
"Bioaccumulation: Test for the Degree of Bioconcentration in Fish"

3. DATA AND REPORTING

• Treatment of results

- (1) The degree of accumulation, expressed by the concentration factor, is calculated from the following equation:

$$BCF_n = \frac{C_{f,n} - C_{f,B}}{C_w}$$

See Definitions and units for clarification of symbols.

- (2) Draw a correlation curve of the values calculated in (1) against time.

• Evaluation of results

The following points should be evaluated:

- Items concerning health of test fish during the test period
 - mortality during the test period
 - abnormality of appearance
 - behaviour in the aquarium
- Test conditions
 - change of the concentration of test water and dissolved oxygen
 - recovery rate of analysis with test fish
 - exposure water

• Test report

The test report must include the following information:

- Information on the test chemicals: name, structural formula, molecular weight, purity, name of impurities, physical chemical properties of test chemical (solubility, colour, etc.), spectral identification data of test chemical.

Acute toxicity test

Test fish

name
average weight
average body length

Test temperature

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Method for the preparation of test solution

48-hr TLM value and the raw data used for the estimation of the 48-hr TLM value

Accumulation test

Test fish

species
average weight
average body length

Methods of disinfection and acclimatisation

Test temperature

Test period

Method for the preparation of test solution: amount of surfactants, solvents

Volume of testing aquarium and number of fish; flow rate of test water

Determination of exposure levels of chemical

Analytical procedure (schematic diagram)

Method of pretreatment for the analysis of test water and test fish

recovery rate of water analysis
blank value of water analysis
recovery rate of fish analysis
blank value of fish analysis
detection limit
conditions of analytical instrument

Results

Table for concentration factor

Correlation curve of the concentration factors according to time

Tables of data which were used to calculate concentration factors

Figure of dissolved oxygen concentration during the test period

Remarks: aquarium-side observations

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5. ANNEX

ACUTE TOXICITY TEST WITH FISH (Japanese Industrial Standard K 0102-1974)

Measurement of TLm (median Tolerance Limit) is carried out as the acute toxicity test with fish. TLm value of waste water is the concentration of waste water at which 50 per cent of the fish survive after they have been kept for a definite time in diluted waste water containing the acute toxic substance. Generally, TLm values for 24, 48 or 96 hours are obtained.

Fifty per cent of the test fish die at the concentration of waste water corresponding to TLm; therefore, TLm is not the acceptable concentration for aquatic life. For example, in order to estimate the acceptable concentration for aquatic life, the TLm for 48 hours obtained using the fish of a fishery in the water area in question is multiplied by a safety factor of 0.1.

In this way, the results of the TLm determination can be utilised not only to ascertain whether the waste water causes damage to living organisms in the discharged water area, but also to judge the effect of the treatment of waste water, to compare the degree of toxicity of various kinds of waste water, and to evaluate waste water which contains complex components difficult to determine chemically.

It is known that the TLm value differs considerably according to biological factors, such as the kind of test fish and their health condition as well as physical-chemical factors, such as the quality and temperature of the diluent water, etc. Consequently, it is desirable that the test fish, diluent water and other factors consistent with the natural conditions of each water area be selected for determining the TLm as a measure of potential hazard from waste water.

Although it would be convenient if the test fish, physical-chemical conditions, etc. were completely unified and standardised for the comparison of various waste waters, such standardisation would not allow the results of the TLm to be applied to the problem of hazard of a particular waste water.

Therefore, the method for determining TLm with fish described below assures adequate availability, reproducibility and uniformity of the results of the test while still maintaining test conditions close to the natural conditions of each water area.

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METHOD

• **Preparations**

Apparatus

Test vessel: Employ a clean glass cistern. There is no need to specify its shape and dimensions, but vessels of the same size should be used for the test carried out concurrently. For example, 10 to 20 cisterns about 2 litres in capacity are enough for the test.

Constant temperatur apparatus: Employ a constant temperature chamber, tank or cistern. The test vessel is placed in it and the temperature of the water kept constant.

Acclimating cistern: For maintaining the test fish, aquaria of 50 to 200 litres in capacity, in which temperature and aeration may be adjusted, are necessary.

