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NOTE:
Daphnia magna are not included with this kit.
See page 14 for Ordering and Care information.

EDVO-Kit

954

Toxicity Detection of Pollutants in Freshwater

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The toxicological effect of pollutants in freshwater is determined by using *Daphnia magna*. This experiment has been adapted from an actual water quality test.

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Safety Data Sheets can be found on our website:
www.edvotek.com/safety-data-sheets

This experiment is designed for 5 student groups.

Experiment Components

Store components A - C in the freezer.

A	Simulated Toxicant Solution Concentrate	Freezer
B	Toxicity Reduction Reagent (EDTA Solution)	Freezer
C	IQ Additive (fluorescent detection substrate)	Freezer

1	Exposure Chamber
12	Wide bore, large capacity transfer pipets
6	Calibrated transfer pipets

NOTE:
Daphnia magna are not included with this kit.
See page 14 for Ordering and Care information.

Requirements

- Longwave ultraviolet light source
- White Light visualization system recommended
- Graduated 1-liter beaker
- UV protective safety glasses
- Additional IQ exposure chambers (as needed)
  Recommended: 1 per group
- Bottled spring water
- Gloves
- 5 ml pipets and pumps
- Test tubes

This experiment is adapted from the IQ Toxicity Test, an actual water quality test. This technology is protected under United States patent # 5,094,944 and is licensed exclusively from Aqua Survey, Inc. by EDVOTEK for education.

Background Information

TOXICITY DETECTION OF POLLUTANTS IN FRESHWATER

The term environment describes everything that surrounds a particular living organism. The environment of a particular organism includes nonliving things, such as soil, air and water, other organisms that live in association with that organism, as well as other factors, such as temperature, humidity and radiation. The term ecotechnology refers to the application of new technologies, such as biotechnology, and how these environmental factors interact with one another. Ecotechnology often focuses on the effect that changes will have on an organism's environment. In order to make sensible decisions about environmental policy, people need to understand how ecological systems work.

Ecological systems, often referred to as ecosystems, are difficult to study because they have so many components, including plants, animals, the cycling of essential materials, transformations of energy, and impact on the human economy. In addition, ecosystems often cover vast areas and have long "lag times" between cause and effect. For this reason, the impact of human disturbance often is not detected until it may be too late to reverse the damage.

Bioassay experiments, such as this one, are commonly used to detect and assess harmful levels of toxic chemicals at hazardous waste sites, spills or in the waste streams from industrial and sewage treatment plants. This experiment will determine what level of a pollutant is unacceptable in a hypothetical body of freshwater. The indicator species for this test is Daphnia magna, a common water flea. It is a freshwater invertebrate, about 0.2 to 3 mm long, which is visible to the unaided human eye.

Despite its common name, Daphnia magna is not an insect; it is a member of the class crustacea, whose relatives include other arthropods such as lobsters and crabs. The most prominent external features of Daphnia magna are a single large compound eye and two pairs of highly branched antennae. The animal uses the antennae for locomotion. The Daphnia magna's thorax and abdomen are protected by a transparent shell. Visible through the shell are 5 to 6 pairs of legs with setae (hairs). The moving legs generate a water current that brings protozoa, algae, bacteria and organic detritus to the mouth.

Many of the tests currently used to measure toxicity require exposing the organism to a toxic substance for periods of 2 to 28 days. In this experiment, however, the Daphnia magna will be exposed to a simulated toxicant for approximately 45 minutes. The Daphnia are then fed a sugar substrate called the IQ Additive. The IQ additive used in this experiment is -D-galactoside sugar with a 4-methylumbellifery fluorescent marker attached ($C_{16}H_{18}O_8$). The bound sugar-marker molecule can not fluoresce. An organism not stressed by toxins has a high enough enzymatic activity level to metabolize the carbohydrate analogue, thus splitting the sugar-marker apart into sugar and marker. The unbound marker becomes free to fluoresce.

