Name _____
APES

Brine Shrimp Toxicology Lab

PART A: LD50 AND MSDS

BACKGROUND-
We handle many materials daily that are toxic. We are often unaware of the degree to which they are toxic. For a variety of reasons, different animals respond differently to the same toxin. Some animals may be very sensitive to a toxin, whereas others are relatively resistant to its effects. Because species of animals vary, it is important to understand that what is toxic to brine shrimp may not necessarily be toxic to other kinds of animals to the same extent.

Many household items that we deal with on a regular basis are toxic materials, but we don't usually think of them as being toxic. It can be instructive to examine several such materials to determine their toxicity.

The commonly used term to describe acute ingestion toxicity is LD_{50}. 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 LD_{50} values are measured from zero up, the lower the LD_{50} the more acutely toxic the chemical. Therefore, a chemical with an oral LD_{50} of 500 would be much less toxic than a chemical with an LD_{50} of 5. LD_{50} 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. For example, if the oral LD_{50} 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.

There are two parts to this activity:

1. Using your OWN MASS in **kg**, figure out how many total g would be required to kill 50% of perfect duplicates of yourself. Be careful about units! You don't need to show work for all of these problems, but write out ONE complete example of your conversion to LD_{50}/person below the table so that I know how you did it. Remember, everyone's answers will be slightly different. (Note: **1 lb. = 0.45359 kg = 453.59g)**

***Your mass =_____kg**

2. Find **a Material Safety Data Sheet (MSDS) for an ingredient in some household substance you have** (e.g. toothpaste, shampoo, mouthwash, junk food additives, etc.) and give its LD_{50} for the oral route for a person in g/person. Assume the LD_{50} of a rat or mouse will be the same as a human. Don't use any of the ones already listed below.

Substance (source or product)	**LD_{50} (mouse or rat)** mg/kg or g/kg	**LD_{50} for you** (g/person)
disodium EDTA (Secret)	2000. mg/kg	
benzaldehyde (Cherry Flavor)*	4.8 mg/kg	
Tetrahydrocannabinol (THC from marijuana)*	110 mg/kg	
Ethyl acetate (Cherry Flavor)*	6100 mg/kg	
propylene glycol (Cherry Flavor)	20 g/kg	
Caffeine *	0.13 g/kg	
malic acid (sour candy)*	1.6 g/kg	
Methanol (wood alcohol)*	5628 mg/kg	
Nicotine (through mouth)*	190 mg/kg	
Botulinum toxin (bacteria)*	3×10^{-8} mg/kg	
potassium nitrate (fertilizer)	190 mg/kg	
sodium fluoride (toothpaste)	52 mg/kg	
parathion (pesticide	6.0 mg/kg	
Vx (nerve gas)	2×10^{-2} mg/kg	
tetrodotoxin (poison from puffer fish)	334×10^{-6} g/kg	
diazinon (ant killer dust)	0.076 g/kg	
amphetamine sulfate	32 mg/kg	
ephedrine	0.600 g/kg	
gamma hydroxybutyrate (date rape drug)	2.0 g/kg	
Showing your work for ONE problem:		

* natural substances

Part B: Bioassay of chemicals on Brine Shrimp

Background-

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 LD-50. For example the LD-50 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. Nicotine has an LD-50 in rats of 0.05 g, which is much more toxic.

A similar measure, the LC-50, (which stands for lethal concentration) is often used. In this lab we will use a small crustacean, the brine shrimp. It is normally found in brackish water and is a very hearty little organism - able to tolerate high salt concentrations.

Materials-

Brine Shrimp (purchased from aquarium store)
Brine (specifically for Brine Shrimp, mixed with aquarium water)
Household Ammonia
Mouthwash
Windex
Garden Fungicide
Dishwashing detergent (Dawn)
Pine Sol or some other household disinfectant
Pipettes (1 mL)
Petri Dishes (6 per group)
Test tube racks
Permanent marker
6 Test tubes with caps
Plastic gloves if handling toxic material

Method (Serial Dilution)

1) Label 5 test tubes as follows: 1:1, 1:10, 1:100, 1:1000, 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. 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.
2) Pipette 1 mL from the 1:10 tube into the tube labeled 1:100. Mix well.
3) Pipette 1 mL from the 1:100 tube into the tube labeled 1:1000. Mix well
4) Pipette 1 mL from the 1:1000 tube into the tube labeled 1:10,000. Mix well.
5) Label 6 petri dishes as follows: 1:1, 1:10, 1:100, 1:1000, 1:10,000, control. Be sure to label the *bottom of the dish, not the cover!* Using a pipette, move 10 brine shrimp into each Petri dish.

This is called "serial dilution"

6) 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.***
7) Count the number of dead brine shrimp after 10 minutes. Leave the shrimp in the dishes and determine how many are dead after 24 hours. Record your data in Data Table A.

If you prefer concentration in percent versus "part per" (part per hundred, etc), the percents would be as follows:

1:1 = 100% (pollutant), 1:10 = 10%, 1:100 = 1%, 1:1000 = 0.1%, 1:10,000 = 0.01%

Data Table A

Material being tested		**# brine shrimp dead after 10 minutes**					**# brine shrimp dead after 24 hours**				
	dilution	1:1	1:10	1:100	1:1000	1:10,000	1:1	1:10	1:100	1:1000	1:10,0000
	concentration	100%	10%	1%	0.1%	0.01%	100%	10%	1%	0.1%	0.01%

Analysis-

Plot a line graph of dilution (X axis) vs. # that died (Y axis) using Excel.
Determine the LD-50 and Threshold Level from your graph. Mark each on graph.

2. 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.

3. What possible sources of error were present in this experiment?

Part C: Toxics in your house/yard scavenger hunt.

In many cases, people often have a variety of substances around their home, which may be caustic, volatile, corrosive, flammable, or even toxic. For this activity, we will focus on materials that are used to combat pests around the home and yard. In this activity, you will hunt around your house/yard and identify any potentially toxic or hazardous materials, identify the major chemical class, describe the associated label warnings, and describe the condition of the container in terms of condition, label present, age, and safe storage. Please identify at least 3 in each area of house. Include a picture of each product. You may include all products from a ***single*** room in one picture. Print out an MSDS for ONE product of your choice.

Make sure not to touch any bottles that appear to be leaking and ask your parents permission before conducting the scavenger hunt, in fact, you may want to encourage your parents to conduct the activity with you. Use the log below to list your results.

http://householdproducts.nlm.nih.gov/

Area of house or yard	**Product(s) found**	**Active ingredient or chemical name**	**Warning listed on label**	**Condition of container, label present, stored safely, age of product**
Kitchen				

Bathroom				
Garage				
Other→ storage shed, home office, bedroom				

Each student will hand in the following, stapled together and typed:

1) A cover sheet with the name of the lab, your name, the date of submission, a picture of your setup and your data table.
2) Properly labeled and titled graph of the data using EXCEL.
3) The answers to the analysis questions and LD50 chart completed according to instructions.
4) Toxics in your house table filled in
5) Pictures of products for each room