APES FORMAL LAB ~ TOXICOLOGY

(from PJ Shlachtman and the APSI)

Remember to include the five SDS (first page only of each) with your formal lab report.

PART A: LD$_{50}$ AND SDS (done independently but must be part of lab report)

Background Info.

Many household items dealt with on a regular basis are toxic materials, but people 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 **LD50 or LD$_{50}$**. LD means **Lethal Dose** or 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 **SDS (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 an SDS:

1) Identification
2) Hazard(s) Identification
3) Composition/Information on Ingredients
4) First-aid Measures
5) Fire-Fighting Measures
6) Accidental Release Measures
7) Handling and Storage
8) Exposure Controls/Personal Protection
9) Physical and Chemical Properties
10) Stability and Reactivity
11) Toxicological Information
12) Ecological Information*
13) Disposal Considerations*
14) Transport Information*
15) Regulatory Information*
16) Other information

* = non=-mandatory

Procedure

1) Calculate your <u>own mass</u> in kg. Calculate the human LD50 for the compounds in the data table on page 2: how many total g would be required to kill 50% of perfect duplicates of yourself. Use the LD50 values in the table on the next page. <u>Show one sample calculation in your report.</u> (1 kg = 2.20462262 lbs)

2) Find a Safety Data Sheet (SDS) for an ingredient in <u>five</u> household substances you on hand (e.g. toothpaste, shampoo, mouthwash, cleaners, junk food additives, etc.) and calculate its LD50 for the oral route for a person in g/person. Find the LD50 listed on the SDS (use the CNTRL-F command and type in LD50) and use it in your calculations as before. Show all five calculations in your report. You may <u>not</u> use examples in the data

table—you must find your own. Search for SDS online and include the printed first page of the SDS for the substances you have chosen.
https://chemicalsafety.com/sds-search/

DATA TABLE 1: LD50 for rodents and humans

Chemical name (main source or product)	LD_{50} oral, rat (mg/kg)	Student's oral LD_{50} (g/person)
disodium EDTA (detergents)	2000.	
benzaldehyde (almond, cherry flavor)	1300	
ethyl acetate (fruit flavoring)	5620	
propylene glycol (moisturizer)	20. g/kg	
caffeine (stimulant)	192	
malic acid (sour candy)	1600	
methanol (wood alcohol)	5628	
nicotine (cigarettes)	1600	
potassium nitrate (fertilizer)	3750	
sodium fluoride (toothpaste)	52	
parathion (pesticide)	4.0	
tetrodotoxin (puffer fish poison)	3.34×10^{-8}	
diazinon (ant killer dust)	139	
pseudoephedrine hydrochloride	371	
phosphoric acid (in sodas)	1530	
FIVE household substances: (list chemical AND main source or product)	**----------------**	**Student's LD_{50} (g/person)**
1)		
2)		
3)		
4)		
5)		

PART B: Bioassay of Ammonia on Brine Shrimp (done in groups)

Background Info.

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; 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 environmental studies, LC values refer to the concentration of a chemical in water rather than a dose of a chemical.

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 (from aquarium store)
brine (specifically for Brine Shrimp, mixed with aquarium water)
household non-sudsy ammonia solution
    OR window cleaner such as Windex
    OR Pine-Sol or some other household disinfectant
graduated cylinders
pipettes
Petri dishes (6 per group)
permanent marker, or labels for Petri dishes
small beakers
test tube racks
test tubes (6 per group)
stirring rods
magnifying glasses/hand lenses

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, 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 magnifying glass, count the number of brine shrimp alive after 10 minutes. Record your data in Data Table 2.
10) Leave the shrimp in the dishes and determine how many are alive after 24 hours. Record your data in Data Table 3.

Data Table 2: Brine Shrimp Survival Rates, 10 minutes

Material being tested	Number of brine shrimp alive after 10 minutes					
dilution	control	1:10,000	1:1000	1:100	1:10	1:1
dilution factor	0	10^{-4}	10^{-3}	10^{-2}	10^{-1}	10^{0}

Data Table 3: Brine Shrimp Survival Rates, 24 hours

Material being tested	Number of brine shrimp alive after 24 hours					
dilution	control	1:10,000	1:1000	1:100	1:10	1:1
dilution factor	0	10^{-4}	10^{-3}	10^{-2}	10^{-1}	10^{0}

Graph

Print the graph paper: http://www.kwanga.net/apesnotes/semi%20log%20graph%20paper.pdf
Look at 2002 FRQ #3 for more information.

1) Plot a line graph of concentration (x axis) vs. mortality (y axis). Use two colors, one each for Data Table 2 data and Data Table 3 data.
2) Determine the LC50 from the graph and label the point on it.

Questions

1) What is (are) the control(s) in this experiment for part B?
2) 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? Explain.
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.