Healthy organisms will have the ability to ingest and digest the sugar compound. Thus, they will glow under long wave ultraviolet light (also known as black light) after the sugar is digested. If a toxin has significantly affected the organism, it will cause its enzymatic activity level to be inhibited. Any Daphnia that fail to glow brightly under long UV light will die within 48 hours. The glow/no-glow ratio makes it possible to estimate lethal concentration (LC) values. An LC50 value is the lethal concentration at which 50% of the organisms die after exposure to the toxic agent.

Background Information

TOXICITY REDUCTION EVALUATION

A major environmental concern is the pollution of our water systems. For some industries, it is common practice to discharge wastes into nearby bodies of water. The discharge is usually an effluent, which is a complex waste material, such as sewage or chemical by-products. Because raw waste can not be directly discharged into waterways, it must treated to make it less toxic, and to avoid exceeding acceptable discharge limits set by the federal government. In order to qualify for a discharge permit, industry must have toxicity tests conducted on their effluent to determine if it complies with the standards allowable for discharge. Organisms such as Daphnia magna are used as sensors, because they are good biological indicators of how effluent affects existing populations of organisms in water which is being contaminated.

Toxicity reduction plays an important role in altering the harmful effects of an effluent and also whether or not a company can continue to discharge the waste that is generated as a result of their production. In general, it is easier to reduce the toxicity of a sample if its contents are known. However, if the components of an effluent are unknown, a Toxicity Reduction and Evaluation (TRE) must be conducted.

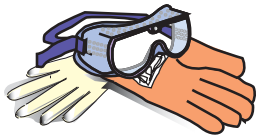
A TRE involves adding certain chemicals to the effluent to alter its toxicity. Depending on what is added, and if there is a significant toxicity reduction, a toxicologist may be able to identify the cause of toxicity. For example, toxicity resulting from heavy metal contamination can be detected by the addition of a divalent cation chelator, such as EDTA. This binds to the metals, thereby making them unavailable to harm the organisms populating a particular body of water. Sometimes an extreme pH value can be toxic to an organism, and the addition of NaOH will raise an acidic pH, and the addition of HCl will lower an alkaline pH. Zeolite is a naturally occurring substance which absorbs different toxic materials, such as ammonia, which is harmful to aquatic organisms.

When toxicity is reduced as a result of adding known chemicals, it becomes possible to determine what may be the harmful ingredient. A toxicologist can then utilize this information to analyze how the toxicity in the effluent may be reduced.

Section II of this experiment utilizes Daphnia magna in a Toxicity Reduction Evaluation by using EDTA to chelate the divalent cation (copper solution toxicant). Two lab groups will add EDTA solution to the toxicant solution, add this combination to the Daphnia, and observe the mortality of Daphnia magna. Lab groups performing Section II will compare their results against lab groups performing Section I (three groups) without EDTA. The entire class will observe and evaluate the potential effectiveness of adding EDTA to reduce pollution by copper or other metals.

EXPERIMENT OBJECTIVE:

The toxicological effect of pollutants in freshwater is determined by using Daphnia magna. This experiment has been adapted from an actual water quality test.



LABORATORY SAFETY

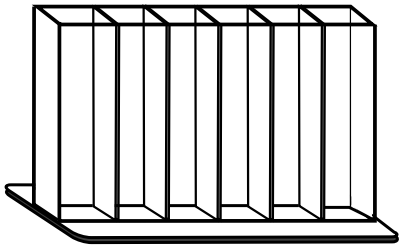
No human materials are used in this experiment. Gloves and safety goggles should be worn as good laboratory practice.

