Name: _____

APES LAB- TOXICOLOGY LC_{50}

Background Information:

Many household items dealt with on a regular basis are toxic materials, but people don't usually think of them as being toxic. The commonly used term to describe acute ingestion toxicity is **LD50**. LD means **Lethal Dose** (deadly amount) and the subscript 50 means that the dose was acutely lethal to 50% of the animals to whom the chemical was administered under controlled laboratory conditions. The test animals (usually mice or rats) are given specific amounts of the chemical in either one oral dose or by a single injection and are then observed for 14 days. Since LD50 values are measured from zero up, the lower the LD50 the more acutely toxic the chemical. Therefore, a chemical with an oral LD50 of 500 would be much less toxic than a chemical with an LD50 of 5. *LD50 values are expressed as milligrams per kilogram (Mg/kg) which means mg of chemical per kg of body weight of the animal. Mg/kg is the same as ppm. The unit can also be expressed as mg/kgbw.* For example, if the oral LD50 of the insecticide parathion is 4, a dose of 4 parts of parathion for every million parts of body weight would be lethal to at least half of the test animals.

An **MSDS (Material Safety Data Sheet)** is a document for each chemical with information on all the physical and chemical properties for that chemical, as well as information on reactions and safe disposal of the chemical waste. The following information can usually be found in a MSDS:

- Identity of the organization responsible for creating the sheet and the date of issue
- The material's identity, including its chemical and common names
- Hazardous ingredients
- Exposure limits
- Physical and chemical hazards and characteristics
- Health hazards
- Emergency and first aid procedures
- Spill and disposal procedures
- Precautions and safety equipment

A bioassay is a toxicity test used to determine the dose or concentration of a toxicant. In dealing with toxins, a frequent relative danger indicator is the LD50. For example the LD50 for sugar in rats is 30 grams that is out of 100 laboratory rats, 50 would be expected to die at levels of 30 grams of sugar/kg of body weight.

A similar measure, the **LC50**, (which stands for **lethal concentration**) is often used. In this lab a small crustacean, the brine shrimp, will be used. It is normally found in brackish water and is a very hearty little organism able to tolerate high salt concentrations.

Materials

- brine shrimp
- brine
- household non-sudsy ammonia solution
  - OR window cleaner such as Windex
  - OR Pine-Sol
- graduated cylinders
- pipettes
- Petri dishes (6 per group)
- permanent marker, or labels for Petri dishes
- test tube racks
- test tubes (6 per group)
- stirring rods

Procedure

1. Label 5 test tubes as follows: 1:1, 1:10, 1:100, 1:1000, and 1:10,000. Take 11 mL of the full-strength material being tested for toxicity from the stock solution and add it to the test tube labeled 1:1.
2. Place 9 mL of brine into each of the other test tubes. Pipette 1 mL of "toxic" material from the 1:1 tube into the tube labeled 1:10. Mix well.
3. Pipette 1 mL from the 1:10 tube into the tube labeled 1:100. Mix well.
4. Pipette 1 mL from the 1:100 tube into the tube labeled 1:1000. Mix well
5. Pipette 1 mL from the 1:1000 tube into the tube labeled 1:10,000. Mix well.

