

Toxicity Tests

Introduction:

An aquatic toxicity test, otherwise called "Bioassay test" is a procedure in which the responses of aquatic organisms are used to detect or measure the presence or effect of one or more substances, wastes or environmental factors, alone or in combination.

Toxicity tests are desirable in water pollution evaluations because chemical and physical tests alone are not sufficient to assess potential effects on aquatic biota.

Toxicity tests are useful for a variety of purposes that include determining:

- Suitability of environmental conditions for aquatic life
- Favourable and unfavourable environmental factors, such as DO, pH, temperature, salinity or turbidity
- Effect of environmental factors on waste toxicity
- Toxicity of wastes to a test species
- Amount and type of waste treatment needed to meet water pollution control requirements
- Effectiveness of waste treatment methods
- Permissible effluent discharge rates and compliance with water quality standards

Terminology:

1. General terms:

a. Acclimate:

To accustom test organisms to different environmental conditions such as temperature, light and water quality.

b. Response:

The measured biological effect of the variable tested. In acute toxicity tests, the response usually is death.

c. Control:

Treatment in a toxicity test that duplicates all the conditions of the exposure treatment, but contains no test material.

d. Range Assay:

Preliminary test designed to establish approximate toxicity of a solution.

e. Definite Assay:

Toxicity test designed to establish concentration at which a particular end point occurs.

2. Toxicity Terms:

a. Dose:

Amount of toxicant that enters the organism.

b. Toxicity:

Potential or capacity of a test material to cause adverse effects on living organisms. It is a result of dose or exposure concentration and exposure time, modified by variables such as temperature, chemical form etc.

c. Exposure Time:

Time of exposure of test organism to test solution.

d. Acute Toxicity:

Relatively short-term lethal or other effect, usually defined as occurring within 4 days for fish and other selected macro invertebrates and 2 days for organisms with shorter life span.

e. Chronic Toxicity:

Toxicity involving a stimulus that continues for a relatively long period of time. It can be measured in terms of reduced growth, reduced metabolism, reproduction, mutation etc. in addition to lethality.

f. Lethal Concentration:

Toxicant concentration of a toxicant that is lethal to 50% of the test organisms in 96 hrs. (LC 50)

g. Effective Concentration:

Toxicant concentration estimated to cause a specific effect which is usually sublethal, such as a change in respiration rate or loss of equilibrium in a given time i.e. 96 hrs EC 50.

h. Maximum Allowable Toxicant Concentration (MATC): toxicant concentration that may be present in receiving water without causing significant harm to productivity or other uses. MATC is determined by conducting long-term tests.

3. Solution Renewal Terms: (Flow Terms)

a. Static Test:

The test solutions and the test organisms are placed in test chambers and kept there for the duration of the test. The 96 hr. bioassay is of the static type.

b. Re-Circulation Test:

Static test with circulation of test solution through test chamber. Test solution may be treated by aeration, filtration, sterilization etc. to maintain the water quality.

c. Renewal Test:

Tests in which organisms are exposed to solutions of the same composition that are renewed periodically during the test period.

d. Flow-Through Test:

Test in which solution is replaced continuously in test chambers throughout the test duration.

Toxicity tests, which are classified according to duration, are short-term tests and long-term tests.

Short-term type of bioassay is conducted for a short period of time, usually 48 or 96 hrs. The 96 hr test period is the most frequently used. These acute definite tests typically use mortality as an end point or other discrete observations to determine effects due to the toxicant. The results of these tests can be used to calculate acceptable concentrations for very short exposures.

Long-term type of bioassay is conducted from 7 days to one or more months depending upon the species used and the type of data desired. These tests are generally used for estimating maximum allowable toxicant concentration (MATC) values or chronic toxicity. This may include full life cycle testing.

Basic Requirements for Lethal Toxicity Tests:

The basic requirements and desirable conditions of lethal toxicity are an abundant supply of water of desired quality, adequate and suitable containers and an adequate source of healthy experimental organisms. Apart from this, due consideration should be given regarding sample size, test concentrations, acclimatization of the experimental animals and maintenance of constant environment.

1. Choice of Sample Size:

Normally, a sample of at least 10 animals per concentration is used. Occasionally, if the test animals are rare or short supply, as few as 6 can be used, but the accuracy of the final results will be very poor and such samples are best avoided. Sample size upto 20 are likely to increase the accuracy of the final result. But, practical experiences shown that, above 20 animals decreases the accuracy of the final result and should be avoided except in certain experimental cases like plankton or other few small organisms.

2. Test Concentrations:

The range of concentrations to be tested is best chosen on the basis of some simple preliminary tests. A minimum of 6 concentrations plus appropriate control is normally required. They should be spaced logarithmically and it is some times necessary to select closely spaced concentrations. A useful and rapid way required is to select the range of concentrations and place them equally on a logarithmic scale. The geometric scale can also be used by multiplying with a constant factor and later it can be converted logarithmically.

3. Acclimatization of Test Organisms:

Acclimatization of the test organisms to the laboratory condition is extremely important. The period of acclimatization varies according to the species to be experimented. The acclimatization period allows animals to recover from the stress of transportation and handling to adopt physiologically to the conditions in the laboratory and to establish normal feeding patterns and enables to establish that the animals are not suffering from disease or any abnormalities. At the end of acclimatization, the test organisms must be in excellent condition to withstand the experiments on them. If the mortality exceeds 20% of the total during acclimatization, it is a good practice to reject the entire batch.

4. Environmental Conditions:

Since the toxicity of any pollutant is greatly influenced by environmental conditions such as pH, temperature and salinity, it is necessary to maintain them at constant levels. In fresh water, hardness is an important variable. In seawater, pH and salinity varies even in small experimental vessels. As far as possible, these variations are controlled within close limits.

Presence of animals itself is responsible for alteration of environmental conditions. Animals utilize oxygen and excrete CO₂, ammonia and solid faecal matter. The pollutants concentration vary during experiment because of absorption by animals and pollutants may also be subjected to chemical and microbiological degradation, absorption or adsorption by particulate matter or on to the sides of the experimental vessel and volatilization particularly if the test vessels are aerated. It is suggested that, if containers are relatively large, the test solution should be regularly renewed. This renewal can be done by siphoning out most of the solution gently and replacing it with fresh solution.

Duration of Lethal Toxicity Tests:

Duration of lethal toxicity is usually 4 Days (96 hrs). Generally, feeding of animals is best avoided as it only contributes to deterioration of environmental conditions and many

pollutants bind readily to free organic matter thus interfering with the test. If the test is last for weeks, feeding is done with extra care by using continuous flow apparatus. It is probably best to offer food once in 2 days.

Preparation of Test Solution:

The test material can be a one or more pure chemical. Test solutions are often prepared by dissolving a test material in water to form a suitable stock solution and then by adding a portion of the stock solution to dilution water. The test solutions are prepared immediately prior to initiation of the experiment.

Data Collection and Analysis:

The observations are made in every 6 hrs upto 48 hrs, for smaller invertebrates and 96 hrs for fish and macro-invertebrates. The collected data is used to find out LC 50 by Probit method.

Probit Method for Finding LC 50:

In probit method of analysis, the data are plotted on logarithmic probit paper, otherwise known as 'semi-log paper'. To construct the graph, the probit values of percentage mortality on the vertical axis against log concentrations along horizontal axis is plotted. Draw a straight line by joining all the points. The concentration where the 50% mortality line cuts the straight line is the LC 50.