

## LESSON:

# Chlorine, Asthma, and... Blackworms?

**Summary:** Students read an article about research showing increased incidence of asthma with time spent at pools. Then students conduct an experiment exposing blackworms to chlorine and make connections between their experimental results and the hypotheses introduced in the article.

**Lesson Type:** Experiment—Students collect, manipulate, and summarize data from an experiment or activity they conduct.

**EHP Article:** “Swimming in Allergens? Pool Use and Asthma”  
*EHP Student Edition*, January 2007, p. A600  
<http://www.ehponline.org/docs/2006/114-10/ss.html>

**Objectives:** By the end of this lesson, students should be able to

1. identify the elements Cl, O, and Na by name and abbreviation;
2. identify the atomic mass/weight for Cl, O, and Na;
3. calculate the molecular mass/weight for sodium hypochlorite (NaClO; bleach);
4. conduct an experiment, and summarize the results in a clear, accurate manner;
5. generate a hypothesis based upon data; and
6. compare their experimental results with results from an epidemiological study described in the article.

**Class Time:** 2.5–3.5 hours

**Grade Level:** 9–12

**Subjects Addressed:** Chemistry, Physical Science, Biology, General Science, Environmental Science, Mathematics

### ► Prepping the Lesson (40–50 minutes)

#### INSTRUCTIONS:

1. Download the entire January 2007 *EHP Student Edition* at <http://www.ehponline.org/science-ed/>, or download just the article “Swimming in Allergens? Pool Use and Asthma” at <http://www.ehponline.org/docs/2006/114-10/ss.html>.
2. Review the Background Information, Instructions, and Student Instructions.
3. Make copies of the Student Instructions and Student Data Sheet.
4. Prepare the bleach stock.
  - Try to avoid bleaches containing other additives. The ingredients on the bleach bottle should be water and sodium hypochlorite (~5.25%).
  - Stock solution (high concentration) = 1:100 dilution (put 4 mL bleach in beaker and fill to 400 mL with distilled water).
  - The stock solution should remain covered, or the chlorine may volatilize out of solution. You may want to divide the stock solution into several large beakers to allow groups easy access when needed.

#### MATERIALS (per student):

- 1 copy of the January 2007 *EHP Student Edition*, or 1 copy of “Swimming in Allergens? Pool Use and Asthma”
- 1 copy of the Student Instructions
- Periodic table
- Goggles
- Gloves
- Apron



**MATERIALS (per class, for a class of 30 working in 6 groups of 5 people each):**

- 1/2 oz blackworms (can be purchased for about \$2 at a tropical fish store)
- 54 large weigh boats, petri dishes, or similar containers that will hold ~50 mL of liquid
- 30 plastic transfer pipettes
- 1 gal distilled water
- 6 500-mL beakers
- 6 100-mL graduated cylinders
- 1 bottle of bleach (sodium hypochlorite)
- 66 labels
- 6 timers
- 6 Student Data Sheets
- 30 plastic spoons
- 6 or more calculators (at least 1 per group)
- 6 magnifying lenses (1 per group)

**VOCABULARY:**

- allergen
- atomic mass/weight
- atopic asthma
- bronchoconstriction test
- chlorine
- chronic
- control
- exposure
- IgE
- inflammation
- molecular mass/weight
- sodium hypochlorite (NaClO)
- trichloramine (NCl<sub>3</sub>)

**BACKGROUND INFORMATION:**

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 activity also helps students make connections between a “laboratory” investigation and “real life.” Students will discuss and analyze their data, and attempt to reach a conclusion about the potential relationship between chlorine exposure from pools and asthma.

Blackworms are an aquatic worm used as fish food and can be purchased at many pet stores that sell fish and aquarium supplies. You may want to call stores and check for availability. It is important to note that most of the worms exposed to the high chlorine concentration will not survive, and some may also die in the “pool” concentration. Some students may be sensitive about this aspect of the experiment, and it is important to honor their beliefs/concerns. These students could be assigned alternative tasks, such as making the stock solutions or the dilutions. They could also focus their observations only on the lower exposure levels, especially the lowest “air-equivalent” concentration.

