A R T O X K I T M

ESTUARINE/MARINE TOXICITY SCREENING TEST

BENCH PROTOCOL

Principle :
The ***Artemia*** **toxicity test-kit** contains all the materials to perform standardized, simple and cost-effective bioassays for screening toxicity in estuarine (brackish) water and seawater. Using instar II-III larvae of the brine shrimp *Artemia franciscana* hatched from cysts, an acute toxicity test is executed in 24 hours. Each ARTOXKIT M provides for 6 complete tests (range finding or definitive 24hr LC_{50}), or 5 bioassays and 1 quality control test with a reference toxicant.

1. Preparing Standard Seawater :
Fill a 1 liter volumetric flask with approximately 800 ml deionized water and add the contents of vial number 1 containing pure NaCl. When dissolved, add the contents of the other vials with concentrated salt solutions, in sequence 2 to 7 as indicated on the flasks. Add deionized water up to the 1000 ml mark and shake to homogenize the seawater medium. The Standard Seawater has a salinity of 35 ppt.

2. Storing the medium :
The 1 liter solution of Standard Seawater suffices for the 6 bioassays of each Toxkit. If all 6 tests are not carried out within a few days after preparation of the medium, store the Standard Seawater in the refrigerator in darkness. In the latter case, the contents should preferably be distributed between several flasks, for separate use.
Take care to bring the cooled medium (gradually) back to room temperature prior to use.

3. Hatching the *Artemia* cysts :
Artemia cyst hatching should be initiated 30 hours before the start of the toxicity test. Empty the contents of one vial of brine shrimp cysts into one of the small petri dishes; make sure most of the cysts are transferred. Add 9 ml Standard Seawater and swirl gently to distribute the cysts evenly. Cover the petri dish, expose it to a light source (1000-4000 lux) and incubate **for 30 hours**.
Hatching will start after about 18-20 hours, after 30 hours most of the larvae will have moulted into the instar II-III stage.

4. Preparing the Toxicant Dilution Series :
Prepare a dilution series of the test compound or effluent according to standard methods (e.g. USEPA, 1985).
Tests can be carried out in "normal" (35 ppt) seawater to determine effects of the toxicant(s) in "marine" conditions, or at reduced salinity to determine effects in "estuarine" (brackish water) conditions. For the latter case, a medium of the desired salinity has first to be prepared, using deionised water and Standard Seawater in the appropriate ratio.

5. Filling the Test Plate :
The bioassay is conducted in a disposable multiwell test plate with 24 (6 x 4) test wells. The wells are labelled as columns 1 to 6 across, and rows A to D down (see figure).
The distribution of the test solutions should always be carried out starting from the control (column 1, left) towards the highest concentration (column 6, right). To fill the control column, add 1 ml Standard Seawater to the four wells of column 1. Repeat this procedure for the other columns with the respective toxicant concentrations, progressing from low to high concentrations in columns 2 to 6.

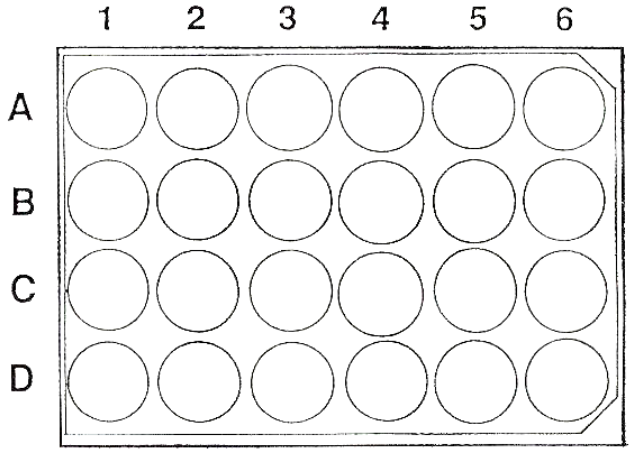

FIGURE : Multiwell test plate composed of 6 x 4 wells ; the 6 wells of row D serve as rinsing wells

6. Adding the larvae :
Using a dissection microscope at magnification 10-12x, transfer approximately 50 instar II-III larvae with a micropipet from the transfer petri dish to each well in row D (rinsing wells*) of the multiwell plate. Subsequently transfer 10 larvae from the rinsing well of column 1 to the three test wells of

this column. Take care, during this operation, to minimize the transfer of medium along with the larvae. Repeat this operation for columns 2 to 6.

* *The intermediate passage of the brine shrimp larvae from the petri dish to the definitive test wells via rinsing wells "washes" the larvae in the appropriate test solution before they enter the actual test well, thus minimizing dilution of the test solution during transfer.*

The test design of the ARTOXKIT **is based on one control and five toxicant concentrations, each with 3 replicates of 10 animals. Each bioassay shall be performed in a new multiwell with a new micropipet.**

7. Incubating the Test Plate and Scoring the results :
Put a strip of Parafilm on the test plate, cover it and incubate at 25°C in darkness. **After 24 hours**, count the dead* larvae in each test well and fill out the results sheet.
Calculate the % mortality** and, for the definitive tests, the LC_{50} using any standard method (e.g. USEPA, 1985).

* *Larvae are considered dead if they do not exhibit any internal or external movement in 10 seconds of observation.*
** *For the ARTOXKIT test to be valid, control mortality (as is the rule in aquatic toxicity tests) must not exceed 10%.*

8. Reference test
It is recommended that every 5 to 10 assays, a quality control test be carried out to check proper adherence to the test protocol, as well as test sensitivity.

Such a quality control test can e.g. be performed with the reference chemical potassium dichromate ($K_2Cr_2O_7$).
When performing this quality control test, the 24h LC_{50} should be within the 95% confidence limits stipulated in the specification sheet.

Preparation of stock solution and dilution series of the reference chemical
Add 100 mg of potassium dicromate to 100 ml of **Standard Seawater** to make a 1000 ppm. Make a dilution series - 100 - 56 - 32 - 18 - 10 mg/l for the quality control test.