Brine Shrimp
“Bioassay” Problems

There are two major problems with the recent brine shrimp exercise of Rice & Maness (March, 2004, 208-215). The term “bioassay” is both misdefined and misapplied, and the wide scattering of the data suggest that the method may be inaccurate. The definition that a bioassay must use a “naive” species is incorrect. Many bioassays use species that are normally exposed to the chemical being assayed. There are several phytohormone bioassays that have been used in teaching including the lettuce hypocotyl elongation bioassay for gibberellins (Reiss, 1994), the oat coleoptile curvature test for auxins, the dandelion leaf disk chlorophyll retention bioassay for gibberellins, and the soybean callus bioassay for cytokinins (Witham et al., 1971). As with many biology terms, the definition of bioassay has been corrupted by some over the years. Dictionaries and textbooks still usually define it similar to the following: appraisal of the biological activity of a substance by testing its effect on an organism and comparing the result with some agreed standard. (http://www.thefreedictionary.com/bioassay)

The brine shrimp exercise lacked the standard curve required for a bioassay. An accurate description of the exercise would be an LC50 determination or simply a dose-response curve. A bioassay is very different. A bioassay quantifies the amount of a chemical, such as auxin, by using a living organism or living tissue as a substitute for an analytical technique or analytical instrument, such as a high performance liquid chromatograph.

Brine shrimp can be used in true bioassays. Michael et al (1956) used brine shrimp to quantify pesticides. They also described a simple technique to easily separate hatched shrimp from eggs using phototaxis. They evaluated results by determining how long it took for the brine shrimp to drop from the top of the solution.

The statistics were incomplete, as there were no correlation coefficients for the fitted curves. The curves seem to fit poorly. The wide data scatter in the three graphs indicates a potential problem with the method. However, there were too many sources of variation to determine if the method itself was the cause of the variability. Each data point represented a different leaf so there could have been substantial variation in toxin content from leaf to leaf. Differences in technique from student to student was another variable in Figures 1 and 3. Possible types of student variation include differences in how finely students cut the leaves before extraction with alcohol, and inconsistency among students in determining whether the brine shrimp were dead or alive.

Figure 1 has several points with zero or very low percent survival at low concentrations, yet several higher concentrations gave 100% survival. The authors did not address this strange result but one possible explanation is a wide variation in the number of brine shrimp per ml. If there are just enough leaf toxins to kill all shrimp at 50 shrimp/ml, there may not be enough to kill any shrimp at 75 shrimp/ml.

To determine the accuracy of the method itself, one person should do five or more tests...