305D
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"Bioaccumulation: Static Fish Test"

1. INTRODUCTORY INFORMATION

• Prerequisites

- Water solubility (high precision value not needed)
- A reliable method for chemical analysis of the test substance in water and fish tissue
- TLm at 96 h four young fish
- Stability and reactivity in water

• Guidance information

- Sorptivity, K_{oc}
- Solubility in solvents other than water
- Volatility
- Partition coefficient, octanol-water
- Purity/composition of test chemical

• Qualifying statements

- The test substance must be soluble in water, at least in the µg per litre range.

- No appreciable loss of test substance must occur in a timespan of eight days from water (not containing fish) to which the substance is added at the concentration(s) used in the accumulation test.

- This implies that the test substance must not be highly volatile, rapidly chemically degradable, readily biodegradable or very apolar.

- In case the test chemical forms metabolites, conduction of a bioaccumulation test with such metabolites must be considered.

- This Test Guideline has been tested in the OECD Laboratory Intercomparison Test Programme (1978-1980).

• Recommendations

As a supplement to this Test Guideline, a simple elimination (depuration) test should be carried out.

Based on results from Ring Test (April, 1980), it was found that the variability of BCF values in several fish species could be reduced by normalising the factors with lipid content of fish. Therefore this procedure is recommended.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

The purpose of this static bioaccumulation test is, firstly, to distinguish test substances with a low or moderate bioaccumulative character from those with a high bioaccumulation character and, secondly, to determine the bioaccumulation factor for those compounds with a low or moderate accumulative character (BCF < 10^3).

For organic substances, the first aim of this test may also be achieved by determination of the n-octanol/water partition coefficient, provided that the substance under consideration does not react with cell material and that active uptake and transport processes and/or metabolism by living animals play only a minor role in the process of accumulation. With new compounds, however, this is seldom known in advance.

In general, it will not be possible to accurately determine the bioaccumulation factor of highly accumulative compounds with this static test method. The upper limit of the bioaccumulation factor which can be determined accurately depends of the ratio biomass: volume of the medium and on the detection limit of the test compound in the medium. The lower limit depends on the detection limit of the test substance in fish tissue.

- Definitions and units

C_f : Concentration of the chemical in the organism, e.g. mg per kg wet weight.

Before the steady state is reached, C_f increases with the exposure time t.

$C_{f,t}$ is the concentration after a certain time t.

$C_{f,s}$ is the concentration at steady state (plateau value).

C_w : Concentration of the chemical in the surrounding medium, e.g. µg per kg medium (~ µg per 1 medium).

$C_{w,s}$ is this concentration in the surrounding medium at steady state (equilibrium).

BCF : Bioconcentration factor is the ratio of the test substance concentration in the test fish (C_f) to the concentration in the test water (C_w) at steady-state.

BCF_t : If no steady state is reached within the time of exposure, the bioconcentration factor reached is given as BF_t, which is the quotient of the concentration of the chemical in the organism after exposure time t and the concentration in the surrounding medium.

TLm : Median Tolerance Limit is the concentration of the test compound expressed in mg/l, at which 50 per cent of the test fish will die in a specified time.

- Reference substances

In some cases when investigating a new substance reference substances may be useful; however, specific reference substances cannot yet be recommended.

- Principle of the test method

Small tropical fish, such as young guppies or zebra fish, preferably bred in the laboratory, are exposed to the medium containing the test substance at a known concentration for 8 days. This medium is not renewed. At regular intervals, samples of the medium are analysed for the test substance.

At the end of the test the fish are also analysed. A blank aquarium containing the medium with test substance and without fish is analysed in the same way. Estimation of loss of the test substance in this blank aquarium permits correction of the values for the aquarium containing fish. This blank loss should not be a significant proportion of the starting concentration.

To prevent deterioration in the condition of the test animals, the maximum test duration should not exceed 8 days. Two concentrations of the test substance should be tested, differing by a factor of at least 3.2, but preferably by a factor of 10.

• Quality criteria

The quality of the test depends mainly on the quality of the chemical analysis of the test substance in water and biological tissue. If the analysis is reproducible, sensitive and specific the test will meet these criteria.

The test is not applicable to very volatile, readily (bio)degradable or strongly bioaccumulating substances; however, the test will qualitatively identify strongly bioaccumulating substances.

The test is easy to standardise. Automation will not be necessary for the biological part, since it is simple; possibilities for automation of the chemical analysis will depend on the test substance.

B. DESCRIPTION OF THE TEST PROCEDURE

• Preparations

1) Collect 100 small fish (e.g. guppies or zebra fish) of about the same wet weight, which should be between 50 and 500 mg per fish.