Another approach for sensitive students is to ask them to write a description of what it feels like when bleach gets on their skin (based on previous experience, rather than putting bleach on their skin) and what happens to their skin after being exposed to bleach or swimming in a pool. They could conduct independent research (with references) on bleach, corrosivity, and cell structure, and describe how bleach disintegrates the cell membrane, which is why our skin feels slimy when we get bleach on it. After the exposure to bleach or chlorine (including in pools), the skin feels dry and flaky from the dead skin layer.

Trichloramine (NCl<sub>3</sub>) is a chlorine-based gas that is produced when chlorine used to disinfect water reacts chemically with organic matter (such as skin and urine in pools). Like chlorine, NCl<sub>3</sub> is corrosive and acts as a strong irritant, especially for the eyes and upper respiratory tract. The article for this lesson discusses an epidemiological research study that investigates the potential relationship between NCl<sub>3</sub> and increased asthma incidence. Although sodium hypochlorite (NaClO; bleach) and NCl<sub>3</sub> are different chemicals, they have similar corrosive/irritating properties, and both contain the highly reactive element chlorine. In this activity, students will be asked to think (and perform chemical calculations) in terms of chlorine to “equilibrate” the chemicals in terms of potential exposure to chlorine in order to make meaningful comparisons.



The article discusses taking measurements of allergen-specific immunoglobulin E (IgE) and finding a correlation between asthma, elevated IgE levels, and time spent at pools. IgE is an immune-related protein made by the body when foreign invaders like allergens (e.g., pollen or mold) enter the body. People who are susceptible to allergies will release allergy-specific IgE antibodies, which in turn causes mast cells (found in connective tissue in the nose, lungs, and other areas of the body) to release inflammatory chemicals and histamines that cause the sneezing, itching, and swelling associated with allergic reactions.

**RESOURCES:**

*Environmental Health Perspectives*, Environews by Topic page, <http://ehp.niehs.nih.gov>. Choose Allergies, Asthma

Agency for Toxic Substances and Disease Registry, ToxFAQs for chlorine, <http://www.atsdr.cdc.gov/tfacts172.html>

Bernard A, Carbonnelle S, de Burbure C, Michel O, Nickmilder, M. 2006. Chlorinated pool attendance, atopy, and the risk of asthma during childhood. *Environ Health Perspect* 114:1567–1573, <http://www.ehponline.org/members/2006/8461/8461.html>

Cleveland Clinic Information Center, Allergy overview, <http://www.clevelandclinic.org/health/health-info/docs/1900/1948.asp?index=8610>

Conversion calculator, “parts per million (ppm)” to “milligram per cubic meter (mg/m<sup>3</sup>)”, <http://www.skinc.com/nioshdbbs/calc.htm>

National Institutes of Health, NIH Institute and Center Resources:

Allergies, <http://health.nih.gov/result.asp/23>

Asthma, <http://health.nih.gov/result.asp/56>

## ► Implementing the Lesson

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**INSTRUCTIONS:**

1. Read “Prepping the Lesson” for the preparation of the bleach stock.
2. Have the bleach stock solution, distilled water, and other lab materials available at a central location for the lab groups to collect. You may want to have several gallons of distilled water and several large beakers of the stock chlorine solution available for groups to easily get their materials. It may be helpful to assign two people per group to assemble their group’s materials.
3. Hand out the Student Instructions and copies of the article.
4. Have the students conduct the experiment.
5. Review the periodic table, chemical properties (especially liquid, gas, and corrosivity), and math calculations as needed (see Step 3 #1c, d, and e, and Step 4 #3 concentration section).
6. Review the answers to questions as needed to advance students’ understanding of the experiment and the connections to the “real-life” impacts discussed in the article. Be sure to provide students with the opportunity to revise their work (especially Step 2 #3) to improve writing skills and their understanding of the experiment.