Test animals

The basic conditions for choosing test fish are their adaptability to maintenance in the laboratory, i.e. water temperature, feed, handling, etc., equality in size, good health, the availability of a large number at a time, etc.

When the test is related to an actual water pollution problem, fish should satisfy the conditions described above. The following fish are generally considered suitable for testing: the salmon family (rainbow trout, brook trout, etc.), carp family (common carp, crucian carp, bitterling, landlocked masou salmon, zacco platypus, etc.), killifish family (killifish, scarlet killifish, etc.) and topminnow family (guppy, topminnow, etc.). The species of test fish must be accurately identified before the test.

At the time of testing the following points should be given special attention:

- (1) The size of fish to be used at the same time should be about the same, with the largest not more than 1.5 times longer than the smallest.
- (2) Small fish, less than 75 mm in average total length, should preferably be used. (When the test fishes are particularly larger, it is necessary to use a large cistern.)

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- (3) Fish to be used for the same test must be procured under the same conditions, i.e. at the same time from the same water area or the same fish farm, etc. The procured test fish are first put into a pond or aquarium and are kept at the proper temperature and quality of water until they are used for the test.

The fish are kept in the acclimating aquarium for least 1 week (10 days or more if possible) to accustom them to the temperature and quality of water (the same quality as the diluent water) for the test. During the acclimation, they are fed once a day if circumstances allow and left without food for 2 days before the test. At the time of the test, the fish used must be healthy; they should not be used if the number of dead or ill fish in the 4 days before the test exceeds 10 per cent. Special attention must be paid to ensure that fish which are ill or of abnormal appearance and behaviour are not used.

Preparation of waste water and diluent water

When the quality of the waste water is not constant it should be sampled from time to time, and the test should be made using the most toxic waste water. The bottle is filled with the waste water sample up to the mouth, sealed tightly and kept under conditions in which the temperature does not rise above that at the time of sampling. In the case of waste water containing organic matter which is easily decomposed by bacteria, it should be kept at 0 to 4°C (care being taken not to let it freeze). Waste water to be used in a single test should be sampled at the same time.

When the study is to be made in an influent area, it is necessary to use water from the area into which the waste water was discharged. If such water is difficult to procure, it should be prepared by adding the necessary components to suitable water taken from another source so that the pH, acid or alkali consumption, hardness, etc. are the same as those of water from the water area in question.

In the components of other waste waters are intermixed, the addition of the same components is necessary. When water from an area containing a great deal of suspended matter is used, the greater part of the suspended matter must first be removed by precipitation or filtration. If the water from the special water area is not considered as the diluent water, soft water of about pH 7, free of the special abnormal component, may be used. It is necessary to dissolve enough oxygen in the diluent water by aeration.

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- Test conditions

- The temperature of the water at the time of the test should be in the range of 20 to 28°C for warm water fish, and 12 to 18°C for cold water fish. During the test, special care should be paid to changes in water temperature, ensuring that the difference between the highest and the lowest temperature is less than 4°C.
- The amount of water in the test aquarium is determined by the weight of the test fish. At least 10 fish are tested at the same time at each concentration; this may be done by putting the fish in several aquaria each of the same concentration, if necessary. (Repetition of each test and the increase in the number of fish improve the accuracy of the test.) The weight of the fish should be less than 2 g per litre of water and it is preferable that it be less than 1 g per litre.
- The concentration of the diluted waste water at the time of the test is decided following a preliminary test. In the preliminary test, a wide range of concentrations is made and the test solution prepared by diluting the waste water so as to make the interval between the logarithmic values of each concentration equal, such as 100, 10, 1 and 0.1 per cent or 100, 32, 10 and 3.2 per cent.

In the preliminary test, the number of fish may be less than 10, and the capacity of the vessel and the amount of water may be reduced according to the weight of the fish.

The concentration to be adopted in this test should lie between the highest concentration at which the greater part of fish survive after 48 to 96 hours and the lowest concentration in which the majority of the fish die in 24 hours. After one preliminary test, a second preliminary test may be carried out at the middle concentration if necessary.

If the concentration range can be obtained from the results of the preliminary test, the test concentration is decided on the basis of the concentration obtained. In this case, the concentration of 5 to 10 steps are chosen so that the intervals between the logarithmic values of every concentration are equal.

