RESET AN AEOP EFFORT aeop

Toxins in the Environment

1 hr teacher preparation time
45-min class periods

Authors

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SUBJECT/GRADE LEVEL

Life Science, Environmental Science
Grades: 9-12

Materials

- Seed corn, any variety, but NOT Roundup® ready
- Resealable plastic sandwich bags, 6 per group
- Sharpie or permanent marker for labeling
- Absorbent paper towels
- Sodium Hypochlorite (bleach) solution (5.25%) to disinfect seed corn, found in most grocery stores
- Roundup® Weed and Grass Killer Concentrate Plus (or any other herbicide with glyphosate as active ingredient), found in most national hardware stores
- Disposable graduated pipettes, 1 per group
- Germination mat or another warm, dry location such as an incubator, sunny windowsill, cabinet, etc.
- Safety equipment (gloves, goggles and lab aprons (if desired)

STANDARDS

Next Generation Science Standards

- HS-LS1-4 From Molecules to Organisms: Structures and Processes: Students who demonstrate understanding can use a model to illustrate the role of cellular division (mitosis) and differentiation
- HS-LS2-1 Ecosystems: Interactions, Energy and Dynamics: Use mathematical and/or computational representations to support explanations of factors that affect the carrying capacity of ecosystems at different scales.

Mathematics Standards | Common Core State Standards

- CCSS. MATH.CONTENT.HSS.ID.A.4 Use the mean and standard deviation of a data set to fit it to a normal distribution and to estimate population percentages. Recognize that there are data sets for which such a procedure is not appropriate. Use calculators, spreadsheets, and tables to estimate areas under the normal curve.
- CCSS.MATH.CONTENT.HSS.ID.B.6.C Fit a linear function for a scatter plot that suggests a linear association.

English Language Arts Standards | Common Core State

- CCSS.ELA-LITERACY.RST.9-10.3 Follow precisely a complex multistep procedure when carrying out experiments, taking measurements, or performing technical tasks, attending to special cases or exceptions defined in the text.

LESSON OBJECTIVE(S)

Students design and run an experiment to test various concentrations of a toxin on the germination rate of a model organism and through analyzing the data and research literature will recommend usage/non-usage of toxins based.

CAREER CONNECTION(S)

Mentor Background:

Dr. Erik Mylroie - ERDC - Vicksburg, MS
Graduated with B.S. in Biology from Millsap College, and PhD in Molecular Biology from Mississippi State University. Dr. Mylroie has been working at ERDC since October of 2016 and has exclusively been working with the ZFET and Molecular Toxicology since starting here. His main research interests include molecular breeding in maize and currently is focusing on ecological toxicology.

Catherine Steward - ERDC - Vicksburg, MS
Graduated with a B.S. in Biological Sciences from Mississippi State University. Mrs. Steward has been working in the ZFET lab since September of 2019, first as an ORISE fellow and now as a Bennett Aerospace Biological Researcher. Along with the PFOS Multigenerational Embryo Study, she is also co-investigating mitochondrial stress responses in zebrafish embryos using the Agilent Seahorse Extracellular Flux Analyzer and optimizing assay parameters.

Dr. Natalia G. Vinas - ERDC- Vicksburg, MS
Graduated with a B.S. in Biology from University of Girona, a M.S. in Nutrition and M.S. in Genetics from the University of Barcelona, and a PhD in Molecular Genetics from the University of Barcelona. Dr. Vinas has been working at ERDC since 2014 and working with the ZFET lab since 2015. She has been working with fish for over 20 years focusing on investigating molecular links in adverse conditions. Her main research interests include: the impacts of sub-lethal contaminants and other stressors on water quality, aquatic organisms and human health, collaborating with U.S. government agencies and International agencies for establishing new regulatory guidelines and approaches for toxicology.

Related STEM Careers:

Toxicologist: a scientist who has a strong understanding of many scientific disciplines, such as biology and chemistry, and typically works with chemicals and other substances to determine if they are toxic or harmful to humans and other living organisms or the environment.

Aquatic Fisheries Manager: a person that raises fish and shellfish in ponds, floating net pens, raceways, and recirculating systems. They stock, feed, and maintain aquatic life used for food and recreational fishing.

