A Preliminary Guide to

BIOASSAYS USING GAMMARID AMPHIPODS

Bioassays of cleaning products

This method can be used to determine acute and chronic LC50 levels for products lacking published ecotoxicology data. Sodium lauryl sulfate (SLS, SDS) is used as a standard for comparison. A six-well plastic culture tray is used for each product test. Amphipods are exposed to four serial dilutions of each product for 30 and 60 minutes (acute), and for 24 hours (chronic). Percent morbidity/mortality at each concentration can be plotted to infer the LC50 for each test.

Statistical reliability of results can be achieved by exposing more amphipods to each test condition, and by repeating the test. We recommend placing five amphipods in each well (more will use up available oxygen too quickly) and repeating each test three times.

Preparation:

Place four plastic culture plates on a light-colored bench top where they can be free from heat, strong light or disturbance overnight. Fill each well with 9 mL of distilled water. Prepare test concentrations of 100, 10, 1 and 0.1 ppm of each test product as follows (this will take about 12 minutes):

1. Add 1 mL of product to 9 mL water in small cylinder (1 mL of this for 100 ppm)
2. Remove all but 1 mL from cylinder, top to 10 mL with water (1 mL for 10 ppm)
3. Transfer remainder from cylinder to large cylinder, add water to 100 mL, mix
4. From large cylinder add 1 mL for 1 ppm and 0.1 mL for 0.1 ppm

Add 1 mL of 1000 ppm SLS to the Standard well (for 100 ppm SLS).

Use a fine mesh aquarium net to transfer amphipods from their holding tank to a shallow light-colored dish. Use a blunted disposable plastic pipette to transfer one amphipod at a time into wells. Avoid unusually large or small animals. This will take 5-8 minutes. Set the timer for 30 minutes and turn off lights to avoid overheating the amphipods.

After 30 minutes, turn on the lights and observe the movement of amphipods. Count the number of amphipods that are no longer swimming, even if gently prodded with a pencil tip or toothpick. Count separately any amphipods that only move if prodded.

Note that these are bioassays of product formulations—combinations of ingredients—and that the concentrations of toxic ingredients are much lower. A 10-ppm concentration of a shampoo containing (for example) cocamide DEA may actually represent as little as 0.1-ppm cocamide DEA. Since people use products *as they are formulated*, however, what is

important is (for example) which shampoo is least toxic at a standard quantity of ordinary daily use: tablespoon by tablespoon, or cup by cup, as used by consumers.

Materials:

Gammarid amphipods (at least 30 per product test)
One 6-well culture dish for each product tested
10-mL syringe for filling wells with distilled water
Disposable plastic 3-mL transfer pipettes for dilution (one for each product tested)
1-1/10-mL glass filter pipette for 0.1 mL aliquots of diluted products (one per product)
10-mL graduated cylinder for dilutions
100-mL graduated cylinder for dilutions
Distilled water (170 mL per product test)
1000-ppm stock solution of SLS with a dedicated 3-mL pipette
Glass rod for mixing dilutions
Lab timer

Determining the persistence of products

Even less is known about the environmental persistence of soaps, cleaners, and "natural" home and garden pesticides and herbicides than about their toxicity. Do they continue to be toxic for days, weeks, or years? This determines the rate at which they accumulate in the environment, and the real threat they pose. Bioassays are a simple way of evaluating persistence under laboratory conditions.

To determine the effective persistence of a household product, create prepare four serial dilutions of the product (1000, 100, 10, and 1 ppm) in loosely capped glass jars or flasks. You will need to prepare at least 100 mL of each dilution. As the baseline, use these four dilutions for an amphipod bioassay (1 mL of the 1000-ppm solution for the 100-ppm well and so on). Then apply one or more real-world conditions to the four dilutions remaining in the jars/flasks: bright light (photolysis), daily shaking (aeration-oxidation), increasing pH to 8.0 with magnesium carbonate, even adding some garden soil for bacteria. Here in San Juan County, our surface waters tend to be "hard" and alkaline. Natural streams and wetlands have good aeration and light penetration, whereas dug ponds and ditches tend to be turbid, poorly aerated, with incomplete light penetration.

Repeat the bioassay after one week. If there is still some inhibition after one week, wait three more weeks to repeat the bioassay. If there is still any inhibitory effect—one month total after your baseline bioassay—the product is pretty persistent. You may want to wait another month and try again. A precise determination of the "half-life" of a product may require weekly testing for months. But distinguishing fast-degrading products (just days) from products that persist and accumulate for weeks is sufficient to make some important consumer choices.

If the class is divided into 4-6 teams, each team can test a different product under similar degradation conditions; or each team can test a different condition on the same product to see what factor accelerates product degradation the most.

