

temperature of the animal room before and during the test should be  $22 \pm 3^{\circ}\text{C}$  and the relative humidity should be 50-70 per cent. With artificial lighting, the sequence should be 12 hours light, 12 hours dark. Conventional laboratory diets may be used for feeding with an unlimited supply of drinking water. The animals should be group-caged but the number of animals per cage should not interfere with proper observation of each animal. Prior to the test, the animals are randomized and divided into groups; 20 animals in each group.

3.2 Test substance Each test substance should be appropriately identified (chemical composition, origin, batch number, purity, solubility, stability, etc.) and stored under conditions ensuring its stability. The stability of the substance under the test conditions should also be known. A solution of the test substance should be prepared just before the test. Solutions with concentrations of 0.5 mg/ml and 10 mg/ml should be prepared. The preferable solvent is 0.85 per cent saline. Where the solubility of the test substance is a problem, a minimum amount of an organic solvent such as ethanol, propylene glycol or polyethylene glycol may be used to achieve solution.

3.3 Test method Twenty animals receive in the back region 1 ml/kg of the solution containing 0.5 mg/ml of the test substance. The number of dead animals is determined within 48 hours and again after 7 days. If the death rate is lower than 10 animals, another group of 20 animals should be injected by the same way with 1 ml/kg of the solution containing 10 mg/ml of the test substance. The number of dead animals should be determined within 48 hours and again after 7 days. If the result is doubtful (e.g. death rate = 10), the test should be repeated.

3.4 Evaluation of the results If the death rate in the first group of animals (receiving a solution containing 0.5 mg/ml) is equal to or higher than 50 per cent, the test substance will fall into the "super-toxic lethal chemical" category. If the death rate in the second group (receiving a solution containing 10 mg/ml) is equal to or higher than 50 per cent, the test substance will fall into the "other lethal chemical" category; if lower than 50 per cent, the test substance will fall into the "other harmful chemical".

#### 4. Data reporting

A test report should include the following information:

- (i) test conditions: date and hour of the test, air temperature and humidity;
- (ii) animal data: strain, weight and origin of the animals;
- (iii) test substance characterization: chemical composition, origin, batch number and purity (or impurities) of the substance; date of receipt, quantities received and used in the test; conditions of storage, solvent used in the test;
- (iv) results: the number of dead animals in each group, evaluation of results.