Farmer/Agricultural Manager: a person that runs farms that are primarily family owned. Those who do not own the land themselves may lease it from a landowner to operate as a working farm. Agricultural Managers take care of the day-to-day operations of one or more farms, ranches, nurseries, timber tracts, greenhouses, and other agricultural establishments for corporations, farmers, and owners who do not

live and work on their farm or ranch. Agricultural managers usually do not participate directly in production activities. Instead, they hire and supervise farm and livestock workers to do most of the daily production tasks. Managers may determine budgets and decide how to store, transport, and sell crops. They also may oversee the maintenance of equipment and property.

Analytical Chemist: a person that determines the structure, composition, and nature of substances by examining and identifying their various elements or compounds. They also study the relationships and interactions among the parts of compounds. Some analytical chemists specialize in developing new methods of analysis and new techniques for carrying out their work. Their research has a wide range of applications, including food safety, pharmaceuticals, and pollution control.

Statistician: a person (mathematician or statistician) that analyzes data using specialized statistical software. In their analyses, mathematicians and statisticians identify trends and relationships within the data. They also conduct tests to determine the data's validity and to account for possible errors. Some help write software code to analyze data more accurately and efficiently.

Soil Scientist: a person that examines the composition of soil, how it affects plant or crop growth, and how alternative soil treatments affect crop productivity. They develop methods of conserving and managing soil that farmers and forestry companies can use. Because soil science is closely related to environmental science, people trained in soil science also work to ensure environmental quality and effective land use.

Environmental Engineer: a person that uses the principles of engineering, soil science, biology, and chemistry to develop solutions to environmental problems. They work to improve recycling, waste disposal, public health, and water and air pollution control. They also address global issues, such as unsafe drinking water, climate change, and environmental sustainability.

Chemical Engineer: a person that solves problems that involve the production of chemicals in food, fuel, drugs, and a wide array of other products through the use of chemistry, biology, physics, and math.

Veterinarian: a doctor that diagnoses illnesses and injuries and provides medical care to animals.

ENGAGEMENT 15 minutes total

Brief Description:

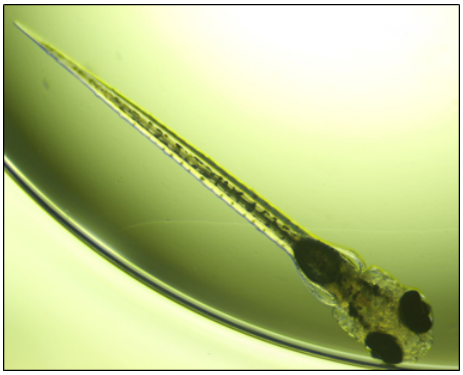
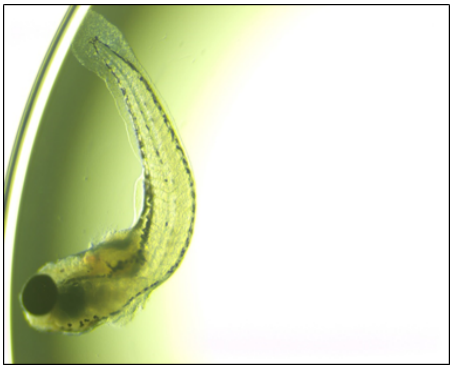
1. Students will compare a zebrafish embryo under normal conditions at 120 hpf to a zebrafish embryo exposed to a toxin (PFOS) at 120 hpf. Students will be asked to describe the differences between the two embryos and provide a possible cause for the differences.
2. Then, students will use a formative assessment probe to elicit their thinking and/or preconceptions about mutations, genes, and embryonic development.

Procedures:

Timing: Students should be given 3 minutes to look at the pictures and 2 minutes to describe the differences.

Next: Students should be given 4 minutes to read the statements, pick a statement they agree with, and use 6 minutes to draft their opinion/argument.

Content Background: Zebrafish embryos go through normal stages of vertebrate development starting with fertilization and forming a diploid zygote. Zygotes mitotically divide and form the blastocoel, internal tissue layers, gastrulation (creating the gastrointestinal channel), and finally neurulation (development of the nervous cord and nerves). The embryo at 24 hpf should show the heart, the blood pumping through the veins and arteries, the yolk sac, and the characteristic "tail flip/twitch" which indicates an active and properly functioning nervous system.

Control embryo 120 hpf	Embryo exposed to PFOS 120 hpf
	

EXPLORATION

Brief Description: Students will expose seed corn to various concentrations of glyphosate, the active ingredient found in Roundup® to determine the effects of glyphosate on seed corn germination rates. Using this data, students will calculate the rate of seed germination at each toxin concentration and determine which concentration still yields productive plants and which toxin concentrations are above the LD50 value.