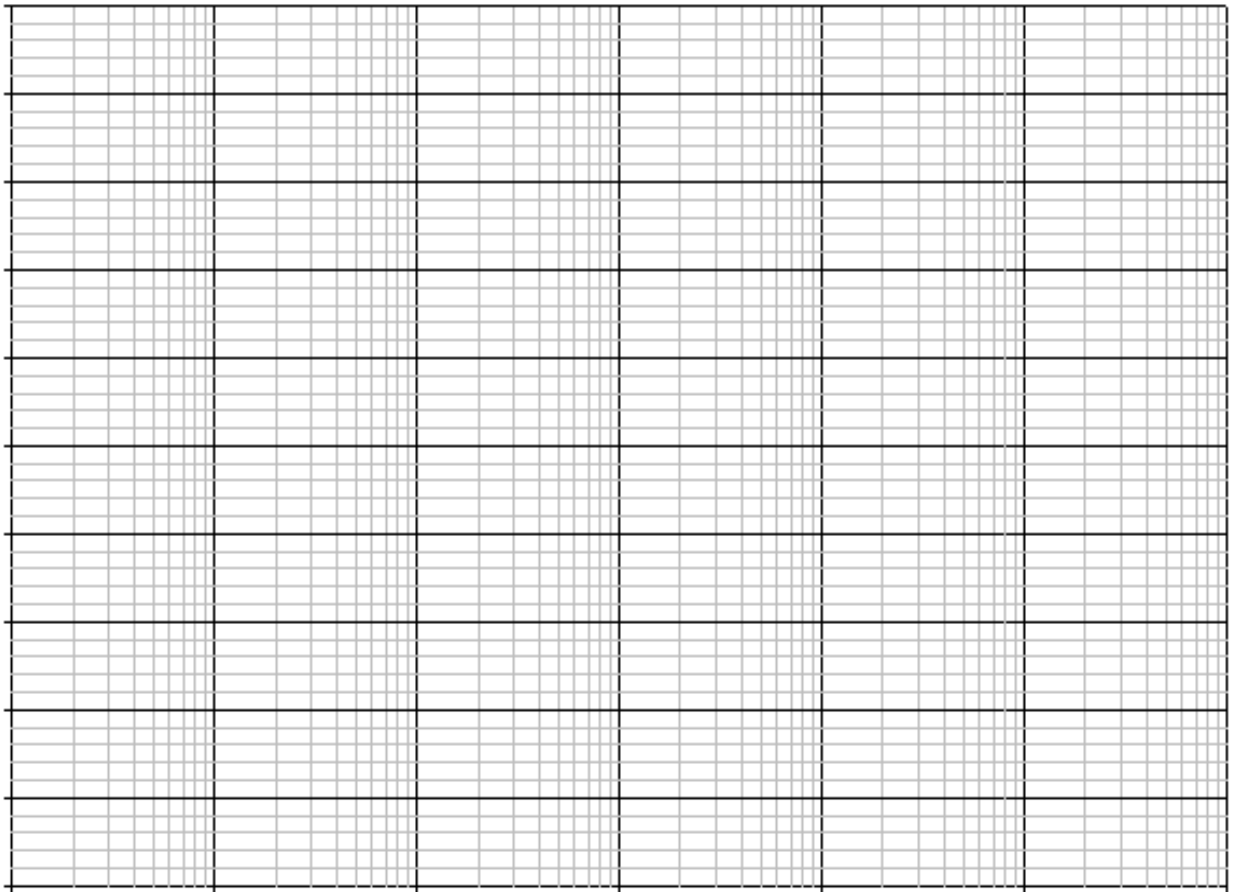
6. Label six Petri dishes as follows: 1:1, 1:10, 1:100, 1:1000, 1:10,000, and control. Be sure to label the *bottom of the dish, not the cover.*
7. Using a pipette, move 10 brine shrimp into each Petri dish.
8. Put 10 mL of brine in the control dish. Pour the contents of each tube into the appropriate Petri dish and observe for 10 minutes. *Be sure to add the appropriate brine solutions as quickly as possible AFTER the brine shrimp are added to the Petri dish.*
9. Using a hand lens or microscope to count the number of brine shrimp alive after 10 minutes.
10. Leave the shrimp in the dishes and determine how many are alive after 24 hours. Record your data in Data Table.

DATA TABLE 2: BRINE SHRIMP SURVIVAL RATES

Material being tested		**Number of brine shrimp alive after 10 minutes**					**Number of brine shrimp alive after 24 hours**				
	Dilution	**1:1**	**1:10**	**1:100**	**1:1000**	**1:100000**	**1:1**	**1:10**	**1:100**	**1:1000**	**1:100000**
	Dilution Factor	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}

Graph

1) Plot a smooth transition line graph for **10 minutes** and **24 hours of concentration** (x axis) vs. mortality (y axis.) Be sure to plot on semi-log graph paper.
2) Determine the **LC-50** from your graph for both times.



Questions

1) What is (are) the control(s) in this experiment? What is the independent variable? What is the dependent variable?

2) Explain the meaning of the term "threshold level of toxicity." Based on your data in this lab, what is the safe concentration for brine shrimp?

3) 10 ppm ammonia is the standard for drinking water for humans. Can brine shrimp survive in our drinking water at this limit of ammonia? Why would this be a concern for humans?

4) Often indicator species are used to study the overall health of an ecosystem. If you were to study an ecosystem containing brine shrimp, would you use it as an indicator species? Why or Why not? Explain your reasoning.

5) Provide one argument for extending these toxicity results to humans and one argument against doing so.

6) Some environmentalists argue that there is no such thing as a threshold for pollution effects. What do they mean?

7) Why is it difficult to establish standards for acceptable levels of toxins?