



The Biotechnology Education Company ®

EDVO-Kit
856

**Environmental Toxicity
Response in *C. elegans***

**Storage: See Page 3 for specific storage
instructions.**

EXPERIMENT OBJECTIVE:

The objectives of this experiment are to observe and compare the effects of heavy metals found in the environment on normal and mutant strains of *Caenorhabditis elegans* (*C. elegans*).

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Environmental Toxicity Response in *C. elegans*

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Material Safety Data Sheets can be found on our website www.edvotek.com

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This experiment is designed for 10 groups.

Experiment Components

A	<i>C. elegans</i> Normal	Room Temperature
B	<i>C. elegans</i> Toxicity mutant	Room Temperature
C	<i>E.coli</i> OP50 Bactobeads™	Room Temperature
D	S- Buffer	4° C Refrigerator
E	CuSO ₄	Room Temperature
F	CdCl ₂	Room Temperature
G	ZnSO ₄	Room Temperature
H	ReadyPour NGM Agar	4° C Refrigerator
I	NGM Medium Salts	4° C Refrigerator

Storage

Store components listed below at room temperature.

- Petri Dishes
- Large Transfer Pipets
- Small Transfer Pipets
- 1.5 ml Microcentrifuge Tubes
- Counting Chamber Microscope Slides
- Sterile Loops
- 15 ml centrifuge tubes

Requirements

- Covered box
- Microscopes
- Timers
- 70% Ethanol
- Waterbath
- Incubator (optional)

Background Information

Caenorhabditis elegans (*C. elegans*)

Caenorhabditis elegans is a small (~1mm) transparent, unsegmented nematode that lives in temperate soils. *C. elegans* is a multicellular organism which goes through a complex growth starting as an embryo, undergoing a morphogenesis and developing into a fully formed adult. It exhibits specific behaviors and is even capable of rudimentary learning. It produces sperm and eggs, mates and reproduces. After reproduction it gradually ages, loses vigor and finally dies. *C. elegans* is a model organism for the study of embryogenesis, morphogenesis, development, nerve function, behavior and aging, and how these processes are regulated by genes. *C. elegans* is a eukaryotic organism having simple membrane bound organelles and is one of the simplest animals with a neural system.

C. elegans makes a good model system for the study of many biological processes. The worms are easy and inexpensive to maintain on solid media in Petri dishes or in liquid culture. They have a life span of 2-3 weeks and progress from egg to fully developed animal in a few days allowing for greatly accelerated studies. Each worm consists of 959 somatic cells whose development have been completely mapped, 300 of these cells are neuronal cells.

C. elegans shares many essential biological characteristics of higher animals and humans. Neural structures include a battery of sense organs in the head which mediate responses to taste, smell, temperature and touch—and although *C. elegans* has no eyes, it might respond slightly to light. Among other neural structures is an anterior nerve ring with a ventral nerve cord running back down the body. (There is also a smaller dorsal nerve cord.) These features have led to an increased use of *C. elegans* in toxicology studies, both in the study of how the toxins affect the neural system and as a high-throughput system to determine the potential toxicity of substances.

Metals In The Environment

Humans require trace amounts of many metals for the correct function of biological pathways. The trace amounts of these beneficial metals can be provided by a normal diet or are in most multi-vitamin supplements. Other metals are acutely poisonous due to their interference with normal biological pathways or the body is unable to remove them efficiently. A subset of metals and some of the more toxic examples, are the heavy metals. Heavy metals are defined as chemical elements that have a density of 4 – 5 times the density of water. This group of toxic metals includes arsenic, cadmium, mercury and aluminum. These elements become toxic when they are not metabolized and accumulate in the tissues. Heavy metals can enter the body through ingestion of contaminated food or water, absorption through the skin due to contact with a contaminated surface or inhalation from contaminant in the air. Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that



Background Information

mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Allergies are not uncommon and repeated long-term contact with some metals or their compounds may even cause cancer. Some of the beneficial heavy metals can also become toxic at high concentration.