A further extension can be created where students plant the germinated seeds and grow the corn into adult plants. They will harvest and germinate the F1 generation to see if any effects of the glyphosate toxin affected the next generation.

*A different model organism to use for a multi-generational study is Wisconsin Fast Plants®.

Procedures:

Teacher: After obtaining the seed corn, the teacher will need to sanitize the seeds by soaking them in a 50/50 solution of bleach for 5 minutes. These seeds should be rinsed two times and then allowed to dry on paper towels. This can be done a day in advance, up to 10 minutes before the seeds are required (like between class transitions).

The teacher will need to purchase Roundup Weed and Grass Killer, Concentrate Plus® from any major hardware store (Home Depot, Lowes, ACE, etc) and make serial dilutions. We used the Roundup Concentrate Plus® with 18% Glyphosate Isopropylamine salt. See ***Appendix A*** for how to measure, calculate and create the serial dilutions stock used for the lab groups. The teacher will need enough of the serially diluted stock solution to perform two rounds of the experiment (corn seed germination and green pea respiration).

Students: Full lab handout available for photocopy/printing in ***Appendix B***

Students will need the following equipment for the exploration (per lab group):

5 teacher created Glyphosate solutions of various concentrations (5 - 10mL each container)
Distilled water for the control group (5-10mL)
6 containers to soak the corn seeds for 5 minutes while labeling bags
One 5mL disposable pipette
6 plastic resealable sandwich bags
Sharpie® or permanent marker
Paper towels
Gloves and goggles
Pencil/pen and lab instructions

EXPLANATION

Brief Description: Students will answer a series of questions within the lab activity to determine data and graph the data

Procedures: Students will share data via Google Sheets, or Microsoft Excel to gather data from the other lab groups and graph the data for their experiment and use the average data to use as a comparison.

ELABORATION

Brief Description: Student will use their understanding of the corn and Roundout (glyphosate) to extend an experimental procedure to test vertebrates and PFOS

Procedures: Students would identify the independent, dependent variables and establish

EVALUATION

Brief Description: Students will do either a lab report or poster gallery walk (similar to a professional poster presentation) where they will explain their materials and procedures, the experimental variables, the experimental control groups, their recorded data and analyzed graphs, a summary of their findings and any other relevant information. A good resource is the University Library System of New York University found at: https://guides.nyu.edu/posters

Procedures: Scientific Poster Presentations are how scientists present their research findings and other related information from their experiment. Usually, scientists present their posters in a formal setting at various conferences that are related to their field of study, their professional organization memberships, or through symposia (a gathering of scientists to present and discuss their research with other scientists). Posters are colorful, have relevant data (even if the data gathering is still ongoing), and have all the basics of a typical lab report.

You are going to create a poster presentation of your lab information. You will include your experimental setup, your data tables, and graphs, your hypothesis, and your analysis. Any pictures you have collected during your experiment can be included in your poster to highlight a particular technique, equipment, or results you feel are helpful to the presentation.

Posters are usually colorful and are about 16 x 18 inches, but you will use any poster-sized paper provided or recommended by your teacher.

REFERENCES

- AAT Bioquest, Inc. (2021, July 15). *Quest Calculate™ Serial Dilution Calculator and Planner .".* Retrieved from https://www.aatbio.com/tools/serial-dilution
- New York University Libraries. (2021, April 1). How to Create a Research Poster: A Guide to creating a research poster. Retrieved from https://guides.nyu.edu/posters
- Kimmel, C. B., Ballard, W. B., Kimmel, S. R., Ullmann, B., & Schilling, T. F. (1995). Stages of Embryonic Development of the Zebrafish. *Developmental Dynamics*, *203*, 253–310.
- U.S. Environmental Protection Agency (EPA). (2017, November). *Technical Fact Sheet - Perfluorooctane Sulfonate (PFOS) and Perfluorooctanoic Acid (PFAS)*. 1–8.
- Helander, M., Pauna, A., Saikkonen, K., & Saloniemi, I. (2019). Glyphosate residue in soil affect crop plant germination and growth. *Nature*, (9), 19653. doi:https://doi.org/10.1038/s41598-019-56195-3

APPENDIX A

To prepare the following serial dilutions, obtain Roundup Weed and Grass Killer Concentrate Plus® (16oz/473mL) which contains at least 18% glyphosate isopropylamine salt.

