LD-50 Lab for Blackworms

Name:_____

AP Environmental Science- Student Notes

Part I (1 class period): LD-50 of Copper sulfate with blackworms

Background Information:

"The dose makes the poison." Paracelsus said this in the 1500s, when he was studying medicine and toxicology, and because of this he is often considered the *father of toxicology.* **What he did mean by this?** Paracelsus realized that many substances can be both beneficial and harmful to humans, depending on their ***dose*****,** or how much of it is consumed. For example, ***if you take one Advil it might help your headache, but if you took a whole bottle it might poison you.***

Since the ***toxicity***, or harmful effects, of a substance is often **determined by its dose**, scientists need to test what dose of various chemicals can be harmful to humans in order to regulate them. Obviously scientists can not directly test toxicity on humans, so this is usually done in the laboratory with rats or mice. Scientists will give a population of rats or mice **different doses (concentrations)** of the chemical in question, and observe changes in behavior in the rats or mice. One of the important pieces of data the scientists collect is called the ***LD-50 (Lethal Dose- 50)*** of the chemical, which is the concentration (or dose) of the chemical where ***50%*** of the rat or mice population dies upon exposure. Similarly, they also often determine the ***ED-50 (Effective Dose- 50),*** which is the concentration of the chemical where they see **negative affects in 50%** of the population. These two values help scientists to determine which levels of chemicals are safe to use in humans, and safe to have in the environment.

There are many chemicals present in the environment that are regulated. Certain metals such as **mercury, arsenic, lead and copper** are very harmful to humans and are often present in polluted waters and soils. In order to determine is water is safe for recreational use, drinking, and other uses, metal concentrations have to be below a certain limit set by the local government. Often, these levels are based on experiments done by scientists to determine the ***LD-50***.

In this lab, you will be the scientist trying to determine the level of copper that might be safe to have in a local water source. You will determine the ***LD-50,*** and ***ED-50*** of blackworms in freshwater with various copper concentrations, and answer follow-up questions related to your investigation.

Write two initial questions you might have about this lab before beginning. This can be anything related to the lab, blackworms, or copper in the environment.

Initial Questions:
1.

2.

Objectives:
- Calculate and titrate various concentrations of **copper sulfate ($CuSO_4$)**
- Collect and graph data for ***mortality rate*** of increasing concentrations of **copper sulfate** with blackworms
- Using copper testing kits to get a baseline reading for copper values in water samples
- Using graph data, determine the ***LD-50 and threshold values*** of copper sulfate for blackworms

Materials:
- Stock 0.0025 M copper sulfate solution
- Distilled water
- Graduated cylinders - *for class use*
- 5 petri dishes (capable of holding at least 25 mL)- *each student group*
- Grease pencil
- 50 blackworms- *each student group*
- Timer- *each student group*

Procedures:
1. Lay out each petri dish on your bench top and ***pre-label them*** with the following concentrations using the grease pencil:
    a. **0% $CuSO_4$**
    b. **0.01% $CuSO_4$**
    c. **0.1% $CuSO_4$**
    d. **1% $CuSO_4$**
    e. **10% $CuSO_4$**