**NOTES & HELPFUL HINTS:**

1. Moving the worms can be challenging. The best way to move them is to let them clump and then spoon the clumps from one container to another. Students should not try to get all of the worms from one container to another, as this will impact the exposure times.
2. The experiment in this activity is presented as a qualitative, observational analysis. You can find student data sheets at [http://www.coep.pharmacy.arizona.edu/events/teacher\\_events/2001/chlor\\_bworm.pdf](http://www.coep.pharmacy.arizona.edu/events/teacher_events/2001/chlor_bworm.pdf) that help make this a quantitative experiment, where students can average data and make graphs.
3. An excellent next step for this activity is to have the students write a protocol for a chronic, low-dose chlorine exposure experiment, implement the experiment, and then discuss how their new protocol relates to the study discussed in the article “Swimming in Allergens? Pool Use and Asthma.”

## ► Aligning with Standards

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**SKILLS USED OR DEVELOPED:**

- Classification
- Communication (note-taking, oral, written—including summarization)
- Comprehension (listening, reading)
- Computation
- Critical thinking and response



- Experimentation (conducting, data analysis, design)
- Manipulation
- Observation
- Research
- Unit conversions

**SPECIFIC CONTENT ADDRESSED:**

- Atomic and molecular mass/weight
- Corrosivity
- Chemical concentrations
- Chlorine
- Asthma
- Toxicology

**NATIONAL SCIENCE EDUCATION STANDARDS MET:****Science Content Standards****Unifying Concepts and Processes Standard**

- Systems, order, and organization
- Evidence, models, and explanation
- Change, constancy, and measurement
- Form and function

**Science as Inquiry Standard**

- Abilities necessary to do scientific inquiry
- Understanding about scientific inquiry

**Physical Science Standard**

- Structure and properties of matter
- Chemical reactions

**Life Science Standard**

- The cell

**Science and Technology Standard**

- Abilities of technical design
- Understanding about science and technology

**Science in Personal and Social Perspectives Standard**

- Personal and community health
- Environmental quality
- Natural and human-induced hazards

**History and Nature of Science Standard**

- Science as a human endeavor
- Nature of scientific knowledge

**▶ Assessing the Lesson****Step 1:** Students conduct the experiment.

Make sure all of the students have a role and actively participate in the experiment. Depending on the experience level of the students, you may need to provide guidance on the types of observations to make. For example, when the students put the worms into the weigh boats containing distilled water, the worms will all have a high level of activity simply because of moving them. Then the worms will settle into their normal clumping behavior. When the worms are transferred to the weigh boats containing chlorine, the worms will also have a high activity level, but students will need to note the differences between the worms' responses in the control and the different chlorine concentrations. Students should note clumping behavior, movement of the individual worms, texture of the worms' skin, color of the worms' skin, and the color of the water in the different chlorine levels and over time.



**Step 2: Data Evaluation Questions**

1. Exposure occurs when the organism comes in contact with a toxicant (in this case, chlorine).

a. What is the exposure frequency (i.e., how often or how many times are the worms exposed to chlorine) for this experiment?

Once.

b. What is the exposure duration (i.e., how long are the worms exposed) for this experiment?

The duration is a total of 6 minutes.

c. What are the exposure concentrations for the experiment (i.e., ppm NaClO)?

500 ppm, 5 ppm, and 0.1 ppm

2. The investigation that you did was a controlled experiment.

a. Describe the control.

The control is the worms exposed only to the distilled water. We move the worms from one container of distilled water to another to account for the impacts of simply moving the worms.

b. Why is a control necessary in a scientific experiment?

To have a "norm" to compare to.

3. Use your own words (not a shared answer from the group) to describe your observations from the experiment. Compare each exposure level to the control, and refer to changes in the worms over time and what you saw after their "recovery." Use a separate piece of paper so you can make edits to produce a clear, concise, flowing summary.