Dilution	Roundup (Glyphosate Isopropylamine salt) (mL)	Distilled Water (or Deionized Water) (mL)	[Roundup (Glyphosate Isopropylamine salt)] (M)
1	466.67 (from 0.65 M stock)	0	0.65
2	46.67 (from dilution 1)	420	0.065
3	46.67 (from dilution 2)	420	6.5×10^{-3}
4	46.67 (from dilution 3)	420	6.5×10^{-4}
5	46.67 (from dilution 4)	420	6.5×10^{-5}

1. Pipette 466.67 mL of 0.65 M Roundup (Glyphosate Isopropylamine salt) into 0 mL of Distilled Water (or Deionized Water). Mix well before continuing. Avoid generating bubbles.

2. Pipette 46.67 mL of dilution 1 (from step 1) into 420 mL of Distilled Water (or Deionized Water) to create dilution 2. Mix well before continuing. Avoid generating bubbles.

3. Pipette 46.67 mL of dilution 2 (from step 2) into 420 mL of Distilled Water (or Deionized Water) to create dilution 3. Mix well before continuing. Avoid generating bubbles.

4. Pipette 46.67 mL of dilution 3 (from step 3) into 420 mL of Distilled Water (or Deionized Water) to create dilution 4. Mix well before continuing. Avoid generating bubbles.

5. Pipette 46.67 mL of dilution 4 (from step 4) into 420 mL of Distilled Water (or Deionized Water) to create dilution 5. Mix well before continuing. Avoid generating bubbles. Discard 46.67 mL from dilution 5 to obtain the correct volume for the final dilution.

APPENDIX B

Toxins in the Environment

Investigating Glyphosate Effects on Corn Seeds

Background: Glyphosate is a commonly used herbicide found in many commercial sprays. The chemical disrupts the ESPS pathway (shikimic pathway) by disrupting the formation of amino acids necessary for protein synthesis in plant cells. Glyphosate concentrates in the meristem tissue of the plant, causing the plant to wither and die. When people use glyphosate on their unwanted plants, some of the spray gets deposited on the ground. When water flows across the ground, the glyphosate can accidentally end up in local bodies of water.

Materials

6 - concentrations of glyphosate solutions (0.65M, 0.065M, 0.0065M, 650ppm, 65ppm)
6 - 50mL beakers
6 – resealable plastic sandwich bags
2 or 3 - Paper towels to sandwich the seeds
1 – disposable 1mL plastic pipet
1 – Permanent marker to label your sandwich bags
24 – corn kernels (corn seeds)

Safety Note: The concentration of glyphosate you are working within this lab has not been found to be toxic to humans. However, this concentration is toxic to plants, aquatic insects, and fish. Use extreme caution when working with glyphosate, use proper personal safety protective gear, and dispose of the waste accord

Pre-Lab

1. Using the materials and procedures, identify the following:

    a) The independent variable:_____

    b) The dependent variable:_____

    c) The control group:_____

2. Explain which concentration you hypothesize will have the greatest or least germination of corn seeds:

3. Why is glyphosate a safety concern?

Procedures

1. Obtain the materials according to your teacher's instructions. Be sure to put on gloves, goggles and aprons (if required).

2. Label your resealable plastic sandwich bags using the permanent marker with the concentrations you are working with. Note that one bag will be labeled 0.0M glyphosate. Make sure to label with your name, partner's name, period, etc...as appropriate.

3. Arrange the corn seeds on the paper towel with space between each kernel.

4. Fold the paper towel so it surrounds the corn seeds and carefully place it in each marked sandwich bag

5. Obtain 5mL of each concentration in separate 50mL beakers

6. Starting with the 0.0M concentration. Use the pipet to moisten the paper towel with 1-2mL of the concentration. DO NOT SOAK the paper towel. You should not have excess liquid in your sandwich bag.

7. Continue adding 1-2mL of the appropriate concentration to each sandwich bag. Go in reverse of concentration. (ex: start with 0.0M, then 65ppm, then 650ppm, then 0.0065M, then 0.065M and the last concentration of 0.65M

