



ESTUARINE / MARINE 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 estuarine (brackish) water and seawater. Using juveniles of the rotifer *Brachionus plicatilis* hatched from cysts, an acute toxicity test is executed in 24/48 hours. The sensitivity of the ROTOXKIT M bioassay is comparable to that of acute tests with other marine invertebrates. Each ROTOXKIT provides for 6 complete tests (range finding or definitive 24/48hr LC₅₀), 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 the 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. 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 24-26 hours prior to the start of the toxicity test, and has to be carried out in seawater of reduced salinity (20 ppt). Prepare 10 ml hatching medium of 20 ppt salinity by mixing 5.7 ml Standard Seawater with 4.3 ml deionized water in a test tube.

Add 2 ml hatching medium to the hatching trough in the test plate (one can also use a 5 cm petri-dish (with 10 ml hatching medium) to perform the hatching).

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 hatching medium to carry over all the cysts into the hatching trough.

Put a strip of Parafilm on the test plate, cover the multiwell and incubate at **25°C for 24-26 hours, under continuous illumination (light source of 3000-4000 lux)**. If hatching is delayed (which in most cases is due to lower temperatures than prescribed), incubate the test plate for another 2 hours.

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).

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 deionized water and Standard Seawater in the appropriate ratio.

5. Filling the Test Plate :

The bioassay is conducted in a specially developed, disposable, PVC "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

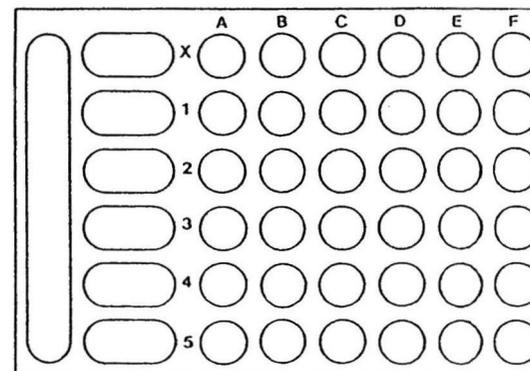


FIGURE : Multiwell test plate composed of (from left to right) : 1 hatching trough, 6 rinsing troughs and 36 test wells.

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 fill control row X add 0.7 ml Standard Seawater (or brackish water) 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.

change (e.g. from 20 ppt in the hatching medium to the salinity in the test medium). 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 operation 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. Each bioassay shall be performed in a new multiwell with a new micropipette.

7. Incubating the Test Plate and Scoring the results :

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

Put the strip of Parafilm back on the test plate, cover the multiwell and incubate at 25°C in darkness. **After 24 and 48 hours**, count the dead* rotifers in each well and fill out the results sheet.

Calculate the % mortality** and, for definitive tests, the LC₅₀ 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.

Each ROTOXKIT M contains one vial with a reference chemical (potassium dichromate K₂Cr₂O₇) to perform an eventual quality control test. When performing this quality control test, the 24h LC₅₀ should be within the 95% confidence limits stipulated on the specification sheet.

8.1. Preparation of stock solution and dilution series of the reference chemical.

Dissolve the contents of the vial with reference chemical into a 100 ml volumetric flask and add Standard Seawater to the 100 ml mark to make a 10 g/l stock solution. Make a dilution series 1000-560-320-180-100 mg/l for the quality control test.