Students should be given multiple opportunities to revise their work. Answers will vary, but should include the following:

- a clear, accurate description of the control worms' look and behavior: when the control worms are moved, their activity level is high, but they settle down and begin clumping together.
- the response of the worms when initially exposed to the different levels of chlorine: worms will wriggle very quickly, especially in the highest chlorine level.
- the response of the worms when exposed to the different levels of chlorine at 3 and 6 minutes: worms typically do not clump together, or there is less clumping behavior compared to the control. This is especially true for the high concentration. In the "Pool" concentration, clumping may decrease over time.
- the look of the worm's skin and color in the different levels of chlorine and changes over time: skin color will appear to become lighter (or bleach out), and the skin will become compromised and begin disintegrating, especially in the high concentration. It will become worse over time (as the exposure increases). The worms may "bleed" and discolor the water. The water becomes thick and slimy from the fatty acids and cell contents entering the water.
- what the worms/water looked like after the "recovery" period: the worms in the high concentration may not recover and just begin to decompose. There may be some variation in worm recovery for the "Pool" and "Air-Equivalent" levels, with greater recovery at the lowest concentration. Look for descriptions that provide good detail. The water is slimy, reddish/brown, and may smell.



**Step 3:** 1. NaClO is the active chemical in bleach. Answer the following questions about NaClO.

a. What are the elements in NaClO?

Na = sodium

Cl = chlorine

O = oxygen

b. What are the atomic masses/weights for each of the elements and for NaClO as a whole? Show your calculations.

Na = 23

Cl = 36

O = 16

NaClO = 23 + 36 + 16 = 75

c. Chlorine is what percent of the total atomic mass/weight for NaClO? Show your calculations.

$36/75 = 0.48 \times 100 = 48\%$

d. The concentration of NaClO in the "Pool" sample is 5 ppm (parts per million), and most pools contain 1–3 ppm chlorine. Does our "Pool" concentration of chlorine fall in the correct range? Show your calculations. HINT: Use the percentage of chlorine in NaClO you calculated in Step 3 #1c.

$5 \text{ ppm NaClO} \times 0.48 \text{ Cl/NaClO} = 2.4 \text{ ppm Cl}$

Yes, our "Pool" sample chlorine concentration falls within the real pool chlorine concentration range.

e. The concentration of NaClO in the "Air-Equivalent" sample is about 0.1 ppm. What is the approximate chlorine level in the "Air-Equivalent" sample?

$0.1 \text{ ppm NaClO} \times 0.48 \text{ Cl/NaClO} = 0.05 \text{ ppm Cl}$

2. Chlorine is corrosive. This means that it "eats away" or "dissolves" cells and tissues.

a. Based on what you saw in your experiment (including the recovery period), why might chlorine (bleach) be a good disinfectant?

Because the bleach eats away the cell's protective coating, which makes it hard for an organism to recover from the exposure (as seen by the poor recovery rate of the worms).

b. Based on what you saw in your experiment, create a hypothesis about why chlorine in pools may increase asthma. (Note: chlorine easily comes out of the water in the form of a gas, which is why we can smell it.)

Answers may range from simple, such as "when the chlorine gas is inhaled it eats away the cells inside of the lungs and causes difficulty in breathing" to more elaborate, offering mechanistic hypotheses such as "when the chlorine gas is inhaled it eats away the cells inside of the lungs and causes cells to leak, putting liquid in the lungs" or "causes scarring" or "causes a layer of dead cells that takes time for the body to heal, making it hard to breathe." There are many possible answers; students who provide additional, logical detail should be given additional credit.



**Step 4:** Read the article “Swimming in Allergens? Pool Use and Asthma.”

1. Write the hypothesis discussed in the article about why pools may increase asthma incidence described in the article.

The cell barriers in the lungs are compromised and allow allergens to enter the lungs, which induces inflammation or an allergic response/asthma. Students may notice that the researchers are hypothesizing that it may be chlorine by-products, like trichloramine ( $\text{NCl}_3$ ), that are contributing to the increased incidence of asthma. Students should be rewarded for catching such a detail.  $\text{NCl}_3$  is discussed in more detail in the Background Information section.

