

## Gatorade Dose Response

This game is designed to illustrate how to conduct a dose response experiment, how to graph a dose response curve, and how to identify an LD50/EC50. It can be conducted as a relay or an in-class activity.

Materials:

- Gatorade (Cool Blue, Fruit Punch, Grape, Lemon Lime, and Orange)
- Alligator dose response cards (“Experiment Data”)
- Water
- Plastic cups (clear is best to illustrate the dilution series)
- Graduated cylinder or other liquid measuring apparatus
- Graph paper
- Markers or writing utensils
- Permanent Markers

## In-Class Format

**Part 1:** Setting up a dose-response experiment. ~10 minutes

Students should break into groups. Groups should be supplied with a single flavor of Gatorade, water, a graduated cylinder, and 6 plastic cups. These supplies will be used to create a serial dilution of Gatorade for the “experiment.” The diluted color throughout provides a nice illustration of sequentially more dilute solutions.

To create the dilution series:

1. **Label your cups.** Seven cups should be labeled with the concentration of Gatorade they contain. You will be diluting by a factor of 2 (1 part solute + 1 part diluent) and will have 7 dilutions and 1 control (the control contains 0% Gatorade). Dilutions will be 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%.
2. **Measure out the diluent into appropriate vessels.** You will be diluting by a factor of 2 (1 part solute + 1 part diluent). Measure 50 mL of diluent (water) into all cups except the one labeled 100%.
3. **Prepare you 100% dilution cup.** Pour 100 mL of your Gatorade into the 100% dilution cup. This is your top standard.
4. **Make the dilution series.** Now you will begin creating serial dilutions. Measure 50 mL of your 100% Gatorade in a graduated cylinder and transfer to the cup labeled “50%.” Mix

by swirling. The Gatorade in this cup is only 50% as concentrated as the first cup, how does this make the color look? Once mixed, measure 50 mL of the 50% Gatorade solution in the graduated cylinder and transfer to the cup labeled 25%. Mix by swirling. Continue doing this until you reach 1.56%. Leave the last cup labeled 0% untouched. This cup contains no Gatorade and represents your control.

5. **Make observations.** How does the Gatorade color in the different cups illustrate the dilution series?

## **Part 2:** Graphing data

Students should be supplied with the experimental data cards that correspond to their color of Gatorade, writing utensils, and graphing paper. Have students create dose-response curves by drawing graphs using this data. Red alligators indicate those that died in the particular dilution, and green alligators indicate those that lived. Students should put the number of mortalities on the Y axis and the concentration of Gatorade on the X axis.

Once the graphs are complete, have students identify the lethal dose 50 (LD50): the dose at which 50% of the animals have perished.

## **Part 3:** Comparing potency

Explain how LD50 values are used to compare the potency of compounds and have students present their graphs to the class. Have the class determine which Gatorade flavor is the most toxic to alligators (lemon-lime) and describe why.

## **Relay Format:**

This fun variation alters *Part 1* of the in-class format and can be done in an open space to get students moving and increase engagement. Students take turns making each sequential dilution in a relay-style game by running from a starting point to the “lab bench” and back.

The “lab bench” can be any flat surface where materials for creating the dilution series are setup. Each station should include 1 plastic cup containing 100 mL of a single Gatorade flavor, 7 plastic cups containing 50 mL of water, a graduated cylinder capable of measuring 50 mL, and the “experiment data.” Teams begin at the starting line, away from the “lab bench.” At the initiation of the relay (READY SET GO!), the first member of the team runs to the table, measures out 50 mL of the 100% Gatorade, and pours it into the cup immediately adjacent (containing 50 mL of water). The student has created a 50% dilution. They then run back to the starting line and sit-down to signify completion. After crossing the starting line, the next team member starts. This time, the 50% dilution is used. The student measures 50 mL of the 50% Gatorade dilution



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and adds this to the next adjacent glass of 50 mL water. They have created a 25% dilution. Again, the student runs back to the finish line and sits down. The pattern continues until the last dilution is made (1.56 %). Students must leave one glass with no Gatorade in it at the end. Explain to students that this is the experimental control and “contaminating” the control with Gatorade results in disqualification. The last person, instead of creating a dilution, grabs the “experimental data” and runs back to the start line. All students must be sitting to signify they have finished the relay and the first team to finish wins!

The remainder of the activities for *Part 2* and *Part 3* can be conducted in the same manner as the in-class format.

Making a dilution series for toxicity testing:

A dilution series

1. Your goal is to determine what percentage of the substance will kill 50% of the brine shrimp. Label the test tubes 1 through 6.
2. Add 10 mls of the substance you are testing to test tube #1.
3. The next step is to set up a serial dilution. Add 9 mls of salt to each of the other 5 test tubes.
4. Remove 1 ml of the substance from test tube #1 and add it to test tube #2. Cover the opening of the test tube and invert it 3 times.
5. Remove 1 ml of the substance from test tube #2 and add it to test tube #3. Cover the opening of the test tube and invert it 3 times.
6. Remove 1 ml of the substance from test tube #3 and add it to test tube #4. Cover the opening of the test tube and invert it 3 times.
7. Continue until you have done the serial dilution for 5 of the 6 test tubes. Do not add liquid from test tube #5 to test tube #6. Instead, remove 1 ml of the substance from test tube #5 and dispose of it. Test tube #6 is your control.
8. Graph your results.