As the intensity of toxicity of the water sometimes changes during the test, a thorough study should be carried out during the preliminary test and, if necessary, proper measure be

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taken as to the conditions of the test so that it can be carried out smoothly. It is necessary that the amount of dissolved oxygen in the water should not be less than 4 mg/l for warm water fish and not less than 5 mg/l for cold water fish so that no problems due to lack of dissolved oxygen occur.

To prevent variation in the components, the diluted waste water is changed continuously or at definite hours. Aeration should also be carried out and controlled to avoid reduction in dissolved oxygen.

The water depth of the test aquarium should not be too shallow to avoid loss of the volatile compound (for example, the depth of water in a 20 l glass cistern should not be less than 150 mm).

• Procedure

First, prepare waste water of 5 to 10 dilutions and transfer to the glass cisterns. Where suspended matter exists in the waste water, it should be mixed uniformly to make it homogeneous. However, it is better not to mix it with air more than is necessary.

When the diluted waste water has been prepared, the test fish are put in the cistern as soon as possible (within 30 minutes). (Record the time required before the test fish are put in the cistern.) Transfer of fish is carried out with a soft scoop net so as not to damage them. Care must be taken not to place fish on a dry surface or to expose them unnecessarily to air for a long time. Fish which have been subjected to unskilled handling at the time of transfer should be removed from the test.

Observe the condition of the fish after 4 and 8 hours from the start of the test. When a fish falls on its side or shows abnormal behavior due to the effect of the toxic substances, this should be recorded.

To confirm that a fish is dead, push slightly with a glass rod and when the fish no longer shows a reaction; it should be recorded as dead and removed from the aquarium immediately.

The number of surviving fish after 24 and 48 hours (or 96 hours) is recorded. A control test with diluent water only must be carried out in parallel with every test carried out at the same time. When the dead and unhealthy fish exceed 10 per cent in the control test, the test results should not be used.

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The volume of the water sample(s) taken for analysis during the test should be as small as possible, and it should be replaced by waste water of the same diluted concentration prepared previously.

The analysis of the waste water should be carried out for dissolved oxygen, pH and major poisonous components. The analysis of the water quality must be carried out at least before and after the test. Measurement of the dissolved oxygen should be carried out as frequently as possible.

DATA AND REPORTING

- Treatment of the results

Calculation of TLm Value

The concentration at which the number of surviving fish is slightly above than 50 per cent and slightly below 50 per cent should be noted. For the calculation of TLm value, the concentration of the waste water is entered on the logarithmic scale of logarithmic paper and the survival ratio on the ordinary scale. The measured survival ratios which are the nearest to 50 per cent among the points higher than 50 per cent and those lower than 50 per cent are marked by dots. These two points are then connected by a straight line which intersects the 50 per cent line at the TLm value.

Expression of TLm Value

The TLm values at 24 and 48 hours (or at 96 hours) are expressed as percentage of the volume of dilution of the waste water. The species of fish and the quality of the test waste water are always noted.

- Test report

The following items must always be recorded during the test:

- Number of fish and survival ratio at each concentration.
- Species of test fish, source, average length, average weight, temperature of the test water.
- Exchange of the diluted waste water, other control methods.

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- Means of acquisition and storage of the waste water before the test and its quality. Means of acquisition of diluent water and its quality, acid or alkali consumption, pH, hardness, presence of chlorine ions and other characteristic turbidity, presence of chemical components or filthy water, etc..
- Amount of water in the testing aquarium, depth of water, number and weight of the fish in each cistern used for the test, time when the fish were put in, condition of fish observed after 4 and 8 hours or at other times.
- Analytical results of water quality carried out during the test, especially dissolved oxygen and pH.

Remarks: Marine fish should sometimes be used as the test fish if required by the object of the test. From the points of view of ease in acquisition and maintenance, the following fish are suitable for the test: young gobies, green puffer, common nibbler and others. When marine fish are handled, special attention should be paid to the decrease in dissolved oxygen, and the contamination of water due to the excretion of fish, half-eaten feed, etc.

It is desirable that a running water aquarium be used for the acclimation of marine fish before the test; if this is impossible, a cyclic filtration type cistern can be used. The test may be carried out in standing water in the same manner as with fresh water fish, but the weight of fish in the testing cistern must be less than 0.5 g in 1 litre.