About amphipods

Gammarid amphipods can be ordered online at least a week before they are needed from Carolina Biological Supply, www.carolina.com, which reliably delivers on whatever date you specify—as long as it is a Wednesday, Thursday, or Friday! Each lot of amphipods costs about $18 including shipping, and can contain from 15 to 20 amphipods. Thus if a class of 10 students will be collectively conducting bioassays of five products, they will need (5 times 30 = 150) amphipods, or 10 lots to be on the safe side.

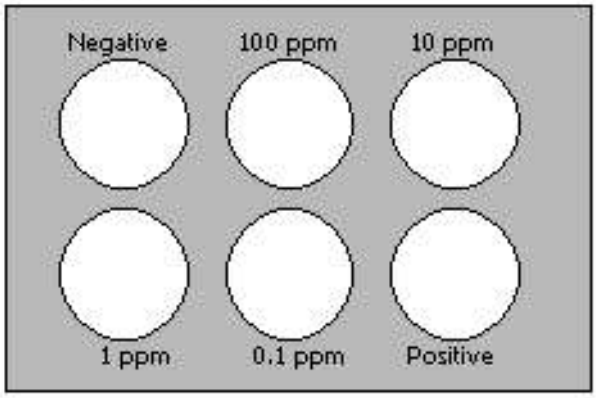
When they arrive, packed in plastic goldfish bags or clear plastic jars, amphipods should be emptied as soon as possible into a clean fish bowl or aquarium together with the plants included by Carolina Biological Supply as food. Strain out a third of the water they came in, and replace it with distilled water, to remove some of the accumulated waste products. Set up a small aquarium aerator and run it for 30 minutes in the morning and again in the afternoon. Amphipods do not require continuous aeration, but they do require cool, dark, undisturbed conditions.

Amphipods often arrive copulating or carrying egg masses. These individuals should be set aside, in a tank with some aquatic plant material (just a few grams) and good aeration. With luck, you can produce enough amphipods for your next round of bioassays! But it will take 2-3 weeks. Over long periods of time (weeks to months) amphipods require the occasional partial water change, and small quantities of fresh green aquatic plants such as pondweed, duckweed, milfoil, or mares-tail.

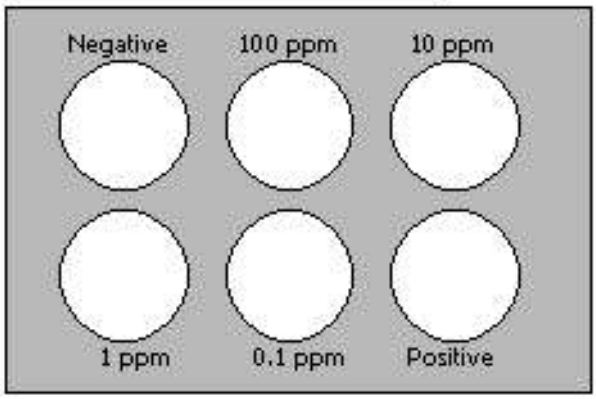
The Gammarid amphipods supplied by Carolina Biological Supply and traditionally used for standard bioassays are an Atlantic freshwater species and are potentially invasive. Be careful to strain amphipods out of the solutions in your test wells after completing assays, and dispose of them as solid waste—never down the drains! Amphipods in the Negative Control wells can simply be returned to their aquarium or holding tank, but ones exposed to household chemicals or SLS should not be mixed with healthy, unexposed animals.

Amphipod product bioassay

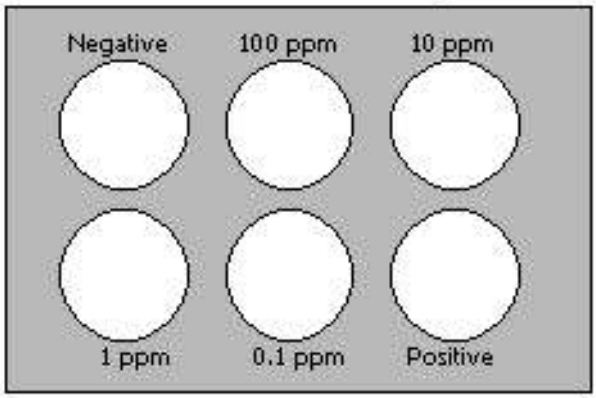
Results at 30 MINUTES:



Results at 60 MINUTES:



Results at 24 HOURS:



Product: _____

Experimenter: _____

Date begun: _____

Coding of wells

Look carefully at the amphipods in each well. Count how many are not swimming at all, even if you poke at them very gently with a toothpick. Then count those that only swim if gently prodded. Write both of the numbers in the corresponding well. So if there were five amphipods in the 10 ppm well to begin with, and at 60 minutes two are not moving, and one is moving only if prodded, write "2+1" in the 10 ppm well. Of course we know that that leaves 2 amphipods that were unaffected.