

LC₅₀ SERIAL DILUTION TOXICOLOGY LAB USING *Tubifex*

PURPOSE: To determine the concentration of a cleaning solution that will kill fifty percent of a given population of *Tubifex* (LC₅₀).

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 **LD₅₀** (lethal dose 50) or the **LC₅₀** (lethal concentration 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 a *Tubifex* worm. *Tubifex* is good for this experiment because it is easy to see and count.

MATERIALS :

Cleaning solution
Live *Tubifex*
Spring water

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, 1ppm, 10ppm, 100ppm, 1,000ppm, 10,000ppm, and 100,000ppm.
3. Carefully rinse each well with spring water and a pipette.
4. Add 5 living *tubifex* 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 serial dilutions.
8. Cover with the lid. Expose the *tubifex* to the experimental conditions for twenty-four hours.
9. After the exposure, carefully count the *tubifex* 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 copper sulfate and the “normal” axis for plotting the percent mortality.
11. The LC₅₀ can be determined by following the 50% mortality line over to where it intersects the mortality curve. The intersection will be your LC₅₀ concentration.
12. Cleaning solutions are commonly rinsed down the drain in the process of being used. This water then makes its way into the environment. Based on the results of this lab, what precautions would you suggest in using the cleaner you tested? EXTRA CREDIT: find a non-toxic alternative to this cleaner.