

APES FORMAL LAB ~ TOXICOLOGY

(from PJ Shlachtman and the APES Summer Institute)

PART A: LD₅₀ AND MSDS (done independently)

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**. 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

Procedure

1) Calculate your own mass 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. Show one sample calculation in your report. (1 kg = 2.20462262 lbs)

2) Find a Material Safety Data Sheet (MSDS) for an ingredient in five household substances you have 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 MSDS (use the CNTRL-F command and type in LD50) and use it in your calculations as befqtg0'

Search for MSDS online and include the printed first page of the MSDS for the substance you have chosen.

<http://www.hazard.com/msds/index.php>

<http://www.msdssearch.com/>

<http://www.ilpi.com/msds/>

<http://www.msds.com/>

DATA TABLE 1: LD50 for rodents and humans

| Substance (main source or product) | LD ₅₀ oral, rat (mg/kg) | Your 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 x 10 ⁻⁸ | |
| diazinon (ant killer dust) | 139 | |
| pseudoephedrine hydrochloride | 371 | |
| phosphoric acid (in sodas) | 1530 | |
| | | |
| FIVE household substances: | ----- | LD₅₀ for you (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, 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 (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
test tube racks
test tubes (6 per group)
stirring rods
dissecting microscopes

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

Data Table 2: Brine Shrimp Survival Rates

| Material being tested | dilution | Number of brine shrimp alive after 10 minutes | | | | | Number of brine shrimp alive after 24 hours | | | | |
|-----------------------|-----------------|---|-----------|-----------|-----------|-----------|---|-----------|-----------|-----------|-----------|
| | | 1:1 | 1:10 | 1:100 | 1:1000 | 1:10,000 | 1:1 | 1:10 | 1:100 | 1:1000 | 1:10,000 |
| | 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 line graph of concentration (x axis) vs. mortality (y axis). Doing the graph on the computer is recommended..
- 2) Determine the LC-50 from your graph.

Questions

- 1) What is (are) the control(s) in this experiment?
- 2) Based on your data in this lab, what is the safe concentration for brine shrimp (**Lowest Observable Effect Concentration** or **LOEC**)?
- 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?
- 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) What possible sources of error were present in this experiment?