2. What are the differences and similarities between your hypothesis and the hypothesis presented in the article?

Answers will vary. Look for logical, clear, accurate comparisons.

3. Compare the differences in exposure route, frequency, and chemical concentration used in your experiment with the exposures of the 40 children discussed in the article. Describe why each of those factors is important.

**ROUTE:** The exposure route differs between the two in that the worms are exposed in the water directly on the skin, and the children are exposed to the gas via inhalation. The importance described by the students may vary depending on their level of background knowledge in toxicology; look for logical answers. Inhalational exposures are typically the most direct route for a chemical to reach the bloodstream (other than injection). Students may note a similarity in that the worms “breathe” through their skin.

**FREQUENCY:** The exposure frequency in the class experiment was only once, while the exposure frequency of the children with asthma was multiple times. The higher the exposure frequency, the higher the rate of asthma.

**CONCENTRATION:** To make reasonable comparisons between bleach (what you used in your experiment) and  $\text{NCl}_3$  (the primary chemical discussed in the article), you first need to convert from the units discussed in the article ( $\text{mg}/\text{m}^3$ ) to parts per million (ppm). Next you need to convert everything in terms of chlorine, and compare the chlorine levels in your experiment with the air chlorine levels cited in the research discussed in the article.

- a. Using the formula  $(\text{___ mg}/\text{m}^3)(24.45)/122$ , convert the range of  $\text{NCl}_3$  gas in  $\text{mg}/\text{m}^3$  (found in the article) to ppm. NOTE: This equation assumes the conditions are  $25^\circ\text{C}$  at 1 atmosphere (because  $\text{NCl}_3$  is a gas). The molecular weight for  $\text{NCl}_3$  is 122.

0.25–0.54  $\text{mg}/\text{m}^3$ , which converts to a range of 0.05–0.11 ppm  $\text{NCl}_3$

- b. Now calculate, in ppm (using your answer immediately above), the approximate range of chlorine in the air samples in the article (show your calculations, units, and cancellations). NOTE: Chlorine is 88.5% of the molecular mass of  $\text{NCl}_3$ .

$$0.885 \text{ Cl}/\text{NCl}_3 \times 0.05 \text{ ppm NCl}_3 = 0.04 \text{ ppm Cl}$$

$$0.885 \text{ Cl}/\text{NCl}_3 \times 0.11 \text{ ppm NCl}_3 = 0.1 \text{ ppm Cl}$$

- c. Compare the chlorine concentrations described in the article with those used in your experiment (use the answers you calculated in Step 3 #1d and e). Are they similar? Why or why not?

The chlorine concentration range for the  $\text{NCl}_3$  air samples is 0.04–0.1 ppm. The concentration of chlorine in the experimental “Pool” sample was approximately 2.4 ppm (Step 3 #1d), and the concentration of chlorine in the “Air” sample was 0.05 ppm (Step 3 #1e). The “Air-Equivalent” chlorine concentration for the experiment falls within the range of 0.04–0.1 ppm measured in the study highlighted in the article.

- d. Recall the response of the blackworms when exposed to the chlorine “Air” concentration and think about the real-life scenario described in the article. Do you think just one exposure at this concentration might cause a susceptible person to get asthma? Why or why not? What do you think will happen after several exposures at this concentration in a given period of time?



Student answers may vary; look for logical, well-explained responses. Most responses may state that just one exposure at the "Air" equivalent concentration will most likely not cause a susceptible person to get asthma if they do not already have the disease (although one exposure could potentially trigger an attack in a person already with the disease). This is evidenced by the minimal damage caused to the worms by this lowest chlorine concentration and the results of the study described in the article. However, some mild damage may have occurred to some of the worms, indicating that repeated exposures may increase damage to the lung lining (worms' skin surface), thereby increasing the potential to develop asthma. The significance of these numbers in the context of the experimental results is that health effects may be occurring in humans at such low concentrations.