Experiment Procedure

Student Experimental Procedures

SECTION I: DETECTION OF TOXICITY

Preparation and Serial Dilution of Simulated Toxicant

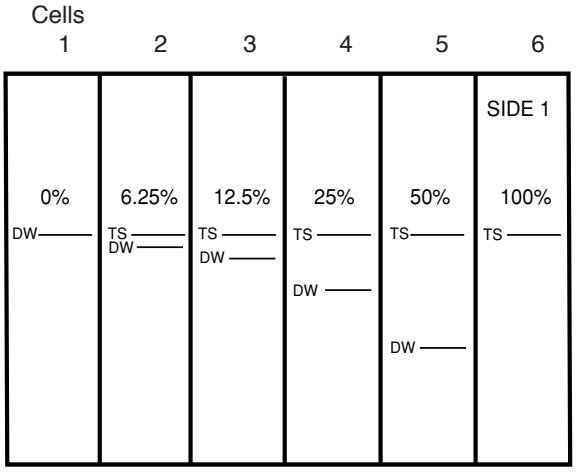


Exposure Chamber

1. Place the Exposure Chamber on a level table with Side 1 facing towards you.
2. With a clean wide bore plastic transfer pipet, quickly transfer six *Daphnia magna* into each of the 6 cells, starting with the control cell (0% toxicant) and ending with 100% toxicant.

   During transfer, carry over as little organism water as possible from the organism beaker or cup (use the pipet to remove any excess water).
3. Use a large, wide bore plastic transfer pipet to add dilution water up to the Dilution Water level (DW) line in each of the cells numbered 1 through 5, as shown in the diagram at lower left.

   Note: Do not add dilution water to Cell #6.



DW = Dilution Water
TS = Toxicant Solution

Exposure of *Daphnia magna* to toxicant

4. Using the same transfer pipet, add toxicant solution up to the Toxicant Solution level (TS) fill lines in cells 2 through 6.

   Note: Do not add toxicant solution to Cell #1.

   The toxicant is a solution which contains copper sulfate ($CuSO_4$). Use the solution as provided by your instructor. At full strength, this solution is 100 parts per million $CuSO_4$.
5. Use the same transfer pipet to stir the toxicant solution and dilution water together in cells 2, 3, 4, and 5.

   Important Reminder: Do not stir Cell #1 (0%) or Cell #6 (100%).

Student Experimental Procedures

In steps 1-3, the serial dilution of toxicant solution ranges between 0 and 100%. The concentrated toxicant solution is serially diluted between 100 and 6.25% by diluting 50% each time. The 0% cell serves as the control.

6. Record the time.

7. Expose the *Daphnia* to the various concentrations of toxicant solution for 45 minutes.

8. After 45 minutes, use a calibrated transfer pipet to add 0.25 ml of the IQ Additive solution (sugar-marker fluorescent substrate) to each of the six cell compartments of the exposure chamber. Carefully and slowly, mix the contents of each cell with a transfer pipet, start with the control cell and work toward the last cell.

9. Allow the *Daphnia* to ingest the IQ Additive for 15 minutes.

10. After 15 minutes, darken the room. Wear protective UV glasses and illuminate the *Daphnia magna* by placing the exposure chamber on top of a source of longwave ultraviolet light (also called "black light").

    CAUTION: Do not use shortwave UV lights for this step. Shortwave UV light can damage eye tissue if eyes are not properly protected.

11. In each cell compartment, count the number of *Daphnia magna* that do not glow at all, or do not glow as brightly as the controls in Cell 1.

12. Record the number in Table 1 on page 12.

13. The concentration at which 50% of the organisms are emitting less light than the controls is the LC50 value for this toxic substance. Estimate the LC50 by following the directions on page 10.

14. Compare your group's results with a group that performs the Toxicity Reduction evaluation.

NOTE:
Daphnia magna have been starved overnight before conducting this experiment.

The best way to illuminate the test chamber is to hold the chamber on top of the longwave UV light source. You may need to slowly move the light up and down the back side of the chamber to accurately count the *Daphnia.*