– The fish should preferably come from a standardised laboratory culture. If they are from a trade source they must be acclimated to the laboratory conditions, which must be the same as those used during the accumulation test (apart from presence of the test substance).

– Check the fish for possible contamination with the test substance. Make a homogenate of 10 fish and analyse it for the test substance (in duplicate if desired).

– Determine the average wet weight of the fish and their fat content. Two samples of 10 fish each are used for this purpose. Drain the fish and determine the wet weight of each fish. Place 10 fish in a small all-glass jar or other suitable, uncontaminated container. Homogenise the fish and determine their fat content.

2) Prepare blank test medium (ca. 150 litres), check for possible contamination by the test substance.

– Any uncontaminated test medium in which the test fish grow well may be used. A recipe for a suitable medium for guppies and zebra fish is given below:

$NaHCO_3$	100 mg
$KHCO_3$	20 mg
$CaCl_2.2H_2O$	200 mg
$MgSO_4.7H_2O$	180 mg
distilled water	1 litre

The equilibrium pH of the medium, after aeration, should preferably be 8.4 ± 0.1, but frequently in practice it is lower, around 8.2 ± 0.2.

3) Take 5 all-glass aquaria, uncontaminated with the test substance or other xenobiotics and large enough to contain 25 litres of medium.

4) The substance is preferably dosed directly into the medium or via a stock solution in this medium, but the method used will depend on its solubility. If a heavy metal is to be dosed, it may be helpful to prepare stock solutions in diluted HCl. In this case the pH of the test medium should be checked and, if necessary, adjusted before the fish are introduced.

   If a non-polar organic substance is to be used, it may be helpful to prepare stock solutions in an organic solvent. These solvents must be non-toxic to the fish at the concentration used, non-biodegradable (to avoid oxygen demand) and miscible with water.

   Not more than 0.1 ml organic solvent per litre of medium should be used.

   Tertiary butanol and dimethyl sulfoxide meet the criteria given above. The use of an organic solvent must not lead to a concentration of the test substance in the medium greater than its solubility.

5) Provision should be made to maintain the medium at 24°C ± 2° for the duration of the test and to supply it with compressed air if oxygen measurements indicate that this is necessary.

6) Tetramin or other suitable dry fish food must be available.

7) Oxygen and pH meters must be available.

• Test conditions

The amount of medium to be used per aquarium must be large enough to determine a BCF of 1000 using the largest fish recommended (500 mg per fish) and with an initial test substance concentration of 5 µg per litre medium.

Calculations indicate that 25 litres medium are suitable.

This amount will in most cases be large enough to maintain the oxygen concentration during the test at >5 mg per litre without aeration.

If no biodegradation of the test substance occurs, the size of the fish and their activity are the main factors which influence oxygen depletion of the medium.

The temperature of the medium during the test should be suitable for the fish species chosen. For guppies or zebra fish 24°C is suitable.

- Performance of the test

1) Place 5 all-glass aquaria, filled with 25 litres of medium, saturated with oxygen, at 24 ± 2°C. Make provisions to supply them with compressed air as soon as this becomes necessary (i.e. if the oxygen concentration is < 5 mg per l).

2) Dose two different levels of the test substance into two aquaria each; the fifth aquarium contains blank medium.

   Both concentrations must be sufficiently high to allow analysis in the medium until the end of the test, provided that the test substance is not highly accumulated by the fish.

   The concentrations must be low enough to avoid adverse effects on the fish, e.g. 1/100 to 1/1000 of the TLm at 96 h.

   The concentrations must not exceed the water solubility.

3) Measure the pH values of all aquaria and, if necessary, adjust them (within 0.2 pH units) to that of the blank medium.

4) Immediately after dosing and mixing, take water samples from all aquaria.

   The sample from the blank aquarium is used as a blank for the analytical procedure. (The medium being previously checked for contamination, see Preparations point 1).

Analyse these samples for the test substance. If desired, the analyses can be delayed until the following three samples of each aquarium have been taken (see point 6).

5) Add 20 fish, randomly chosen from the stock, to the blank aquarium (biological blank) and to one of each of the two aquaria with the same concentration of test substance.

One aquarium with each level of test substance remains without fish (chemical blanks).

The ratio of the wet weight of animals per litre medium should be as low as possible.

Feed the fish at t = 2, 4, 6 days using 20 mg dry food for each gram fish with Tetramin or other dry fish food, after samples have been taken.

6) Take a sample of the medium from each aquarium containing the test substance after 6, 24, 48, 96, 144 and 192 h, and after 192 h (at the end of the test) also from the biological blank.

Analyse the concentration of the test substance, preferably on the same day but at least within 48 h.