### ► Authors and Reviewers

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**Author:** Stefani Hines, University of New Mexico, Community Environmental Health Program (Note: This activity is based on "Toxicants and California Blackworms," a lesson originally developed by the Center for Chemical Education at Miami University. Modifications using chlorine were developed by Stefani Hines.)

**Reviewers:** Susan M. Booker, Erin Dooley, Lisa Pitman, Wendy Stephan, Joseph Tart, and Kimberly Thigpen Tart

**Give us your feedback!** Send comments about this lesson to [ehpscienceed@niehs.nih.gov](mailto:ehpscienceed@niehs.nih.gov).





## STUDENT INSTRUCTIONS:

# Chlorine, Asthma, and... Blackworms?

**Step 1:** Chlorine is one of the primary chemicals used for disinfecting pools to prevent the spread of disease. However, research is showing that chlorine (and some of its chemical by-products, such as trichloramine [NCl<sub>3</sub>]) may contribute to the increased incidence of asthma. You are going to conduct an experiment exposing aquatic worms known as blackworms to chlorine, then generate a hypothesis about why chlorine may contribute to asthma.

In this experiment you will make visual observations and identify behavioral changes that occur when blackworms are exposed to three different concentrations of chlorine through a controlled experiment. At the end of the investigation, you will analyze your data and present a hypothesis about why chlorine in pools may increase asthma.

### **Materials (per group of students)**

~50 blackworms (1 medium-sized clump)  
5 pipettes or eyedroppers  
9 weigh boats or petri dishes  
1 500-mL beaker  
1 100-mL beaker  
distilled water  
bleach solution  
magnifying lens  
marking pen  
11 labels  
100-mL graduated cylinder  
plastic spoons  
calculators  
data sheet

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

1. Select two people in your group to gather beakers, pipettes/eyedroppers, weigh boats/petri dishes, spoons, a graduated cylinder, and labeling materials as needed.
2. Label the 500-mL beaker as "Distilled Water" and the 100-mL beaker as "Chlorine." One student from each group completely fills the 500-mL beaker with distilled water and fills the 100-mL beaker halfway with the bleach stock solution.
3. Label each weigh boat/petri dish as follows: Control, Water Control, Air, Pool, High, Water 1, Water 2, Water 3, and Extra Pool.



4. Arrange your weigh boats as follows (for now, set aside the weigh boat labeled "Extra Pool"):

Distilled Water	Chlorine
Water 1	High
Water 2	Pool
Water 3	Air
Water Control	Control

5. Fill each of the weigh boats labeled Control, Water Control, Water 1, Water 2, and Water 3 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.)
6. Pour some of the Cl—high concentration solution into the weigh boat labeled "High."
7. Using the 100-mL graduated cylinder and pipettes, make your bleach dilutions as described below. This dilution will take you from the "stock" high bleach concentration (500 parts per million, or ppm, of sodium hypochlorite [NaClO]) down to the pool concentration (5 ppm NaClO) and the "Air-Equivalent" concentration (0.1 ppm NaClO).

#### Pool

- Using a pipette, transfer 1 mL of stock (Cl—high concentration solution) into a 100-mL graduated cylinder, then add 99 mL distilled water (filling to 100 mL total).
- Pour some of the diluted mixture into the weigh boat labeled "Pool." Fill the weigh boat approximately half-full.
- Pour 10 mL of the pool solution in the weigh boat labeled "Extra Pool." This will be used for your next dilution.
- Pour out any solution remaining in the graduated cylinder and rinse well. Do a final rinse with some distilled water.