Student Experimental Procedures

SECTION II: TOXICITY REDUCTION EVALUATION

Preparation & Serial Dilution of Simulated Reduced Toxicant

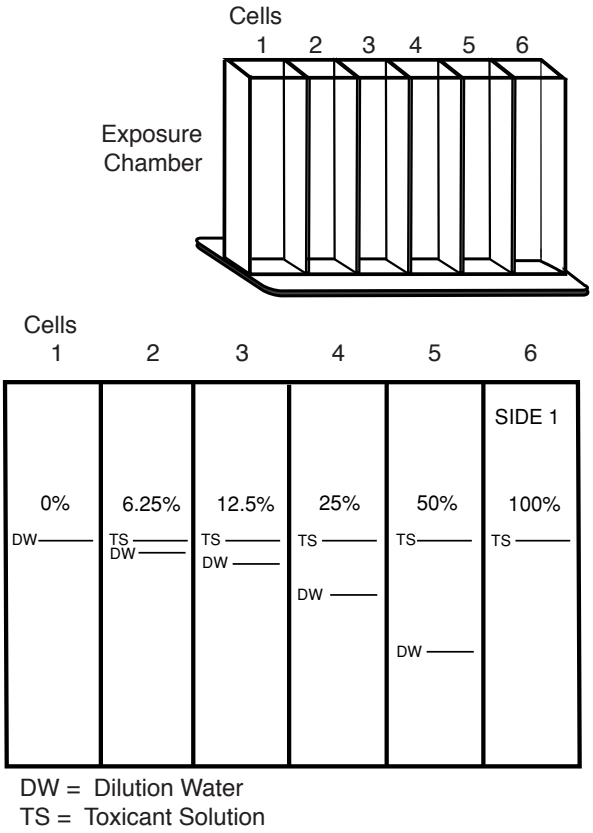
1. Place the Exposure Chamber on a level table with Side 1 facing towards you.

2. With a clean wide bore plastic transfer pipet, quickly transfer six *Daphnia magna* into each of the 6 cells, starting with the control cell (0% toxicity/reduction) and ending with 100% toxicity/reduction.

   During transfer, carry over as little organism water as possible from organism beaker or cup (use the pipet to remove any excess water).

3. Use a large, wide bore transfer pipet to add dilution water up to the Dilution Water level (DW) line in each of the cell compartments numbered 1 through 5, as shown in the diagram at left.

   Note: Do not add dilution water to Cell #6.



Exposure of *Daphnia magna* to Toxicant

4. Using the same transfer pipet, add toxicity reduction solution up to the Toxicant Solution level (TS) fill lines in cells 2 through 6.

   Note: Do not add toxicity reduction solution to Cell #1.

   The toxicity reduction solution is a solution which contains a copper sulfate ($CuSO_4$)/EDTA complex. At full strength, this solution is 100 parts per million $CuSO_4$:100 parts per million EDTA.

5. Use the same transfer pipet to stir the toxicity reduction solution and dilution water together in cells 2, 3, 4, and 5.

   Important Reminder: Do not stir Cell #1 (0%) or Cell #6 (100%).

In steps 1-3 above, the serial dilution of toxicity reduction solution ranges between 0 and 100%. The concentrated toxicant solution is serially diluted between 100 and 6.25% by diluting 50% each time. The 0% cell serves as the control.

Experiment Procedure

Student Experimental Procedures

6. Record the time.

7. Expose the *Daphnia* to the various concentrations of toxicity reduction solution for 45 minutes.

8. After 45 minutes, use a clean calibrated transfer pipet to add 0.25 ml of the IQ Additive solution (sugar-marker fluorescent substrate) to each of the six cell compartments of the exposure chamber. Carefully and slowly mix the contents of each cell with a transfer pipet. Start with the control cell and work toward the last cell.

9. Allow the *Daphnia* to ingest the IQ Additive for 15 minutes.

10. After 15 minutes, darken the room. Wear protective UV glasses and illuminate the *Daphnia magna* by placing the exposure chamber on top of a source of longwave ultraviolet light (also known as "black light").

    CAUTION: Do not use shortwave UV lights for this step. Shortwave UV light can damage eye tissue if eyes are not properly protected.

11. In each cell compartment, count the number of *Daphnia magna* that do not glow at all, or do not glow as brightly as the controls in Cell 1.