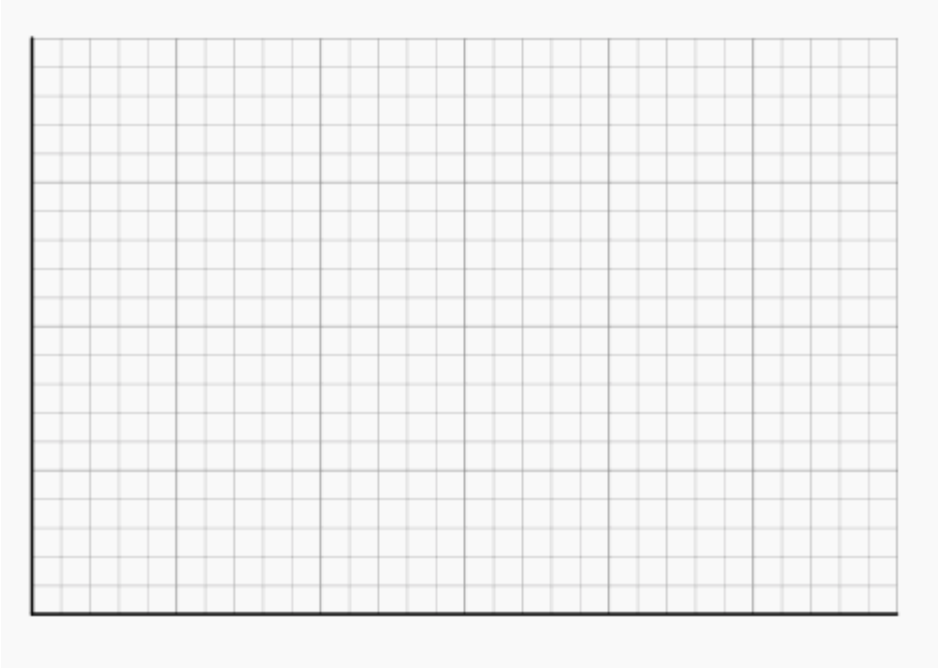
2. Place **22.5 mL of distilled water** into the **0, 0.01, 0.1, 1 and 10% $CuSO_4$** petri dishes. You will be doing a serial dilution for the $CuSO_4$ concentrations in this lab. To start, add 2.5 mL of the **$CuSO_4$ standard (found on the lab bench for the class to share)** to the **10% $CuSO_4$** petri dish. Next, take 2.5 mL of the 10% $CuSO_4$ petri dish and place it in the 1% dish. Then take 2.5 mL of the 1% dish and place it in the 0.1% $CuSO_4$ petri dish. Then take 2.5 mL of the 0.1% dish and

place it in the 0.01% $CuSO_4$ petri dish Take 2.5 mL from the 0.01% dish and discard that into the waste bin. **All the beakers should now have 22.5 mL of solution in them**.

3. Place **10 blackworms into the 0, 0.01, 0.1, 1 and 10% $CuSO_4$ solutions** and record your initial observations into the table below in the column labeled *"Initial Observations"*. Gently prod and poke the blackworms and note how they move, etc… **Observe them for about 1 minute.** Next, observe the blackworms after **5, 10, 15, 20 and 30 minutes**. Record your observations for each petri dish in the columns labeled with the appropriate time. Note how many blackworms in each treatment are moving, and how they look. ***Be specific and quantitative.*** For example, you might record for the 1% solution that 5 of the worms are moving, and 4 are clumped together, and 1 has died. ***At the end of the class period, count how many blackworms have died and record that in the column labeled "Final."***

		Time (min)					
Treatment	**Initial Observations**	**5**	**10**	**15**	**20**	**30**	**Final**
0%							
0.01%							
0.1%							
1%							
10%							

4. **Graph your data** from each treatment from the "Final" observations using the axes shown below. Plot the concentration of the **$CuSO_4$** on the x-axis and the **% of blackworms that died** on the y-axis. For example, in your final data if you had 2 blackworms dead and 8 alive in the 1% $CuSO_4$ solution, you would plot 20% blackworms dead for the 1% $CuSO_4$ concentration**.**



5. Answer the following follow-up questions after completing the lab activity.

Follow-up questions:

1. Review your initial questions before starting the lab. Did you answer these after finishing the lab? **Explain.**

2. Look at the graph you made above of the % of blackworms that died at each concentration of copper sulfate. **What is the LD-50 that you would calculate from this data?**

3. How would your answer change if you were trying to determine the ED-50 **(effective dose, or dose where 50% of the blackworms changed behavior)?**

Hint: Think about what concentration of copper made about half of the blackworms stop moving unless poked, etc…Is this value less than the LD-50? Why?

4. If you were the policy-maker in charge of determining the highest level of copper that should be allowed in San Diego Bay, *what concentration would you choose? Why?*