It is almost impossible to avoid all contact with toxic heavy metals in the environment, they exist at low levels normally and they are used in the creation of many products. Exposure can occur due to heavy metal contamination in the environment, use of products produced with heavy metals or in products where the heavy metal exists as a contaminate in the raw materials. There are government regulations that restrict the amount of heavy metal exposure is allowed in each of these exposure methods. Many groups monitor the environment for heavy metal contamination in food, water or the air. Companies using heavy metals in processes have to comply with regulations to prevent exposure to workers and the environment. Studies with model animals are used to better understand the toxicity of heavy metals.

C. elegans As A Model Organism For The Study Of Heavy Metal Toxicity

C. elegans has been used as a model organism for toxicity testing of pharmaceuticals, chemicals and compounds for several years. With a short lifespan and simple and inexpensive culture conditions *C. elegans* is well suited for the study of the effects of exposure to the neural system and potential developmental effects.

The increasing exposure of the environment to an ever increasing number of man-made and natural chemicals effects all the organisms in the ecosystem. Testing with *C. elegans* provides a high throughput bioassay platform that is accepted by the OECD (Organization for Economic Cooperation and Development) and EPA (Environmental Protection Agency).

C. elegans is a valuable toxicity model as it's results are predictive of outcomes in higher eukaryotes. There is a large body of evidence that at both the level of genetic and physiological processes there is a high level of similarity between *C. elegans* and higher eukaryotes. Many of the basic physiological processes and stress responses that are observed in higher organisms (e.g., humans) are conserved in *C. elegans*. *C. elegans* is an important model in the study of neurotoxicology and genetic toxicology often as the first set of experiments testing the toxicology of new compounds.

Experiment Overview

BEFORE YOU START THE EXPERIMENT

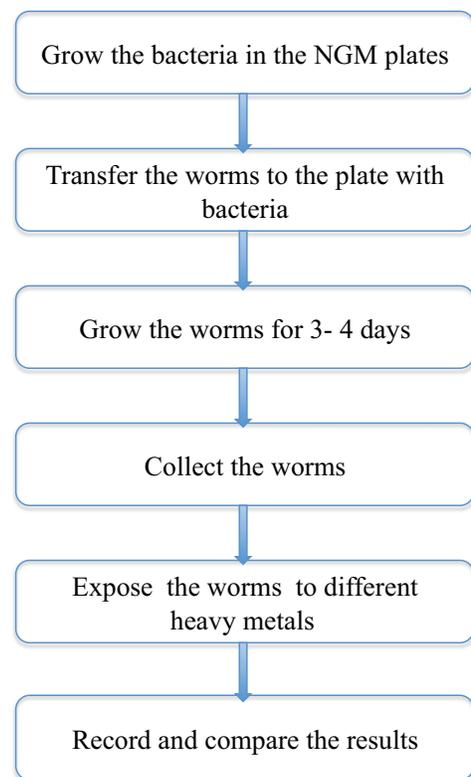
1. Read all instructions before starting the experiment.
2. Culture *C. elegans* for several days to increase the number of animals for the experiment.
3. Write a hypothesis that reflects the experiment and predict experimental outcomes.

EXPERIMENT OBJECTIVE:

The objectives of this experiment are to observe and compare the effects of heavy metals found in the environment on normal and mutant strains of *Caenorhabditis elegans* (*C. elegans*).

BRIEF DESCRIPTION OF EXPERIMENT:

In this experiment, each group of students will treat *C. elegans* worms with heavy metal solutions and record the movements of an individual worm when exposed. The results for several groups can be compared to illustrate the toxicity of heavy metals.



Student Experimental Procedures

LABORATORY SAFETY



Important READ ME!

This experiment involves the use of toxic materials. Care should be taken in aliquoting, mixing and handling any solutions containing heavy metals. Always use personal protective equipment when using hazardous materials. These chemicals can be toxic if taken internally.

