ENVR 431: Techniques in Environmental Health Sciences  
Fall 2007  


Algal Toxicity Bioassay

Lab skills:  
Use of hemacytometer  
Measurement of cell growth rates

INTRODUCTION

The introduction of toxic material into aquatic systems (wetlands, streams, rivers, lakes) can cause adverse effects on the entire ecosystem. The phytoplankton community includes the primary producers, photosynthetic organisms (usually plants) that store energy from sunlight by converting carbon dioxide and water to biomass. The phytoplankton form the basis of food webs and are essential to ecosystem stability and health. Changes in the phytoplankton community by toxicity (death) or reduction in growth rate will reduce or alter the food supply for organisms in other trophic levels (animals) as well as affecting water quality parameters such as dissolved oxygen, pH, alkalinity, color, suspended matter, and taste.

Chemicals may either stimulate or inhibit algal growth and sometimes the same chemical can have both effects, depending upon concentration (the dose makes the poison…). The purpose of an algal toxicity test or bioassay is to determine the relative toxic or stimulatory activity of an agent on a representative algal species (EPA/600/4-91/002). Commonly measured endpoints are death and growth rate inhibition (growth can be inhibited by slowing down or stopping cell division). These endpoints can sometimes be measured in the same assay. The simplest test uses batch cultures of a single species of algae at a specified initial concentration (and growth condition) in a chemically defined medium incubated at specified conditions of temperature, photoperiod (time and cycle of light and dark hours per day), light source and mixing. The test consists of replicate culture flasks: one or more negative controls, and other flasks receiving different concentrations (dilutions) of the test material (such as a chemical, a waste discharge). The growth of algae in each flask is measured by determining cell concentration at time = 0 and then at intervals over a week of incubation. The algae concentrations over time are used to construct a growth curve for each sample. The growth curves are then used to estimate the test concentration that causes a 50% reduction in growth rate (the EC$_{50}$ or 50% effective concentration).

In this laboratory exercise, the EC$_{50}$ of two test agents, copper sulfate and phenol, will be determined in a static assay on unicellular algae. *Selenastrum capricornutum* is the algal species historically used for this assay, sourced from Carolina Biological in Greensboro.
MATERIALS

Culture of algae, to be diluted into algae growth medium as directed.

Test chemicals: 200 mM phenol solution
3 mM copper sulfate solution

Diluent for test chemicals: algae growth medium

Algae growth medium: modified EPA formulation

Medium bottle, 500-ml capacity

Graduated cylinder

Culture flasks: 125 ml capacity Erlenmeyer flasks, eight (8) per group of students

Pipets: 25, 5, and 1 ml

Dilution tubes: four (4) per group.

Light microscope

Hemacytometer

Pasteur pipets

Others: Test tube rack
Pipet bulb
Hand tally
Paper towels and wipes
Disinfectant
Incubator (at 25°C) containing light source and a platform shaker for algae culture flasks.

PROCEDURES

PREPARATION OF ALGAE CULTURE
Using the graduated cylinder, dispense 240ml of algae growth medium into the 500ml capacity medium bottle. Then add the specified number of ml of stock algae culture (this is specially determined by the TA on the morning of the experiment) to this medium and mix briefly by swirling. Dispense 27ml volumes of this algae culture into each of the eight (8) Erlenmeyer flasks. Save the rest of the diluted algae culture for counting the initial cell density (day 0 cell count).
PREPARATION OF TEST CHEMICAL DILUTIONS
Prepare four (4) dilution blanks of 4.5ml each. Dilute each test chemical (copper sulfate or phenol) 10-fold by adding 0.5 ml of stock chemical to a 4.5ml dilution blank and mixing well. Dilute each chemical 100-fold by adding 0.5ml of the 10fold dilution to a 4.5ml dilution blank and mixing well.

INOCULATION OF ALGAE CULTURES WITH TEST CHEMICAL
For each test chemical (copper sulfate or phenol), add 3 ml volumes of the following to a flask of algae culture:
- growth medium (diluent) only (negative control)
- undiluted chemical
- 10-fold diluted chemical
- 100-fold diluted chemical

Mix the contents of the flasks by swirling briefly.
Place the flasks on the platform shaker apparatus in the incubator, try to make sure they each are exposed to light, let them grow, let them grow, let them grow...

COUNTING OF ALGAE CELLS USING A MICROSCOPE AND HEMACYTOMETER

Day zero (0), the beginning of the experiment:
Prepare hemacytometer and coverglass by rinsing under cold tap water and blot/wiping dry.
Place coverglass on the counting platform. Mix the algae culture by swirling and take up a small volume using a Pasteur pipet. Quickly transfer some of the liquid from the Pasteur pipet to each counting chamber. Count the number of algae cells per field under the microscope. Each field measures 1mm x 1mm x 0.01mm depth.
Days 1, 2, 5, 6, and 7:
Remove each algae culture flask from the incubator, use a Pasteur pipet to remove a small volume of culture from the flask, and count the cell concentration according to the counting instructions given above for day zero (0). Remember to put the culture flasks back in the incubator if you want them to keep on growing...

RESULTS

DATA COMPILATION AND REDUCTION

For each test chemical and each sampling time, determine the mean (average) number of algae cells per ml. If possible, compare the results for the whole class.

ESTIMATION OF 50% EFFECTIVE CONCENTRATION (EC<sub>50</sub>)

1. For each chemical concentration and control, plot a graph of the log<sub>10</sub> of the cell concentration against time, in days (which is the dependent variable, which is the independent variable ?). For each of these, draw a smooth curve describing the growth curve.

2. From each growth curve, estimate the percentage growth at each test chemical concentration relative to the negative control. That is, divide the final cell density of each test chemical concentration by the final cell density of the corresponding control flask and multiply by 100.

3. For each chemical, plot the log<sub>10</sub> test chemical concentration versus its percent growth (arithmetic) relative to the negative control. OR plot the graph on semi-log paper, using the logarithmic axis for the chemical concentration. From this graph, estimate the chemical concentration at which the percent growth relative to the control is 50%. Record and report this value.

QUESTIONS

1. HOW FAST DO HEALTHY ALGAE GROW?
From your negative control flasks, estimate how long it takes for a population of “normal” algae to double in density.

2. RELATIVE TOXICITY OF THE CHEMICALS.
Which chemical is more toxic to the algae on a molar basis? Which is more toxic on a weight basis?

3. RELATIVE SENSITIVITIES OF THE CELL TYPES.
How do the estimated EC<sub>50</sub> values of these chemicals for algae compare to their estimated EC<sub>50</sub> values for mammalian (CHO) cells? Is one cell type more sensitive than the other? Which one? Can you provide some anatomical, physiological, or other biological explanations for any observed differences in the toxicity to mammalian compared to algae cells?