A Brine Shrimp Bioassay for Measuring Toxicity and Remediation of Chemicals

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Marya Lieberman
Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556;
Marya.Lieberman.1@nd.edu

This article describes a laboratory project that uses a bioassay on brine shrimp to measure LD_{50} values for a variety of insecticides. This project was the centerpiece of a five-week unit on toxicology and chemical remediation in my course for non-science majors, Chemistry and Public Policy. The 22 students in the class were first-year liberal arts majors, most with fairly solid high school chemistry backgrounds. Two 3-hour lab periods are required, the first for toxicity testing, and the second for remediation and retesting.

Any chemical that can be dissolved or dispersed into water can be studied in this bioassay: plant extracts or other natural products, real or simulated "hazardous waste", metal ions, agricultural chemicals, food additives, home cleaning products, or pharmaceuticals. Although the bioassay is simple enough to use with students who have had little lab experience, it could also be integrated into an environmental, organic, medicinal, or biochemistry lab course.

This set of labs helps students learn how toxicity is measured and how harmful molecules can be decomposed to harmless products. Ethical issues are introduced in a discussion of animal testing. Students learn to search the literature and predict reactivity from structure; this aspect of the lab could be emphasized more for students with more chemistry knowledge.

The bioassay and remediation procedures described in the supplementary material could be used as straight labs or "set up" as a multi-week project. In one such project, students were given a letter from the owner of a dog-grooming salon, who was worried that the insecticide products she uses in the salon are leaching into a nearby stream. She asks the students to test the flea products to determine their toxicity to aquatic life, and to devise some method that she could use to make the wastewater less toxic before she pours it down the drain.

The students were then divided into groups of 3 or 4 and given an insecticide product. They measured the LD_{50} value of their insecticide on brine shrimp; all seven groups were able to complete this lab in a three-hour lab period. Over the next two weeks, the groups researched the chemicals present in their insecticide product. During this time, they studied basic organic chemistry. On the basis of the structure and reactivity of the main active ingredient, each group decided on a method of remediation to test in lab. In the second lab period, the remediation procedures were carried out and the remediated material was assayed on brine shrimp. The project culminated in a poster session at which each group presented its results.

Experimental Methods

The Bioassay

There are several published whole-animal bioassays for assessment of chemical toxicity (*1*). Bacteria are commonly used for quantitative tests, and there are some procedures for qualitative tests on *Blepharisma* (a one-celled pond organism) and *Daphnia* (water fleas).

Brine shrimp have been used as a "benchtop bioassay" for the discovery and purification of bioactive natural products (*2*), and they are an excellent choice for elementary toxicity investigations of consumer products. Brine shrimp, *Artemia* species, also known as sea monkeys, are marine invertebrates about 1 mm in size. Freeze-dried cysts are readily available at aquarium stores. The cysts last for several years and can be hatched without special equipment (directions are given in the supplementary materials[W]). Toxicity testing is carried out by adding different doses of material to small numbers of the shrimp; the entire dose–respose curve for a substance can be generated in a 3-hour lab period. The supplementary material gives a general procedure for this bioassay.[W]

The assay does involve killing brine shrimp. There is a reasonable controversy over use of animals for such purposes, and some people have ethical or religious objections to killing even "lower" organisms. Although a few of my students had formed opinions about the Draize test or about dissecting frogs in high school, most had not thought much about the ethics of what they were being asked to do in this lab. Before the experiment, students investigated a variety of pro- and anti-animal-testing Web sites (*3*). This was followed up by a pre-lab discussion about animal testing and alternatives, and which species the students would be willing to use as experi-

Table 1. Toxicity Data for Some Flea Products on Brine Shrimp

Group	Product	Active Ingredients	LD_{50} / ppm	LE_{50} / ppm
I	Zodiac Pyrethrin Dip	Pyrethrins 0.3% Piperonyl butoxide, tech. 3.00%	40–418	1–32
II	Zodiac Limonene Dip	*d*-Limonene 78.2%	28–100	1–3
III	Hi-Yield Diazinon 4E spray	Diazinon 47.5%	4–63	4–9.5
IV	Releve Flea and TickConditioning Shampoo for Dogs	*d-trans*-Allethrin 0.10% *N*-Octyl bicycloheptene dicarboximide 0.15%	>1670	16
V	Flea Stop Plus Shampoo	Linalool 3.7%	700–900	0.6–2.4
VI	Tomlyn Flea, Tick, Lice Shampoo	Pyrethrins 0.054% Piperonyl butoxide, tech. 0.108% *N*-Octyl bicycloheptene dicarboximide 0.18%	1400–>1670	10–25
VII	Flea Stop 42-Day Dursban Spray	Chlorpyrifos 0.50%	>1670	418–1000

NOTE: Data give the range of LD_{50} and LE_{50} values obtained by each group of 3–4 students.

mental animals under different circumstances. It was particularly informative to pass around some actual brine shrimp at this discussion—some of the students had rather overblown expectations derived from sea monkey ads in comic books!

