

## **The dose makes the poison: Measuring ecotoxicity using a lettuce seed assay**

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### **Introduction**

The goal of green chemistry is the design of materials and processes that are inherently safer for human health and the environment. Currently, few undergraduate chemistry students are trained about toxicity of chemicals and even fewer experience the laboratory investigation of chemical toxicity.

Toxicity is a complex topic encompassing both human and ecotoxicity. Ecotoxicity is also a broad topic. Common assays are available for aquatic and terrestrial ecotoxicity. This experiment introduces students to terrestrial ecotoxicity of different alternative starting materials commonly used for the preparation of biodiesel. Also students can later make informed choices for the preparation of their biodiesel using these results to guide them. Lettuce seed assays were used to compare the ecotoxicity of methanol, ethanol, and isopropanol by these alcohols' effects on germination rate and root elongation. The experiment is simple, fast, inexpensive, reproducible and possible to perform with standard equipment available in most chemistry teaching laboratories.

It is well known that methanol is a toxic substance; however, it will be interesting in this terrestrial ecotoxicity assay to compare methanol to other alcohols.

### **Procedure**

Obtain six plastic Petri dishes<sup>4</sup>, 12 filter papers large enough to cover the bottom of the Petri dishes, and 240 lettuce seeds<sup>5</sup>. Prepare the dishes by putting a piece of filter paper and 40 lettuce seeds into each dish. The seeds should be evenly distributed on the filter paper, then cover the seeds with another piece of filter paper.

Obtain 20 mL of 10% alcohol solution in a 25 mL graduated cylinder. Note: different groups will be assigned different alcohols. We will test methanol, ethanol and isopropanol. Other materials that are water soluble could be substituted at the discretion of the instructor.

Using a plastic pipet, carefully transfer exactly 10 mL of the alcohol solution to a 10 mL graduated cylinder. Pour the solution onto one of the Petri dishes, cover the dish *immediately*, and seal it tightly with a strip of parafilm around the edge of the dish, and label it "10%." Note: these alcohols are volatile.

Add 10 mL of deionized water to the remaining alcohol (diluting it one-half of its original concentration). Using the same plastic pipet from the previous step, mix the liquid well. Again, transfer 10 mL to the small graduated cylinder and pour onto one of the Petri dishes, sealing and labeling this dish “5%.”

Repeat as above, for 2.5%, 1.3%, and 0.6% concentrations of the alcohol.

Finally, in the sixth dish, place 10 mL pure deionized water and label this dish “Control.” Place the dishes in your locker so that they germinate in an undisturbed environment for one week.

### **Obtaining data after one week**

Wearing laboratory gloves, open each dish. If the dish has dried out, discard the seeds and paper into the waste container and do not record any data for the seeds. However, if the filter paper is still wet, record the data:

- Count the number of seeds germinated in each Petri dish and record in the table as shown on the next page. (\*Use forceps; be careful not to break roots! If part of a root breaks, ignore that root in the following step.)
- Measure the length of root of all germinated seeds with unbroken roots. Record the data.

### **Measuring protocol**

Before measuring, dry all the seeds by patting them with Kimwipe to remove as much moisture as possible. For each sprout, measure the *root* length (not the shoot or seed itself) to the nearest mm. Measure when the root is straight. (If the root is curved, try to measure it as you roll it along a ruler.) Calculate the average length (mean) for each plate of seeds. Do not include seeds that did not germinate or seeds with broken roots when calculating average.

Dispose filter papers and seedlings in the waste container provided by your instructor.

### **Postlab Questions**

1. Collect every group’s results and find the mean of root elongation and number of germinated seeds for each alcohol. Based on the data, which alcohol seems to be the most toxic to lettuce seeds?
2. Is the result that you found in question 1 expected? Why? Why not? [Hint: Look up the safety information and/or HFR value for methanol]
3. What does the information you found to answer question 2 tell you about using ecotoxicity data to estimate human toxicity?

## References

1. EPA website:  
<http://epa.gov/greenchemistry/pubs/principles.html> (accessed May 15, 2008)
2. Greene, J. C.; Bartels, C. L.; Warren-Hicks, W. J.; Parkhurst, B. R.; Linder, G.L  
*Protocols for short term toxicity screening of hazardous waste sites*, EPA/600/3-88/029, 1988
3. Private communication, Marissa A. Weaver, Gordon College, April 22, 2007
4. For example, Fisherbrand, 100x15mm, Cat. 08-757-12, Fisher Scientific,  
<http://www.fishersci.com> (accessed May 15, 2008)
5. For example, *Latuca sativa* (Tropicana), Product #2485, Johnny's Selected Seeds,  
<http://www.johnnyseeds.com> (accessed May 15, 2008)

## Lettuce Seed Terrestrial Ecotoxicity Assay Data Report Form

Name (both partners)
Alcohol studied
Date assay began
Date seeds were measured

**\*Table 1 – Germination**

	Petri dish 1 (control)	Petri dish 2 (0.6%)	Petri dish 3 (1.3%)	Petri dish 4 (2.5%)	Petri dish 5 (5%)	Petri dish 6 (10%)
# of seeds germinated (count)						
percentage germinated/ total seeds						

**\*Table 2 – Mean root elongation for germinated seeds**

	Petri dish 1 (control)	Petri dish 2 (0.6%)	Petri dish 3 (1.3%)	Petri dish 4 (2.5%)	Petri dish 5 (5%)	Petri dish 6 (10%)
Mean root length of seeds						
Standard Deviation						