7) Measure the oxygen concentration in all aquaria at least daily, more frequently when it is critical (< 6 mg per litre). If necessary, aerate all the aquaria (also those without fish) with approximately the same amount of air. Avoid this for (moderately) volatile compounds by using smaller fish.

8) Measure the pH at the end of the test in all aquaria.

9) Take two samples of 10 fish from each dosed aquarium after 192 h. Drain the fish and quickly determine the wet weight of each fish. Place each sample of 10 fish in a small all-glass jar or other suitable uncontaminated container. Homogenise the fish and place the homogenate in a tightly closed container and keep deep-frozen until analysis. A combined sample of 10 fish is made to minimise the effect of variation between individuals.

10) Use the fish in the blank aquarium to determine the average wet weight and fat content of the fish at the end of the test. Those parameters are assumed not to have changed greatly during the 8-day test.

3. DATA AND REPORTING

- Treatment of results

1) Tabulate the results for the concentration tested as follows:

Dosed concentration ..... µg/l.

Time	aquarium without fish			aquarium without fish			
(h)		medium			medium		fish
	pH	O_2 conc. mg/l	conc. test substance (C_w) µg/l	pH	O_2 conc. mg/l	conc. test substance (C_w) µg/l	conc. test substance (C_f) µg/kg
0							
6							
24							
48							
96							
144							
192							

Remarks, e.g. air supply, concerning the blank aquarium with fish (pH, O_2, contamination, condition of fish):

2) Draw a graph of test substance concentration in the medium, C_w, versus time: one graph for each concentration tested with two curves, one curve for the aquarium with fish and one for the aquarium without fish.

Calculate the losses of test substance in both aquaria and the amount of test substance taken up by the fish.

Estimate whether equilibrium was reached during the test. In this case the curve of C_w versus time in the aquarium with fish must be parallel to the time axis.

3) If equilibrium was reached, calculate:

$$BCF = \frac{C_{f,s}}{C_{w,s}}$$

If equilibrium was not reached, calculate:

$$BCF_{8\ days} = \frac{C_{f,\ 8\ days}}{C_{w,\ 8\ days}}$$

• Evalution of results

- pH and oxygen concentration must not have been unsuitable for the fish during the test and the fish must not have shown any signs of ill-health.

- The losses of test substance in the chemical blanks must not be so high that a possible uptake of test substance by the fish cannot be clearly distinguished and calculated.

- The decrease in the amount of test substance in the medium with fish, corrected for losses in the chemical blanks, must approximately equal the amount taken up by the fish.

- An accurate determination of the test substance in the medium must have remained possible until the end of the test if a bioaccumulation factor is to be calculated.

- If the uptake of test substance by the fish was so high that determination of the test substance in the medium was not possible at the end of the test, at least the minimum bioaccumulation factor can be estimated, provided that the concentration of the test substance in the aquaria without fish was still relatively high at the end of the test.

- An accumulation factor at steady state conditions, BCF, can only be calculated if equilibrium was reached and if the amount of test substance taken up by the fish is in accordance with the amount lost from the medium (corrected for blank losses).

  If such equilibria are not reached, and there are not doubtful analyses or other findings, at least a concentration factor after 8 days, $BCF_{8\ days}$, may be calculated; the BCF will always be greater than this.

- Test report

– Give a description of the test method as follows:

SAMPLE TEST REPORTING FORM

Type of test : Static bioaccumulation test

Test substance : concentrations tested:

Test animals species :

age at the start of the test:
weight at the start of the test:
fat content at the start of the test:
origin: laboratory culture/trade, if from trade source:
date of arrival in laboratory:
acclimatisation time: temp: °C food:
other information:

Date of the start of the test :
Test duration :
Temperature :
Type of water :
hardness : pH:
Contents of test vessel : litres of water
and test animals

Food :
Type of test vessel :
Aeration :
Solvent for the test substance : amount:

Other information:

– Summarise the results in tabular form (see Treatment of results, part 3) and add the graphs mentioned.

– Record the BCF or $BCF_{8\ days}$, if they could be calculated accurately, taking into account all qualifications given in section on Evaluation of results.

– Record the fat content of the fish, and the average weight per fish with its standard deviation at the start and at the end of the test. Indicate any irregularities found during the test or any reasons to doubt the results.

- Interpretation of results

Compare the results of the accumulation test as far as possible with relevant physico-chemical properties of the test substance, e.g. water solubility, n-octanol/water partition coefficient, volatility.

Indicate whether sufficient information about the accumulative behaviour of the test substance was gathered or whether more tests are needed to determine the BCF accurately, for example, a confirmatory continuous-flow test.