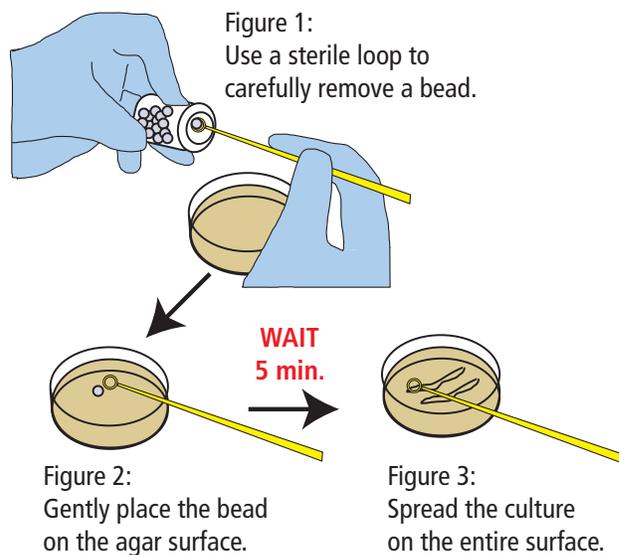
1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment which is used in conjunction with the heating and/or melting of reagents.
3. Do not mouth pipet reagents - use pipet pumps or bulbs.
4. The *C. elegans* used in this experiment is not considered pathogenic. Although it is rarely associated with any illness in healthy individuals, it is good practice to follow simple safety guidelines in handling and disposal of materials contaminated with worms.
5. Properly dispose materials after completing the experiment:
 - A. Wipe down the lab bench with a 10% bleach solution or a laboratory disinfectant.
 - B. All materials, including petri plates, pipets, transfer pipets, loops and tubes, that come in contact with worms should be disinfected before disposal in the garbage. Disinfect materials as soon as possible after use in one of the following ways:
 - Autoclave at 121° C for 20 minutes.
Tape several petri plates together and close tube caps before disposal. Collect all contaminated materials in an autoclavable, disposable bag. Seal the bag and place it in a metal tray to prevent any possibility of liquid medium or agar from spilling into the sterilizer chamber.
 - Soak in 10% bleach solution.
Immerse petri plates, open tubes and other contaminated materials into a tub containing a 10% bleach solution. Soak the materials overnight and then discard. Wear gloves and goggles when working with bleach.
6. Wear gloves, and at the end of the experiment, wash hands thoroughly with soap and water.

Student Experimental Procedures

Note: Modules I and II will be done during Week 1, and Modules III and IV will be done during Week 2 of the experiment. See page 13 for more details.

MODULE I: GROWTH OF BACTERIA

1. Obtain a NGM plate.
2. With the help of sterile loop, place a bead into the hole of the loop (Figure 1).
3. Carefully remove 1 single bead and gently place it on the agar surface (Figure 2).
4. Wait for 5 minutes until the bead dissolves on the agar surface.
5. Use the loop to spread the culture on the entire surface of the agar (Figure 3).
6. Incubate the plate at room temperature overnight.

MODULE II: CULTIVATION OF *C. elegans*

1. Obtain one NGM plate containing the overnight growth of *E. coli* OP50 cells. Each group will use one plate to grow the worms (Normal or Toxicity mutant).
2. Remove a chunk of agar from the assigned *C. elegans* (Normal or Toxicity mutant) plate. Use a sterile loop to cut a small portion of the agar with worms on the surface and transfer the "chunk" to the center of the freshly seeded labeled NMG plate (Figure 4). (Check plate under microscope to confirm the presence of worms).
3. Set the plate aside to grow at room temperature inside of a box for 3-4 days until you have enough worms to start the exposure experiments.