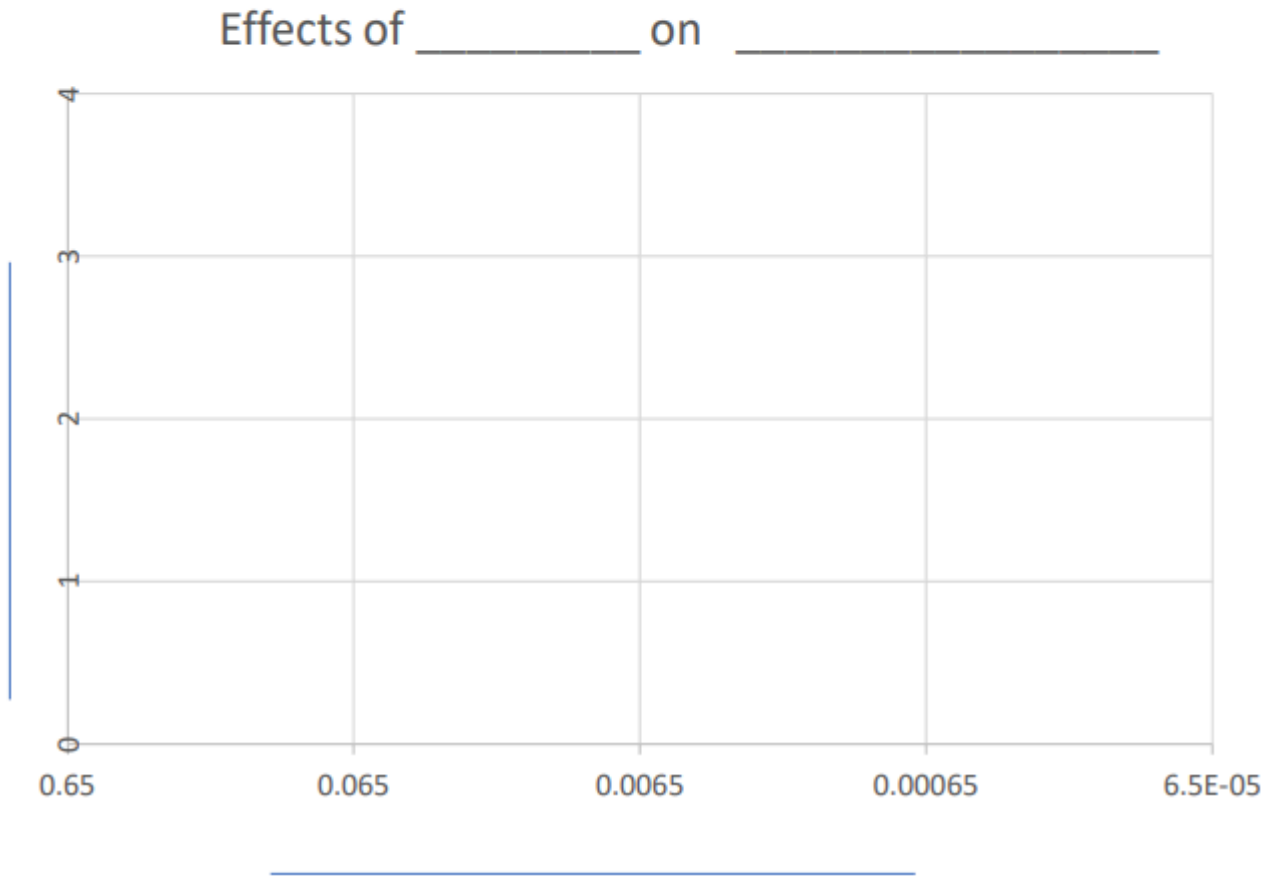
8. Close and seal the sandwich bag, and place it in a dry, but warm area. 9. After 4-5 days, check on your corn seeds to determine how many seeds germinated at each concentration

When my setup is completed, my leftover glyphosate concentration solutions goes where? (hint: ask your teacher)

Period:_____

Data:

Glyphosate Concentration (M)	24hrs	48hrs	72hrs	96hrs	120hrs	Group Average	Class Average
0.0M							
0.65M							
0.065M							
0.0065M							
650ppm							
65ppm							



Toxins in the Environment

Investigating Glyphosate Effects on Corn Seeds

Name:_____
Date:_____
Period:_____

Analysis

Use your data table and graph to answer the following questions. Refer to your data when supporting your claims.

1. Which concentration(s) of glyphosate resulted in the least amount of germination?

2. Which concentration(s) of glyphosate resulted in the most germination?

3. Refer to your hypothesis and compare your hypothesis to the results. Was your hypothesis supported or not supported? Use specific values from your data.

4. PFOS, a fluorinated compound used in industrial manufacturing, shows similar patterns of adverse reactions in animals as glyphosate does in plants. How would you design a similar experiment using PFOS to show the effects on a vertebrate animal?

a) Independent Variable:_____

b) Dependent Variable:_____

c) Control Group:_____