12. Record the numbers in Table 2 on page 12.

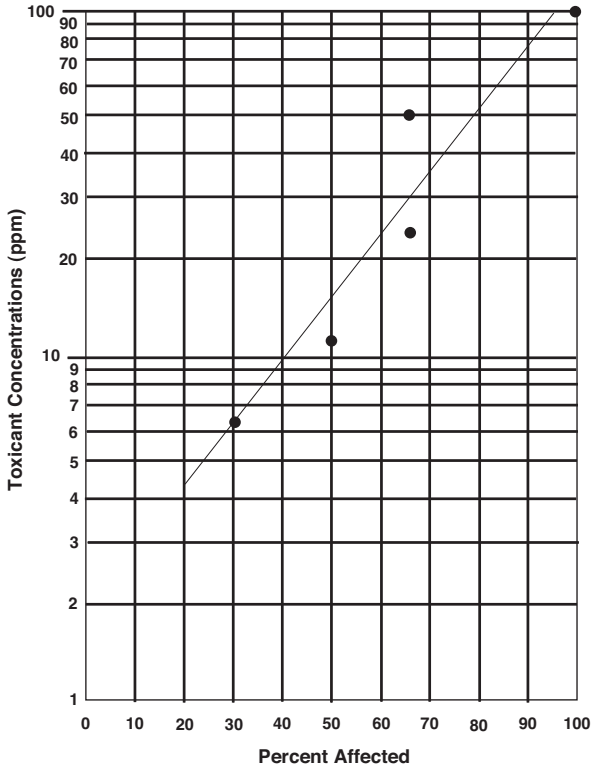
13. The concentration at which 50% of the organisms are emitting less light than the controls is the LC50 value for the copper solution. Estimate the LC50 by following the directions on page 10.

14. Determine whether or not there has been an actual reduction in toxicity by comparing your group's results with a group that performed the experiment without adding EDTA.

The best way to illuminate the test chamber is to hold the chamber on top of the longwave UV light source. You may need to slowly move the light up and down the back side of the chamber to accurately count the *Daphnia.*

Student Experimental Procedures

Example



Concentration of toxicant and Daphnia magna mortality		
% Dilution of Exposure Chamber Cell	Concentration of toxicant Copper solution (ppm)	Number of Daphnia magna emitting less light than controls
0% (Control)	0	---
6.25%	6.25	2
12.5%	12.5	3
25%	25	4
50%	50	4
100% (undiluted)	100	6

SECTION III: DETERMINATION OF LETHAL CONCENTRATION

The lethal concentration at which 50% of the organisms are emitting less light than the controls is the LC50 value for a toxic substance.

