ROTOXKIT F

FRESHWATER TOXICITY SCREENING TEST

BENCH PROTOCOL

Principle :
The **rotifer toxicity test-kit** contains all the materials to perform standardized, simple and cost-effective bioassays for screening toxicity in freshwater. Using juveniles of the rotifer *Brachionus calyciflorus* hatched from cysts, an acute toxicity test is executed in 24 hours. The sensitivity of the ROTOXKIT F bioassay is comparable to that of acute tests with other aquatic invertebrates. Each ROTOXKIT 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 Freshwater :
Fill a 1 liter volumetric flask with approximately 800 ml deionized water and add the contents of the five* vials of concentrated salt solutions, in the sequence 1 to 4 as indicated on the flasks. Add deionized water up to the 1000 ml mark and shake to homogenize the medium.

** Note that there are 2 vials with $CaSO_4$, both of which must be used !*

2. Storing the medium :
The 1 liter solution of Standard Freshwater 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 Freshwater 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 rotifer cysts :
Rotifer cyst hatching should be initiated 1 day before the start of the toxicity test.
Add 2 ml Standard Freshwater to the hatching trough in the test plate.
Empty the contents of one vial of rotifer cysts into the hatching trough of the test plate (see figure); one should rinse the cyst vial with 0.5 ml Standard Freshwater to carry over all the cysts into the hatching trough.

Put a strip of Parafilm on the test plate, cover the multiwell and incubate the plate at 25°C **for 16-18 hours, under continuous illumination (light source of min. 3000-4000 lux)**. If hatching is delayed (which in most cases is due to lower temperatures than prescribed), check the cysts hourly after 18 hours incubation to insure collecting the test animals within 2 hours of hatching. It is indeed important to have 0 to 2 hour old test animals to start the bioassay.

4. Preparing the Toxicant Dilution Series :
As the rotifers are hatching, prepare a dilution series of the test compound or effluent according to standard methods (e.g. USEPA, 1985).

5. Filling the Test Plate :
The bioassay is conducted in a specially developed, disposable, polycarbonate "multiwell" test plate. Each plate has one hatching trough, 6 rinsing troughs, and 36 test wells (see figure). The rinsing troughs and the test wells are labelled as columns A to F across, and rows X and 1 to 5 down.
The distribution of the test solutions should always be carried out starting from the control (X, top row) towards the highest concentration (5, bottom row). To

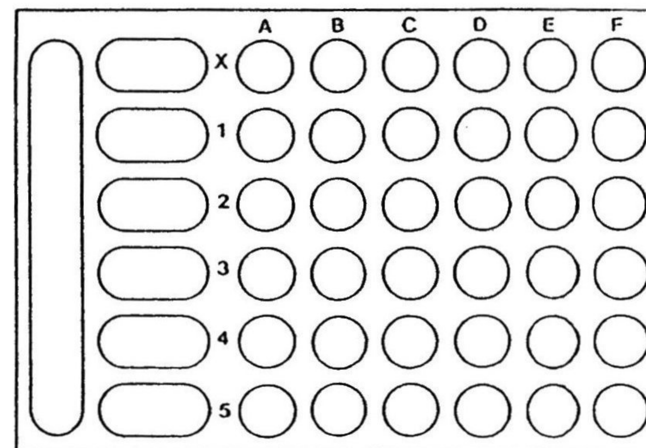

FIGURE : Multiwell test plate composed of (from left to right) : 1 hatching trough, 6 rinsing troughs and 36 test wells.

fill control row X add 0.7 ml Standard Freshwater to the rinsing trough and 0.3 ml to each of the six test wells. Repeat this procedure for the other rows with the respective toxicant concentrations, progressing from low to high concentrations in rows 1 to 5.

6. Adding the Rotifers :

Using a dissection microscope at magnification 10-12x, transfer approx. 50 rotifers with a micropipet from the hatching trough to the rinsing trough of control row X (top row). Subsequently transfer 5 rotifers from the rinsing well to each of the six test wells of row X. Take care, during this operation, to minimize the transfer of medium along with the rotifers. Repeat this two-step transfer procedure for rows 1 through 5*.

* *The intermediate passage of the rotifers from the hatching trough to the wells via a rinsing trough "washes" the neonates in the appropriate test solution before they enter the actual test well, thus minimizing dilution of the test solution during rotifer transfer.*

The test design of the multiwell plate is based on one control and five toxicant concentrations, each with 6 replicates of 5 animals. <u>Each bioassay shall be performed in a new multiwell with a new micropipet.</u>

7. Incubating the Test Plate and Scoring the results :

To avoid spilling of the hatching medium into the test wells during transportation of the multiwel plate, it is advised to empty the hatching trough after completing the transfer of the rotifers; this operation can easily be performed with the micropipet.

Put the strip of Parafilm back on the test plate, cover the multiwell and incubate at 25°C in darkness.
After 24 hours, count the dead* rotifers in each well and fill out the results sheet.

Calculate the % mortality** and, for definitive tests, the LC_{50} using any standard method (e.g. USEPA, 1985).

* *Rotifers are considered dead if they do not exhibit any internal or external movement in 5 seconds of observation.*

** *For the ROTOXKIT 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 on the specification sheet.

8.1. <u>Preparation of stock solution and dilution series of the reference chemical.</u>

Add 100 mg of potassium dichromate to 100 ml of deionized water to make a 1000 ppm stock solution. Make a dilution series 32 - 18 - 10 - 5.6 - 3.2 mg/l for the quality control test.