

## 12.

### Bioassay for Cytotoxicity

Brine shrimps (*Artemia salina*) belong to the class Crustacea and are able to tolerate high salt concentrations. However they are very sensitive to environmental changes. Hence the brine shrimp assay is considered as a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides and cytotoxic testing of dental materials. Extracts, fractions and pure compounds are routinely evaluated in a test for lethality to brine shrimp larvae.

Brine shrimp eggs are readily available in aquaria and remain viable for years when kept dry. Once kept in sea water, eggs will hatch after 12-24 hours and free swimming larvae (nauplii) can be observed. After 48 hours they become the 2<sup>nd</sup> instar nauplii, having three pairs of appendages. This stage is the most important stage for the bioassay because at this stage of their life cycle the nauplii exhibit their greatest sensitivity to test compounds.

The brine shrimp assay has advantages of being rapid, low cost and does not require either expensive instruments or aseptic conditions. It easily utilizes a large number of organisms for statistical validation and a relatively small amount of the test compound is needed.

#### Brine Shrimp Cytotoxicity Assay

##### *Preparation of sea water*

Dissolve NaCl (24.73 g), KCl (0.66 g), CaCl<sub>2</sub> (4.70 g), MgCl<sub>2</sub> (1.90 g), MgSO<sub>4</sub> (6.30 g) and NaHCO<sub>3</sub> (0.18 g) in distilled water to make 1.0 L of artificial sea water.

(Marine Biologists Laboratory Formula

<http://www.mbl.edu/BiologicalBulletin/MMER/ECK/EckApp4.html>)

##### *Hatching of Brine Shrimp Eggs*

Keep sea water in a small two-chamber tank separated by a net which would allow movement of shrimp only. Add shrimp eggs (1.5 g mL<sup>-1</sup>) to one

chamber of the tank. Maintain constant temperature (35±2)°C, sufficient light and oxygen supply to give sufficient aeration. After 48 hours, mature shrimp as nauplii can be collected and used for the experiment.

### **Test**

Prepare vials for testing. For each compound, test initially at 1000, 100, 10 ppm of sample in seawater. Prepare three vials at each concentration for a total of nine vials. Transfer 10 shrimps per vial and adjust the total volume to 5 ml. Place vials, uncovered, under the lamp. Be sure that vials are not overheated by the lamp. Count number of survivals after 24 hours using a magnifying glass. Analyze the data for probit analysis to determine the LC<sub>50</sub>. Additional dilutions at less than 10 ppm may be needed to determine the LC<sub>50</sub> values for potent materials and intermediate concentrations at 750, 500 and 250 ppm can be prepared and tested to narrow the confidence intervals. Atropine, potassium dichromate, berberine chloride can be used as positive control.

### **References**

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Marine biologists laboratory formula -

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