Assaying the Toxicity of Insecticides

Students examined seven products, listed in Table 1. These products all contained insecticide and a variety of inert ingredients, such as solvent or detergents. The students measured all concentrations in terms of the formulated product, rather than the active ingredient. Although "inert" ingredients such as toluene or detergents are less toxic than the actual insecticides, they are generally present in higher concentrations and hence could be responsible for some of the toxicity of these products to brine shrimp.

Each product was assayed by 3 or 4 students. They measured the dose required to kill half of a group of shrimp (LD_{50}) and the dose required to cause sublethal behavioral effects (slow swimming rate) in half of a group of shrimp (LE_{50}). The values varied within an order of magnitude owing to a variety of factors: poor pipetting technique, mixing the tubes too vigorously (this can literally shake the shrimp to death and should be suspected if a student gets moderate levels of mortality regardless of dose), difficulty in counting the shrimp or observing sublethal effects, and errors in constructing smooth curves and interpolating the LD_{50} and LE_{50} values, which are on a log scale. Despite the range of error, some products are clearly more toxic to brine shrimp than others; several of the products were not lethal even at the highest doses tested. Contrary to the students' expectations, "natural" insecticides such as *d*-limonene and pyrethrins were no less toxic than "artificial" ones like diazinon.

Remediation of Insecticides

Students were given procedures for four methods of remediation: oxidation/hydrolysis with bleach, Cu(II)-catalyzed hydrolysis, photolysis, and adsorption onto activated carbon (these are described in the supplementary material).[W] Another option would be to use a published wastewater treatment procedure (*4*). To choose an effective procedure, students need to know how to read Lewis structures and shorthand organic structures, use the structures to predict molecular polarity and solubility, and recognize simple functional groups such as esters, phosphate esters, and hydrocarbons. The following equipment and supplies are needed:

Equipment and Supplies Required

Brine Shrimp Bioassay

Brine shrimp cysts, sea salts (aquarium store)
Air bubbler (aquarium store or can use compressed air)

Remediation Procedures

Sunlight or UV lamp (borrow from Organic lab)
Cation exchange resin, e.g., Bio-Rex 70

The instructor met with each group of students for 20 minutes to discuss their choice of method, what they planned to vary in the method, and appropriate control experiments, and to make sure they understood the experimental procedure that each one would follow in lab. In preparation for the meeting, each student was required to find and interpret toxicity information about her or his pesticide using the Merck Index, RTECS, and online MSDS sheets. They also read articles on pesticides and decontamination of chemical weapons (*5*). These meetings were essential for the unstructured lab that followed.

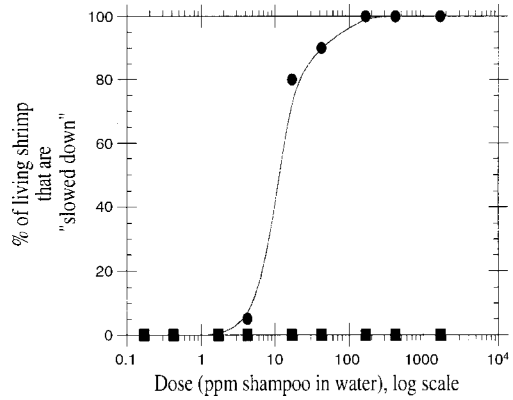

Figure 1. Student data for Releeve Flea and Tick Conditioning Shampoo before and after remediation. The untreated shampoo at a dose of about 16 ppm causes sublethal effects (lethargic motion) in brine shrimp(●). Treatment with activated carbon completely eliminates this effect, even at doses of 1670 ppm (■).

Group results were reported in a poster session. Remediation usually lowered the LD_{50} and LE_{50} values significantly. Even if a product's LD_{50} value was larger than the highest concentration assayed, the students were still able to measure changes in the LE_{50} value before and after remediation. Figure 1 shows data on the remediation of a pyrethrin-containing dog shampoo. The shampoo caused lethargic swimming in brine shrimp at a dose of 16 ppm. After treatment with activated charcoal, the shampoo did not cause any observable effect on brine shrimp even at 1670 ppm, the highest dose tested.

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Note

[W]Supplementary materials for this article are available on *JCE Online* at *http://jchemed.chem.wisc.edu/Journal/issues/1999/Dec/abs1689.html*. They include the chemical background for the experiment, detailed experimental materials and procedures, student handouts, instructions for preparing lab reports and group posters, a grading sheet for the posters, and further literature references.

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