Environmental Science 160 Lab
Environmental Toxicology

From Joe Balczon

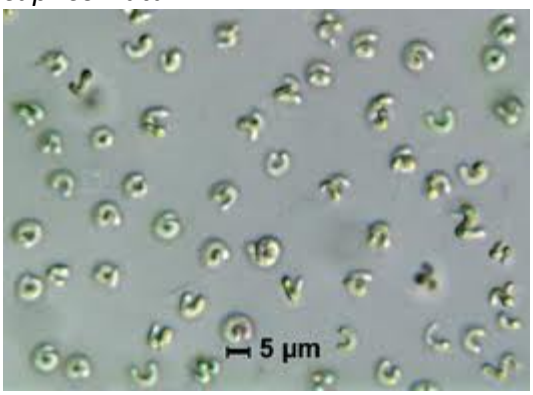
The number of new chemicals produced in the US each year is staggering. As of 2011, 83,000 chemicals were registered in the Toxic Substances Control Act (TSCA) Chemical Substances Inventory, and new chemicals are added at the rate of several hundred per year. For the vast majority of these substances, there is little known about their potential effects on humans (or their surrogates, like mice, rats, beagles, or pigs) let alone their potential effects on populations, communities, and ecosystems. As part of the TSCA, the Environmental Protection Agency (EPA) is responsible for developing standardized procedures for assessing the potential toxicity of chemicals to ecological communities.

These standardized tests usually involve a single species tested in isolation from other species and their natural environment. These single species include representatives from several trophic levels, such as *Selenastrum capricornutum* (an alga), *Daphnia magna* (a crustacean herbivore), *Ceriodaphnia dubia* (another crustacean herbivore) and *Pimephales promelas* (a predatory minnow. Such tests usually involve an endpoint such as mortality, reduced growth, or reduced reproduction to estimate the relative toxicity of substances.

There are several limitations of single species test results. First, there is no good evidence that they are applicable to higher levels of biological organization. Because communities are composed of many species, and each species may have a different level of sensitivity to the substance in question, some may be affected at low concentrations, and some may not be affected until concentrations are very high. Second, the standardized test species may not even be relevant to the ecosystem in question. If the species that are the standardized test organisms are never found in the ecosystem in question, then any extrapolation to the existing community is tentative at best. Third, single species tests ignore the possibility that there may be indirect effects (community-level effects) of the potential toxin. Finally, testing a species in isolation from its natural habitat ignores the possibility that substances may change appreciably by physical and biological processes once they enter an ecosystem. For example, many potentially toxic materials bind to other substances (*e.g.*, sediments or soils) or are metabolized by microbes upon addition to natural ecosystems. Therefore, the substance may actually be biologically unavailable to cause effects upon organisms.

In an attempt to account for these shortcomings, environmental toxicologists have developed multi-species test systems (microcosms) to test potential toxins in more realistic settings. These tests have the advantage of testing many naturally occurring species in one experiment, including potential indirect effects (even if they are not measurable!) and can provide information that is relevant to ecosystem processes, such as production, respiration, and nutrient cycling. The disadvantages of these tests are their complexity, the expertise required to identify the potential target organisms, and the lack of standardization.

Today, you will design an experiment to test the potential effects of some substance on a model organism. Your model organism will be the easily cultured alga known as *Selenastrum capricornutum.*



(From silicasecchidisk.conncoll.edu)

It is a single-celled alga that is a standard toxicity test organism meant to represent *primary producer* (*i.e*., the base of the food chain). You will place them into containers with growth media, and you will introduce known quantities of potentially toxic substances. You must decide which substance of interest that you will add to the containers (heavy metals and pesticides are always good options!). You will allow them to incubate for two weeks, and then you will make measurements on the samples. You will be required to measure the density of the organisms, which will require you to count samples. **Today, you will learn to use a hemacytometer and microscope to count samples of organisms**. You may decide that there are additional measurements that you could make to assess the effect of the substance on the populations of *Selenastrum*, and that would be outstanding! After you have collected the data from your experiment, you will statistically analyze your data to determine if there are significant differences among your treatments, and at what concentrations of pollutant those effects are first apparent.

You should find more information about your substance and its known effects from the literature and internet to supplement your laboratory report. You should begin this research between now before you actually assemble the experiment. Use the literature to find what concentrations of your chosen substance are known to cause effects in other organisms, or that are found in the environment already. In designing your study, remember to identify your null hypothesis, make predictions that follow from your hypothesis, and consider all elements of a good experiment. You need a decent sample size for each treatment, and you need to randomize the study to minimize any sources of bias in the study.

Before you leave today, make sure that you can use the hemacytometer to count organisms, make sure that you discuss your experimental design with me, and that you give us a list of what materials your group need. I will collect the material for you, but it will be entirely up to you and your group to assemble the experiment, and conduct all elements of the study itself.