Effects of toxic chemicals on developing seeds

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Important concepts for this lab:
Using assays
Making serial dilutions
Indicator species
Mean, standard deviation, and coefficient of variation
Assessing effects: LD50, ED50, NOEL

Toxicity Assays
In toxicity assays, we attempt to determine the dose of a chemical substance required to cause acute effects on living organisms. In performing toxicity assays, there are many considerations in experimental design.

When selecting a species for toxicity testing, usually "indicator species" are chosen. An indicator species is a species of organism that is used in the laboratory to act as an indicator for what may be happening in a more complex ecosystem. The choice of indicator organisms varies depending on the problem. For example, if we were concerned with water pollution, we would use aquatic organisms. If we were concerned about a chemical leaking into the soil, we might use a plant. For human toxicity, ideally we'd use humans, but since this is obviously not ethical, we use "model species." A model species is used as a surrogate for the species of interest. Often rats and mice are used as model species for humans.

Once a species is chosen, the dosing method must be determined. Chemicals can be applied dermally (through the skin), given in food or water, injected, or supplied in the air or water in which the organisms are living. When using living organisms, there are also different endpoints that can be considered. Usually, the easiest is death. We can also use other endpoints such as growth rate, birth rate, birth defects, a biochemical change (*e.g.* hormone levels) or cell function.

When examining the results of a toxicity assay, we can use simple statistical methods to evaluate data. For example, the average (mean) is calculated. This average can be the number of surviving organisms, average shoot length, average life span, average egg laying rate, etc. In addition, we can calculate a standard deviation and the coefficient of variation (CV) for each average. The CV is the standard deviation divided by the mean and is a relative measure of data dispersion compared to the mean. The smaller the CV, the more reliable the data. In toxicology, we generally like the CV to be less than 5%. It is then common to use graphical methods to display the data. The most common way to display data for toxicity assays is a dose-response curve, where dose is plotted on the x-axis and response is plotted on the y-axis. From this type of graph we can estimate a number of variables. The **LD50** is the dose of chemical that causes death in 50% of the population. Similarly, the **ED50** is the dose of chemical that causes an effect in 50% of the population. In addition, the **NOEL** is the concentration of chemical that causes no effect (no observed effect level).

Experimental Procedure

You will work in groups of 2-3 for this experiment. Each group of students will test **two** chemicals on one seed variety. At the conclusion of the test, which will occur next week, we will compare data from all groups and make some observations regarding the influence of chemical properties on toxicity with regards to germination of seeds.

Chemical	Common Use	Environmental Relevance
1% Caffeine	stimulant	stimulant
Clorox cleaner	disinfectant	water pollutant, toxicity

1. Use safety glasses and gloves during this procedure.
2. Prepare chemical solutions. Read directions carefully. We will prepare 3 initial solutions then use them to make subsequent dilutions. This is called making serial dilutions.
3. LABEL EVERYTHING (use masking tape)
4. Serial solution preparation:

   Rinse glassware with DDI water- use a large beaker and rinse at small sinks.
   - 20% solution:
     - Measure 20 ml of **one chemical** in a 100-ml graduated cylinder.
     - Add deionized (DDI) water until solution comes to the 100-ml mark.
     - Cover the graduated cylinder with parafilm and mix contents.
     - Pour into a labeled Erlenmeyer flask.
   - 10% solution
     - Measure 10 ml of **the same chemical** in the same 100-ml graduated cylinder.
     - Add DDI water until solution comes to the 100-ml mark.
     - Cover the graduated cylinder with parafilm and mix contents.
     - Pour into a labeled Erlenmeyer flask.
   - 5% solution
     - Measure 5 ml of **the same chemical** in a 100-ml graduated cylinder, *etc.*
   - 1% solution
     - Measure 10 ml **of the 10% solution** in a 100-ml graduated cylinder, *etc.*
   - 0.5% solution
     - Measure 5 ml **of the 10% solution** in a 100-ml graduated cylinder, *etc.*
   - 0.1% solution
     - Measure 10 ml **of the 1% solution** in a 100-ml graduated cylinder, *etc.*

5. Select 1 seed type for the test.
   - It is best to use a medium-sized seed so that shoots are easily measurable.

6. Set up petri dishes. We will work in as **sterile a manner as possible** for this section. Touching the seeds, filter paper or petri dishes with bare hands can lead to mold growth in the test. **This is best avoided.**

7. Use tweezers to handle seeds and supplies. Before you use tweezers make sure they have been sterilized in 70% alcohol. The filter papers have been sterilized in the autoclave, to minimize contamination. Also, minimize laying the petri dish lids on the counter, as they will pick up contamination there as well.

   - You will have 1 petri dishes for each solution concentration (7 dishes per chemical) for a grand total of 13 dishes per group. (Control dish only needs to be done once)

- Dish 1: **control** (distilled water only, no chemical)
- Dish 2: 20% chemical in water
- Dish 3: 10% chemical in water
- Dish 4: 5% chemical in water
- Dish 5: 1% chemical in water
- Dish 6: 0.5% chemical in water
- Dish 7: 0.1% chemical in water

8. Place clean plastic petri dishes on counter. Using a wax pencil or tape, label each set of dishes with your group's name or initials, date, seed type, dish number and solution type. Write this info on the smaller half of the petri dish as this will be the top half.
9. Sterilize the tweezers. Using the sterilized tweezers, place one piece of sterile filter paper in each petri dish. The paper fits best in the larger half of the petri dish.
10. Carefully measure 10 ml of each solution (in a 10-ml graduated cylinder) and pour into the appropriate petri dishes. **Begin with the lowest concentration solution and work up to the most concentrated.** That way you won't contaminate your solution concentrations.
11. Place 5 seeds of one type in each dish with tweezers in a circular fashion, being careful to maintain a maximum distance between each seed and its neighbors and keeping them away from the sides of the dish and on top of the filter paper.
12. Important- Wrap all dishes with parafilm or tape so that the moisture will not evaporate.

13. Repeat steps 6 through 9 using the second chemical. You can switch with another group (assuming you trust they made their dilutions correctly!) Please note the names of the group you switched with.
14. Pile dishes carefully in a warm dark place to grow. Leave for 1 week.
15. Do all your dishes
16. Rinse all glassware in DDI water and clean up (rebuild) your station the way you found it.

What to turn in for this lab next week.

1. An introduction (1-2pages) and method (1 page) section that sets up the experiment, describes what seed(s) and chemical you used and any complications or assumptions about your experiment. "Human error" will not be an acceptable source of error. Next week you will hopefully have results and will be able to make graphs of your data and interpret the data. Make sure to explain what a toxic assay is and some information about your chemical. The introduction should not just be a re-hash of the lab handout but should show some research from the primary literature. Make sure to cite any sources of information. You should write your report independent of you lab partner but please include your partner's name. Watch you writing carefully especially your tense. You can use a style like this: "We did this and we did that, we determined…etc. More information will be given in lab.