

**MEPC IX/INF.2.**  
**International Maritime Organization (IMO).**

**METHOD FOR ACUTE TOXICITY DETERMINATION OF LC<sub>50</sub>**  
**(Lethal Concentration 50 percent).**

**STABILITY.**

The products shall have a store life of at least five years.

**TOXICITY**

**9.1. General.**

**9.1.1. Principle of method.**

The method is based on determination of acute toxicity determination of LC<sub>50</sub> (Lethal Concentration 50 percent).

This is the concentration of the product at which 50 percent of the animals used in the test survive for a given time (24, 48 or 96 hours). The concentration is ascertained first by a preliminary test, followed by a final one. The preliminary test services to bracket the approximate range of concentration of the LC<sub>50</sub> value, while the final test enables the value to be pinpointed.

The "Level of toxicity" of the product is derived from the 96-hour LC<sub>50</sub> value.

**9.1.2. Storage of samples.**

About 500 ml of product are required to run the test. The samples are stored in completely full, sealed containers.

**9.2. Equipment.**

The toxicity tests are run in glass or plastic tanks. Six tanks each having a capacity of at least 10 litres are required for the preliminary test, and five, with a capacity of at least 25 litres, for the final one.

The shape of the tanks is not of great importance, provided that the depth of liquid is not less than 15 cm. Each tank must be complete with an air-bubbling system which ensures good oxygenation and uniform distribution of the liquids throughout the whole of the tank.

During the tests and the holding period prior to the tests, the water temperature must be kept at 20°C +/- 1, by means of water bath or thermostatic chamber.

Tanks are also required in which to keep the animals prior to use. The water in these tanks must be brought gradually to the test temperature, care being taken to ensure that it remains clear (by recycling over carbon or by being continuously changed) and is adequately oxygenated (70 to 100 percent saturation) by bubbling air through it.

In the case of tanks working on the recycling principle, the frequency with which

In the case of tanks working on the recycling principle, the frequency with which the water has to be replaced will depend on the number of animals kept and their needs. Some useful pointers on frequency can be obtained by analysis of certain physical characteristics of the water (e.g. N-NH<sub>3</sub>) performed at given intervals. However, this system of holding animals prior to the test should only be used when there is no possibility at all of the water being continuously changed. Under such circumstances the holding period should be reduced to the absolute minimum required.

### **9.3. Experimental animals and solution.**

#### **9.3.1. Experimental animals.**

The species selected for performance of the tests is *Liza aurata* (Risso, 1810) and *Sin. Mugil auratus* (Risso, 1810), "Golden-grey mullet", a kind of mullet common around the coasts of Italy and readily obtained from breeders. It adapts well to aquarium conditions.

The fish, whose overall average length will be 8 to 10 cm, must remain in the holding tanks for at least 15 days before the test is started. If, during this time the natural death rate exceeds 10 percents, the whole batch of fish must be put on one side until the rate drops.

During the holding period the fish are fed regularly until the day before the start of the test, during which time they must not be fed.

#### **9.3.2. Number of animals and volume of liquid.**

In the preliminary test, four animals are used for each dilution in the 10-litre tanks. In the final test, instead, ten animals are used per 25-litre tank. In both cases the tanks must be properly aerated to ensure an oxygen-saturation level of not less than 70 percent and to keep the product under test evenly distributed throughout the mass of the dilution water.

#### **9.3.3. Dilution water.**

This can be seawater taken from points offshore where it is certain there is no pollution, or "artificial" seawater, the salts for which can be bought off the shelf already batched in the right proportion.

### **9.4. Procedure.**

#### **9.4.1. Preliminary test.**

This is designed to indicate the concentration range of the 24-hour LC<sub>50</sub>. Six 10-litre tanks are prepared with product concentrations (in seawater) of 1, 10, 100 and 10.000 ppm, and one containing only seawater to act as control. Twenty-four hours after, the fish are put into the tanks it will be possible to ascertain the dilution range in which all or many of the fish (more than half) have died, and that where none or only some of the fish (less than half) have died.

#### 9.4.2. Final test.

This test is designed to indicate the concentrations at which the percentage of survivors is below 50 percent (though not nil) or higher than 50 percent (but not all), after 24, 48 and 96 hours. Concentrations intermediate between the two bracketed by the preliminary test are prepared in 25-litre tanks, using logarithmic intervals. For instance, if the preliminary test has indicated a range of between 100 and 10 ppm for the LC<sub>50</sub>, the concentrations prepared for the final test will be 100, -50.1, -22.4 and 10 ppm, plus the control.

The relevant survival percentages are noted after 24, 48 and 96 hours, care being taken to remove dead fish from the aquariums as soon as possible.

If the desired results are obtained after the times indicated, it is then possible to calculate the LC<sub>50</sub>, otherwise it is necessary to repeat the test by selecting other dilution intervals.

#### 9.5. Graphic determination of LC<sub>50</sub>.

The percentage of survivals observed at two successive concentrations (on the logarithmic scale) after 24 hours are plotted on log-normal paper. The two points (which should be located above and below the 50 percent level) are joined by a line and a perpendicular is dropped to the "concentration" axis from the points where the plotted line crosses the 50 percent level: this gives the 24 hours LC<sub>50</sub> value.

The same procedure is adopted for the 48 and 96-hour survival percentages, in order to obtain the relevant LC<sub>50</sub> values.

#### 9.6. Toxicity evaluation.

The level of toxicity of the product is derived from the 96-hour LC<sub>50</sub> value by reference to the following scale:

96-hour LC <sub>50</sub>	Level of toxicity
< 1 ppm	Highly toxic
1-10 ppm	Toxic
10-100 ppm	Slightly toxic
100-1000 ppm	Virtually nontoxic
> 1000 ppm	Innocuous

#### Presentation of results.

The results of the toxicological test are expressed in terms of 96-hour LC<sub>50</sub> (in ppm). However, it is advisable to indicate the 24 and 48-hour LC<sub>50</sub> values too.