

# LD<sub>50</sub> SERIAL DILUTION TOXICOLOGY LAB

## USING *ARTEMIA SALINA*

**PURPOSE:** To determine the concentration of a sodium fluoride (NaF) solution that will kill fifty percent of a given population of *Artemia salina*. In short: to find the NaF - LD<sub>50</sub> for *Artemia salina*.

**BACKGROUND:** There are many ways to evaluate health threats from toxic substances in the environment. Toxins may affect cells, tissues, organs, organ systems, whole organisms, populations, and ecological communities. Toxins may be either synthetic or naturally occurring.

In the science of **toxicology** (the study of toxic substances), bioassay techniques are often used to evaluate the direct lethal effects of substances on organisms. A **bioassay** is a test that uses living organisms as an indicator. A common measure of acute (lethal) toxicity is called the **LD<sub>50</sub>** (lethal dose 50). This is the experimentally determined concentration of the substance that will kill fifty percent of the individuals exposed to it.

The reason that some, but not all of the exposed individuals are killed is twofold. First, there is genetic variation among individuals in a population. Some individuals are more susceptible and some are more resistant to the lethal effects of any substance. Second, exposure to the toxin is uneven. The toxin is likely not to be evenly distributed in the food or environment of the individuals. One of the challenges of a good experiment is making sure that all the experimental individuals are evenly exposed to the toxin.

Also, it is extremely important to remember that different species of animals will vary in their response to a particular toxin. What may be extremely poisonous to one animal may have no harmful effect on another. This is why test studies using laboratory animals cannot be extrapolated to humans with 100% certainty. Generally speaking, the more closely related two animals are, the more the results can be extrapolated from one to another with confidence.

Universities and government agencies use vertebrate animals such as rats, rabbits, and chimpanzees for drug and toxicology testing. Since it is illegal for high schools to use vertebrate animals for toxicology testing, we will be using a small invertebrate species called *Artemia salina* commonly known as the sea monkey. *Artemia salina* is good for this experiment because it is easy to see and count. Sea monkeys are crustaceans in the order Anostraca.

### MATERIALS :

Sodium fluoride stock solution 1:10,000

Live *Artemia salina*

Salty spring water (0.003g NaCl/ml)

Six-cycle Semi-Log graph paper

1 24 well plate with lid

1 pipette

### PROCEDURE:

1. Obtain a 24 well plate with lid.
2. Label 7 wells as follows: Control, 1ppb, 10ppb, 100ppb, 1ppm, 10ppm, 100ppm.
3. Carefully rinse each well with spring water and a pipette.
4. Add 5 living sea monkeys to each well. Try to pick ones of a consistent size.
5. Remove most of the water from the control well; then add 3 ml of spring water.
6. Gently mix and repeat step 5 (the goal is to replace most of the water without stressing the sea monkeys).
7. Repeat steps 4 through 6 with each of the prepared serial dilutions.
8. Cover with the lid. Expose the sea monkeys to the experimental conditions for twenty-four hours.
9. After the exposure, carefully count the sea monkeys to determine the percent mortality.
10. Graph the mortality rate on six-cycle semi-log graphing paper. Use the log axis for plotting the concentrations of sodium fluoride and the "normal" axis for plotting the percent mortality.
11. The LD<sub>50</sub> can be determined by following the 50% mortality line over to where it intersects the mortality curve. The intersection will be your LD<sub>50</sub> concentration.
12. The concentration of sodium fluoride in toothpaste is about 1:238. In your opinion, is that a safe amount? Why or why not?
13. Some water systems add sodium hexafluorosilicate, hexafluorosilicic acid, or hydrofluorosilic acid to the water for additional cavity protection. The maximum level is 2ppm. In your opinion, is this a good idea? Why or why not?

