the observations made by each observer on the Student Data Sheet.

Observers (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).

11. Transfer the worms and start the clock immediately. TRANSFER THE WORMS AS CLOSE TO THE SAME TIME AS POSSIBLE.

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

13. After 6 minutes, gently remove your worms from each dish and place in back into the dishes labeled W Control, Water1, Water2, and Water3. This begins the recovery time for the worms.

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

15. 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 Question Sheet.

Bleach Concentration Calculations

1. The highest bleach solution was made by mixing 10 ml of bleach with 90 ml of water, which is a 1:100 dilution. What does 1:100 mean? Describe in words and show mathematically.

2. Bleach is composed of 95% water and 5% sodium hypochlorite. Calculate the concentration of chlorine (sodium hypochlorite) for all of the solutions you will use. Use the units parts per thousand (ppt) and/or parts per million (ppm) in your answer. Show your work.

    a. High concentration 1:100

    b. Medium concentration 1:1,000

    c. Low concentration 1:10,000

    d. Drinking water 1:1,000,000

Chlorine & 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 (Get concentration information from your "Chlorine Concentrations Calculations" worksheet.). 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. 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

Bleach & California Blackworms Questions, pg. 2

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

6. 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?

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

8. Chlorine (bleach) is corrosive. This means that it "eats away" or "dissolves" cells and tissues. Based on what you saw in your experiment, why might chlorine (bleach) be a good disinfectant?

9. 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). Based on your results of the different exposure levels of chlorine to the worms, what would your recommendations be about levels of chlorine to use in drinking water?

10. How would you design an experiment to determine safe drinking water levels of chlorine? What things might you consider?

Student Data Sheet

Timer: ______________________
Data Collector: __________________
Worm Movers: ____
Observers: ____

Directions: Circle an activity rating at each time interval (see rating scale below). Record any additional observations under "other."

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

EXPOSURE

Swimming Behavior	Control	Drinking Water Level	Bleach Low	Bleach Medium	Bleach 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	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	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	6 min 0 1 2 3 4
NOTES					
Notes – clumped, not clumped, bleeding, curled, straight, etc.	0 min	0 min	0 min	0 min	0 min
	3 min	3 min	3 min	3 min	3 min
	6 min	6 min	6 min	6 min	6 min

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							
Drinking Water	0 min							
	3 min							
	6 min							
Solution Low	0 min							
	3 min							
	6 min							
Solution Medium	0 min							
	3 min							
	6 min							
Solution High	0 min							
	3 min							
	6 min							

COMPILED DATA CHART

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

Exposure Chart

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

TIME (minutes)