Log Concentration Verses Percent Mortality Method

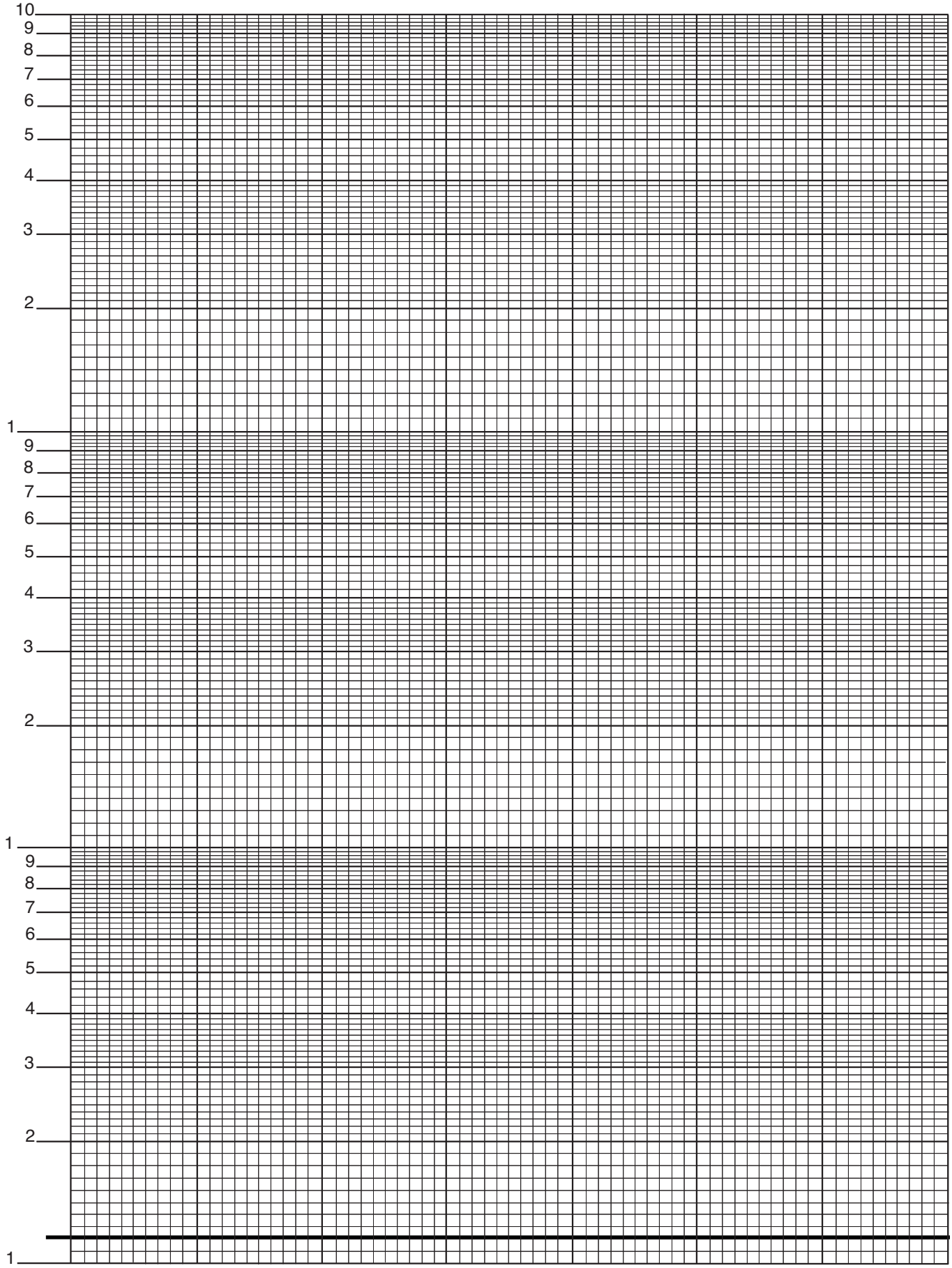
Evaluating the IQ Toxicity Test™* data involves plotting of the data using 2-cycle semi-log graph paper.

1. Label the vertical (logarithmic) y-axis for toxicant concentration (ppm).
2. Label the horizontal (linear) x-axis for percent adversely affected.
3. For each test concentration, plot the corresponding percent affected:

$$\frac{x}{6} \times 100$$

(X = number of *Daphnia magna* emitting less light than controls)

4. Draw the best average "best fit" straight line through all the points (see example at left)
5. From the 50% affected point on the x-axis, draw a vertical line until the it intersects the graph line.
6. From the point of intersection, draw a second line horizontally to the y-axis and determine the LC50 toxicant concentration (in ppm):
7. At what concentration of toxicant (in parts per million) did half the *Daphnia magna* show lethal effects?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Data Record Tables

Table 1: Concentration of toxicant and *Daphnia magna* mortality

% Dilution of Exposure Chamber Cell	Concentration of toxicant Copper solution (ppm)	Number of Daphnia magna emitting less light than controls
0% (Control)	0	
6.25%	6.25	
12.5%	12.5	
25%	25	
50%	50	
100% (undiluted)	100	

Table 2: Toxicity Reduction with EDTA and *Daphnia magna* mortality

% Dilution of Exposure Chamber Cell	Concentration of toxicant Copper solution (ppm)	Concentration of EDTA solution (ppm)	Number of Daphnia magna emitting less light than controls
0% (Control)	---	---	
6.25%	6.25	6.25	
12.5%	12.5	12.5	
25%	25	25	
50%	50	50	
100% (undiluted)	100	100	

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. What concentration of the simulated pollutant in this experiment should be considered "unacceptable"? Why?
2. If the control organisms do not glow brightly, what might be the cause?
3. What toxicological information is obtained from LC50?
4. Does EDTA chelate copper specifically?

Instructor's Guide

Notes to the Instructor & Pre-Lab Preparations

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK® web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800- EDVOTEK (1-800-338-6835).