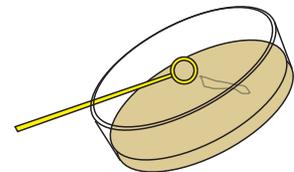


Figure 4

Student Experimental Procedures

MODULE III: COLLECTION OF *C. elegans*

1. Check growth of worms under a microscope. Worms are visible at 40X magnification. Place on an inverted microscope and observe. If the plate contains 50 or more worms, proceed with the experiment. If the plate contains less than 50 worms, continue incubation. (When using a standard compound microscope, turn the plate upside down on the stage and focus through the agar to view the worms on the surface.)
2. Transfer 3.5 ml S-buffer to the Petri dish with a large transfer pipet (Figure 5).
3. Rinse the dish with the buffer and transfer the worms to 15 ml centrifuge tube with a large pipet (Figure 6).
4. Allow the worms to settle to the bottom of the tube (~15 minutes) and remove 3 ml of the cleared buffer with a large pipet (Figure 7). (Be careful to avoid removing the worms, leave ~500 μ l of worms and buffer in the bottom of the tube, check tube graduations.)
5. Mix to resuspend the worms and transfer 1 drop of the worms (~20 μ l) into each labeled 1.5 ml tube with a small transfer pipet (Figure 8). Label tubes Control, CU, CD and ZN. (Note: each tube will take ~10 minutes to count) so the number of samples can be adjusted based on the time available. Keep tubes at room temperature.

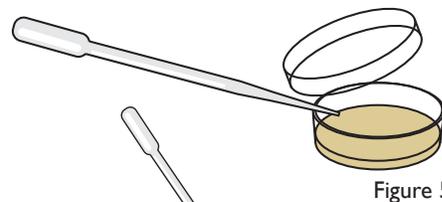


Figure 5

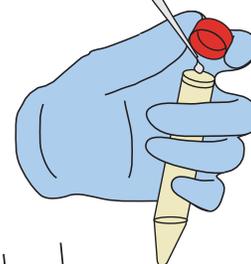


Figure 6



Figure 7

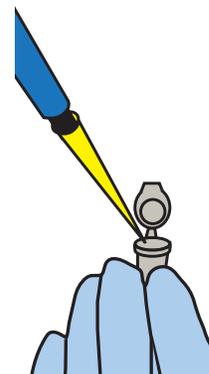


Figure 8

Student Experimental Procedures

MODULE IV: EXPOSURE OF *C. elegans* To Heavy Metal Solutions

Important READ ME!

Complete steps 1-4 for each tube individually. Only add the heavy metal solutions to one sample at a time and complete the experiment for that sample before proceeding to the next sample.

1. To a tube add 100 μ l sample (S-Buffer or heavy metal solution).
2. Cap the tube and mix by tapping the bottom of the tube.
3. With a small pipet transfer 1 drop to the opening of one chamber on the counting plate. Allow the solution to move into the chamber by capillary action.
4. Observe movement of a single worm under the microscope. Count the number of "thrashes" that occur for 30 seconds. Wait 2 minutes and repeat the count for a total of three counts (use the same worm for each count). For each group one member will observe and count the "thrashes" on the animal and another member will time the count.
5. Check the chambers after all the samples have been counted and note the appearance of the worms.
6. Record all your observations in your notes including the counts and a description of the appearance of the worms.

	Thrash Normal Control	Thrash Mutant Control	Thrash Normal CdCl ₂	Thrash Mutant CdCl ₂	Thrash Normal CuSO ₄	Thrash Mutant CuSO ₄	Thrash Normal ZnSO ₄	Thrash Mutant ZnSO ₄
Trial #1 (30 seconds)								
Wait 2 min								
Trial #2 (30 seconds)								
Wait 2 min								
Trial #3 (30 seconds)								

Student Experimental Procedures

LABORATORY NOTEBOOK DATA RECORDING:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

- Record (draw) your observations, or photograph the results.

Following the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Answer these questions **BEFORE** analyzing your results.

1. How would you expect the worms to react to the heavy metal solutions? Explain.
2. Which heavy metal solution will have the greatest reaction on the worms? Explain.
3. What factors could affect the worm's reaction to the heavy metal solutions?

Data Collection

- Compare the movement and behavior of the worms in each chamber.
- What do you notice about how the worms move?
- What do you think is happening to the worms?
- What differences do you observe in the worms reaction to heavy metal solutions (compare large and small worms, different concentrations)?
- What are the effects of each heavy metal solution on the worms? How do these effects change over time?