#### Air-Equivalent

- Using a pipette, transfer 1 mL solution from the weigh boat labeled "Extra Pool" into the 100-mL graduated cylinder, then add 49 mL distilled water (filling to 50 mL total).
  - Pour some of the "Air-Equivalent" mixture into the weigh boat labeled "Air." Fill the weigh boat approximately half-full.
8. Take the 500-mL beaker and partially fill one-quarter to one-half full with distilled water. You may use any leftover distilled water already in the beaker. Use a spoon to transfer at least 50 worms to your beaker (a medium-sized clump).
9. Using a spoon, transfer about 10 worms (a small clump) from the beaker to the "Control" dish, about 10 worms to Water 1, about 10 worms to Water 2, and about 10 worms to Water 3. The numbers of worms do not have to be exact. HINT: It is much easier to move the worms when they are clumped.
10. Observe the worms for a few minutes. Look at their behavior (e.g., clumping, activity level), color, and skin texture, and the color of the water. Familiarize yourself with the behaviors listed on your Student Data Sheet.



11. Decide who in your group will have the following assignments (some of you may have multiple assignments):
  - Timer (1 person): Lets the group know during the experiment when 0, 3, and 6 minutes have passed.
  - Worm Movers (1–3 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., Water 1 to High; Water 2 to Pool; Water 3 to Air; Water Control to Control). HINT: 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 chlorine to the containers holding only distilled water or dilute the chlorine solutions). You do not have to move all of the worms from one container to the other.
  - Data Collectors (1–2 people): Records the observations made by each observer on the Student Data Sheet.
  - Observers: Everyone in the group will observe the behavior and appearance of the worms once they are moved from one container to another. The data collectors will be writing and will rely on the other group members to accurately and descriptively report the activity rating, clumping behavior, and any other observations. *Be sure to observe the worms' behavior immediately upon entering the solution (time 0).*
12. Transfer the worms from the water dishes to the corresponding "Chlorine" dish (e.g., Water Control to Control, Water 1 to High), and start the clock immediately. *Transfer the worms as close to the same time as possible.*
13. Observe and record the worm behavior and appearance at time 0, 3 minutes, and 6 minutes.
14. After 6 minutes, gently remove your worms from each dish and place them back into the dishes labeled Water Control, Water 1, Water 2, and Water 3. This begins the recovery time for the worms.
15. Observe the worms during recovery at 3 and 6 minutes in all the dishes, including the control. Record the recovery levels and other visual observations of the worms.
16. Leave the worms in their weigh boats overnight. Observe again the next day, and record your observations. Be sure to record the number of deaths that might have occurred. NOTE: If possible, refrigerate the worms or keep them in a cool area. They should at least be kept away from heat or warm areas.

## Step 2: Data Evaluation Questions

1. Exposure occurs when the organism comes in contact with a toxicant (in this case, chlorine).
  - a. What is the exposure frequency (i.e., how often or how many times are the worms exposed to chlorine) for this experiment?
  - b. What is the exposure duration (i.e., how long are the worms exposed) for this experiment?
  - c. What are the exposure concentrations for the experiment (i.e., ppm NaClO)? HINT: The exposure concentrations can be located in the "Procedure" section.



2. The investigation that you did was a controlled experiment.
  - a. Describe the control.
  
  
  
  
  
  
  
  - b. Why is a control necessary in a scientific experiment?
  
3. Use your own words (not a shared answer from the group) to describe your observations from the experiment. Compare each exposure level to the control, and refer to changes in the worms over time and what you saw after their "recovery." Use a separate piece of paper so you can make edits to produce a clear, concise, flowing summary.

**Step 3:** Answer the following questions

1. NaClO is the active chemical in bleach. Answer the following questions about NaClO.
  - a. What are the elements in NaClO?
   
Na =
   
Cl =
   
O =
  
  - b. What are the atomic masses/weights for each of the elements and for NaClO as a whole? Show your calculations.
   
Na =
   
Cl =
   
O =
   
NaClO =
  
  - c. Chlorine is what percent of the total atomic mass/weight for NaClO? Show your calculations.
  