ORGANIZATION OF THIS EXPERIMENT

This experiment is organized into three sections:

Section I:	Detection of Toxicity
Section II:	Toxicity Reduction Evaluation
Section III:	Determination of Lethal Concentration

Three groups will perform (Section I) the detection of toxicity. Two other groups will perform the toxicity reduction evaluation part of the experiment. The results from their respective experiments can be shared. All five lab groups should complete the graphing activity in Section III with the data they collect.

Safety Data Sheets can be found on our website:
www.edvotek.com/safety-data-sheets

Pre-Lab Preparation of Reagents and Biologicals

Ordering and Care of Live *Daphnia magna*

We recommend purchasing live *Daphnia* from Connecticut Valley Biological Supply Company or another reputable living organism supplier. In order to ensure that the live *Daphnia magna* are healthy enough for the experiment, we recommend scheduling the experiment for the day after they are received. Upon receipt, the *Daphnia* should be prepared for the experiment (see below).

DAY BEFORE EXPERIMENT

Starvation of *Daphnia magna*

Daphnia magna should be starved overnight before conducting this experiment.

1. On the day before the experiment (mid-late afternoon) strain off and discard the shipping water (a clean, fine mesh fish net works well). Quickly suspend Daphnia in fresh spring water into a clean container.

Dilution Water

2. Supply each of the 5 lab groups with a labeled beaker or plastic cup containing 60 ml of Dilution water (bottled spring water). Do not cover the water.

Toxicant Solution

3. Rehydrate the Toxicant pellet by adding 0.5 ml dilution water to tube A and vortex or shake vigorously to dissolve the pellet completely. Transfer all of this solution to 200 ml of Dilution water (bottled spring water).

    - Stir the solution. This is the 100% test solution, which is 100 parts per million toxicant (copper).

    . - Provide 25 ml of this solution in a labeled beaker or plastic cup for the three groups performing the Detection of Toxicity. Place 50 ml of this solution into a clean beaker for the two groups performing the Toxicity Reduction portion of the experiment.

Toxicity Reduction Reagent (EDTA Solution)

4. The two student groups that will perform the Toxicity Reduction portion of the experiment should prepare the Toxicity Reduction solution.

    - Thaw and add 50 µl Toxicity Reduction Reagent (B) (EDTA solution) to 50 ml of the Toxicant Solution prepared in step 2. Mix on stir plate overnight or mix occasionally by hand.

Pre-Lab Preparation of Reagents and Biologicals

DAY OF EXPERIMENT

IQ Additive (fluorescent detection substrate)

5. Add the IQ Additive powder [fluorescent detection substrate (C)] to 15 ml spring water. Rinse the tube with the spring water to remove any remaining powder.

6. Mix for 30 seconds.

7. Label five tubes "IQ Additive"

8. With a clean pipet, dispense 2 ml of IQ Additive suspension into each tube for the 5 lab groups.

Distribution of *Daphnia magna*

9. Supply each of the 5 lab groups with a beaker or plastic cup containing at least 40 *Daphnia magna* in dilution water.

   - Start by pouring 30 ml of dilution water into each of the 5 containers.
   - Using a wide bore pipet, transfer *Daphnia* into each container. Count at least 40 *Daphnia* per container.
   - Allow the organisms to acclimate in these containers for at least 30 minutes before students transfer them into the exposure chambers.

Groups performing Detection of Toxicity should receive:

60 ml Dilution water
25 ml Diluted toxicant solution
2 ml IQ Additive

1 beaker/cup containing 40 Daphnia magna
2 Wide bore plastic transfer pipet
1 Calibrated transfer pipet
1 Exposure chamber

Groups performing Detection of Toxicity should receive:

60 ml Dilution water
25 ml Toxicity Reduction solution
2 ml IQ Additive

1 beaker/cup containing 40 Daphnia magna
2 Wide bore plastic transfer pipet
1 Calibrated transfer pipet
1 Exposure chamber

NOTE TO INSTRUCTOR

Students can be encouraged to develop strategies for introducing their own toxicants in laboratory experiment extensions. Examples include cations of iron, zinc, manganese, acidic and basic solutions.

Please refer to the kit insert for the Answers to Study Questions