Study Questions

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

1. *Canorhabditis elegans* is used as a model system for the study of the nervous system. Why?
2. What other major system is *C. elegans* a model system for?
3. What are some of the benefits of using *C. elegans* in toxicology studies?
4. What is the visible result of exposure to the toxic solutions for a short period (30 sec.) versus longer term exposure (greater than 5 minutes)?
5. How do these results apply to humans?

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Instructor's Guide

Experiment

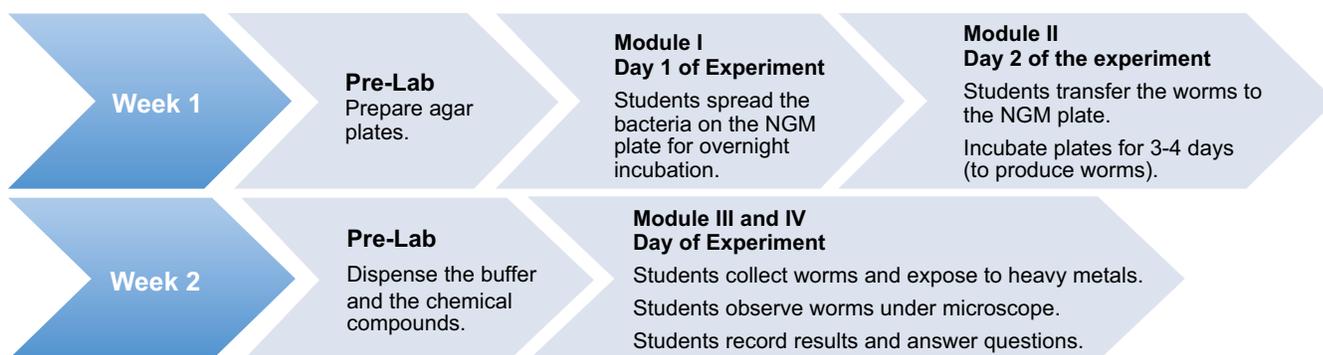
Important READ ME!

This experiment involves the use of toxic materials. Care should be taken in aliquoting, mixing and handling any solutions containing heavy metals. Always use personal protective equipment when using hazardous materials. These chemicals can be toxic if taken internally.

ORGANIZING AND IMPLEMENTING THE EXPERIMENT

Class size, length of laboratory sessions, and availability of equipment are factors which must be considered in the planning and the implementation of this experiment with your students.

The guidelines that are presented in this manual are based on ten laboratory groups consisting of two, or up to four students. The following are implementation guidelines, which can be adapted to fit your specific set of circumstances. If you do not find the answers to your questions in this section, a variety of resources are available at the EDVOTEK web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call 1-800-EDVOTEK for help from our knowledgeable technical staff.



*The INSTRUCTOR decides which day of the week to begin the experiments, taking into account the total time required for the experiments.

Notes to the Instructor

NATIONAL CONTENT AND SKILL STANDARDS

By performing this experiment, students will develop skills necessary to do scientific inquiry, learn new techniques using a microscope, and will learn standard procedures used in *C. elegans* cultivation. Observation and analysis of the experiments will provide students the means to transform an abstract concept into a concrete explanation.

LABORATORY NOTEBOOKS

It is highly recommended that students maintain a laboratory notebook to formulate hypotheses and to record experimental procedures and results.

- Guidelines for keeping a laboratory notebook are available at the EDVOTEK web site.



Pre-Lab Preparations

ONE WEEK BEFORE THE LAB

Plates can be prepared one week prior to the day of the lab. Store plates in the refrigerator at 4° C).

- For optimal results, prepare plates two days prior to plating and set aside the plates inverted at room temperature.
- If they are poured more than two days before use, they should be stored inverted in the refrigerator. Remove the plates from the refrigerator and store inverted for two days at room temperature before use.