  
  
  
  
  
  
  - d. The concentration of NaClO in the "Pool" sample is 5 ppm (parts per million), and most pools contain 1–3 ppm chlorine. Does our "Pool" concentration of chlorine fall in the correct range? Show your calculations. HINT: Use the percentage of chlorine in NaClO you calculated in Step 3 #1c.
  
  
  
  
  
  
  
  - e. The concentration of NaClO in the "Air-Equivalent" sample is about 0.1 ppm. What is the approximate chlorine level in the "Air-Equivalent" sample?



2. Chlorine is corrosive. This means that it “eats away” or “dissolves” cells and tissues.
  - a. Based on what you saw in your experiment (including the recovery period), why might chlorine (bleach) be a good disinfectant?
  
  
  
  
  
  
  
  
  
  
  - b. Based on what you saw in your experiment, create a hypothesis about why chlorine in pools may increase asthma. (Note: chlorine easily comes out of the water in the form of a gas, which is why we can smell it.)

**Step 4:** Read the article “Swimming in Allergens? Pool Use and Asthma.”

1. Write the hypothesis discussed in the article about why pools may increase asthma incidence described in the article.
  
  
  
  
  
  
  
  
  
  
2. What are the differences and similarities between your hypothesis and the hypothesis presented in the article?



3. Compare the differences in exposure route, frequency, and chemical concentration used in your experiment with the exposures of the 40 children discussed in the article. Describe why each of those factors is important.

ROUTE:

FREQUENCY:

CONCENTRATION: To make reasonable comparisons between bleach (what you used in your experiment) and  $\text{NCl}_3$  (the primary chemical discussed in the article), you first need to convert from the units discussed in the article ( $\text{mg}/\text{m}^3$ ) to parts per million (ppm). Next you need to convert everything in terms of chlorine, and compare the chlorine levels in your experiment with the air chlorine levels cited in the research discussed in the article.

- a. Using the formula  $(\text{--- mg}/\text{m}^3)(24.45)/122$ , convert the range of  $\text{NCl}_3$  gas in  $\text{mg}/\text{m}^3$  (found in the article) to ppm. NOTE: This equation assumes the conditions are  $25^\circ\text{C}$  at 1 atmosphere (because  $\text{NCl}_3$  is a gas). The molecular weight for  $\text{NCl}_3$  is 122.
- b. Now calculate, in ppm (using your answer immediately above), the approximate range of chlorine in the air samples (show your calculations, units, and cancellations) NOTE: Chlorine is 88.5% of the molecular mass of  $\text{NCl}_3$ .
- c. Compare the chlorine concentrations described in the article with those used in your experiment (use the answers you calculated in Step 3 #1d and e). Are they similar? Why or why not?
- d. Recall the response of the blackworms when exposed to the chlorine "Air" concentration and think about the real life scenario described in the article. Do you think just one exposure at this concentration might cause a susceptible person to get asthma? Why or why not? What do you think will happen after several exposures at this concentration in a given period of time?



### Student Data Sheet

Timer: \_\_\_\_\_ Data Collector: \_\_\_\_\_

Worm Movers: \_\_\_\_\_

Observers: \_\_\_\_\_

**DIRECTIONS:** Record your qualitative observations of the worms at time 0 (i.e., the worms' response when they are first moved from the distilled water dishes to their corresponding exposure dishes), 3 minutes of exposure, and 6 minutes of exposure. Consider the worms' activity level, their clumping behavior, the color of their skin, the condition of their skin, the color of the water, the smell, and other features you observe.

EXPOSURE LEVEL	OBSERVATIONS AT EACH EXPOSURE
Control	0 minutes:
	3 minutes:
	6 minutes:
Air-Equivalent	0 minutes:
	3 minutes:
	6 minutes:
Pool	0 minutes:
	3 minutes:
	6 minutes:
High	0 minutes:
	3 minutes:
	6 minutes:

#### Recovery Notes

Control	Air-Equivalent	Pool	High