Heat the ReadyPour™ Medium

1. Equilibrate a waterbath at 60° C for step 5 below.
2. Loosen, but do not remove, the cap on the ReadyPour™ medium bottle to allow for the venting of steam during heating.
3. Squeeze and vigorously shake the plastic bottle to break up the solid agar into chunks
4. Heat the bottle of ReadyPour™ medium by one of the methods outlined below. When completely melted, the amber-colored solution should appear free of small particles.
 - A. Microwave method:
 - Heat the bottle on High for two 30 seconds intervals.
 - Using a hot glove, swirl and heat on High for an additional 25 seconds, or until all the ReadyPour™ medium is dissolved.
 - Using a hot glove, occasionally swirl to expedite melting.
 - B. Hot plate or burner method:
 - Place the bottle in a beaker partially filled with water.
 - Heat the beaker to boiling over a hot plate or burner.
 - Using a hot glove, occasionally swirl to expedite melting.
5. Allow the melted ReadyPour™ medium to cool. Placing the bottle in a 60° C water bath will allow the agar to cool, while preventing it from prematurely solidifying.

When the ReadyPour™ medium reaches approximately 60° C, the bottle will be warm to the touch but not burning hot.

6. Add 12 ml of NGM Medium Salts. Swirl the medium to mix.



Wear Hot Gloves and Goggles during all steps involving heating.

Caution:

Failure to loosen the cap prior to heating or microwaving may cause the ReadyPour medium bottle to break or explode.

Pre-Lab Preparations

Pour the Plates

Note: The single bottle of agar medium will be used to make the required number of plates.

7. Use a 10 ml pipet and pipet pump to pour the plates 8 ml each, with the ReadyPour medium.
8. Allow the agar to cool and resolidify.

If you have extra sterile petri plates on hand, use any remaining medium to pour additional plates for the optional activity described on page 18.

Note: If plates will be used within two days, store in a sealable plastic bag so the plates will not dry out. Store at room temperature, inverted.

Reminder:

Follow proper procedures for disposal of contaminated materials.

Quick Reference: Pouring Agar Plates

- Use a sterile 10 ml pipet with a pipet pump to transfer the designated volume of medium to each petri plate. Pipet carefully to avoid forming bubbles.
- Rock the petri plate back and forth to obtain full coverage.
- If the molten medium contains bubbles, they can be removed by passing a flame across the surface of the medium.
- Cover the petri plate and allow the medium to solidify.



Pre-Lab Preparations

WEEK 1

Day 1 of the Experiment - Module I: Growth of Bacteria

1. Dispense 1 Bactobead™ into each microcentrifuge tube just prior to the start of the experiment. Distribute one tube per student group.
2. Distribute to each student group: one NGM agar plate and one sterile loop.

Day 2 of the Experiment - Module II: Cultivation of *C.elegans*

1. There are two source plates – one *C. elegans* Normal and one *C. elegans* Toxicity mutant plate.
2. Students can be divided into 10 groups. Five student groups are designated to share the *C. elegans* Normal plate, and the other five student groups are designated to share the *C. elegans* Toxicity mutant plate for their experiment.
3. Distribute the appropriately labeled *C. elegans* plate to the groups of students. (They will get a chunk of the agar containing the *C. elegans* for their experiments.)
4. Distribute to each student group one loop.

WEEK 2

Day of the Experiment - Module III & IV: Exposure of *C.elegans* to Chemicals in the Environment

1. Dispense 5 ml S-Buffer into ten microcentrifuge tubes labeled "S-Buffer". (This buffer is for recollecting the worms in Module III.)
2. Dispense 100 µl of each heavy metal (CuSO₄, CdCl₂ and ZnSO₄) into ten microcentrifuge labeled tubes (Module IV).
3. Dispense 100 µl dH₂O into ten microcentrifuge tubes labeled "dH₂O" (Module IV).
4. Distribute to each group 2 large sterile pipets, 8 small pipets, a counting chamber, four microcentrifuge tubes and one tube of each heavy metal, dH₂O and S-Buffer (Module IV).

Pre-Lab Preparations

OPTIONAL ACTIVITY

The experiment can be repeated using a different compound or chemical to test the toxicity of the chemical or compound on *C. elegans*. This experiment would require preparation of additional plates of worm cultures. The worms can be maintained by passaging ("chunking" a portion of a culture from an old plate to a new plate). The counting plate contains enough chambers for 10 experimental counts.



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