OECD GUIDELINE FOR TESTING OF CHEMICALS

302A

Adopted:
12 May 1981

"Inherent Biodegradability: Modified SCAS Test"

1. INTRODUCTORY INFORMATION

• Prerequisites

– Water solubility

– The organic carbon content of the test material must be established

• Guidance information

Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level".

Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

• Qualifying statements

The method is only applicable to those organic test materials which, at the concentration used in the test,

– are soluble in water (at least 20 mg dissolved organic carbon/l),
– have negligible vapour pressure,
– are not inhibitory to bacteria,
– do not significantly adsorb on glass surfaces, and
– are not lost by foaming from the test solution.

This test has been found suitable by the OECD Expert Group Degradation/Accumulation for determining the inherent biodegradability of organic chemicals under aerobic conditions.

• Recommendations

Test chemicals giving a result of greater than 20 per cent loss of DOC in this test may be regarded as inherently biodegradable, whereas a result of greater than 70 per cent loss of DOC is evidence of ultimate biodegradability. The use of a compound specific analytical technique on ^{14}C-labelled test substance may allow greater sensitivity. In these last cases a lower level may be regarded as evidence of inherent biodegradability.

- Standard documents

This Test Guideline has been based on the paper cited in reference 1, Section 4, literature.

2. METHOD

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

The method is an adaptation of the Soap and Detergent Association semi-continuous activated sludge (SCAS) procedure for assessing the primary biodegradation of alkyl benzene sulphonate. The method involves exposure of the chemical to relatively high concentrations of micro-organisms over a long time period (possibly several months). The viability of the micro-organisms is maintained over this period by daily addition of a settled sewage feed.

Because of the long detention period (36 hours) and the intermittent addition of nutrients the test does not simulate those conditions experienced in a sewage treatment plant. The results obtained with the test substance indicate that it has a high biodegradation potential, and for this reason it is most useful as a test of inherent biodegradability.

Since the conditions provided by the test are highly favourable to the selection and/or adaptation of micro-organisms capable of degrading the test compound, the procedure may also be used to produce acclimatised inocula for use in other tests.

The test is applicable to water soluble, non-volatile, organic chemicals that are not inhibitory to bacteria at the test concentration.

- Reference substances

In some cases when investigating a new substance, reference substances may be useful; however, specific reference substances cannot yet be recommended. Data on several compounds used in ring tests are provided (see Annex) primarily so that calibration of the method may be performed from time to time and to permit comparison of results when another method is employed.

- Principle of the test method

Activated sludge from a sewage treatment plant is placed in an aeration (SCAS) unit. The test compound and settled domestic sewage are added, and the mixture is aerated for 23 hours. The aeration is then stopped, the sludge allowed to settle and the supernatant liquor is removed. The sludge remaining in the aeration chamber is then mixed with a further aliquot of test compound and sewage and the cycle is repeated.

Biodegradation is established by determination of the dissolved organic carbon content of the supernatant liquor. This value is compared with that found for the liquor obtained from a control tube dosed with settled sewage only.

- Quality criteria

Reproducibility

The reproducibility of this modification of the method based on removal of dissolved organic carbon has not yet been established. When primary biodegradation is considered, very precise data is obtained for materials that are extensively degraded. The results reported in reference (1) suggest 95 per cent confidence limits of less than ± 3 per cent, and this includes interlaboratory tests. As would be expected, wider confidence limits are obtained for less biodegradable materials.

Sensitivity

The sensitivity of the method largely depends on the precision of the determination of dissolved organic carbon and the level of test compound in the liquor at the start of each cycle. At the end of the aeration period about 10 mg/litre of dissolved organic carbon remain in the supernatant liquor of the control experiment. Assuming that the dissolved organic carbon determination is within ± 5 per cent and a level of 20 mg/litre of carbon as test material is added at the start of the aeration period, then the assessment of the extent of biodegradation should be within ± 6 per cent for the range 80-100 per cent biodegradation.

Specificity

The method is applicable to any non-volatile, water soluble, organic compound.

Possibility of standardisation

Since the method uses a feed of real settled sewage, absolute standardisation is not possible unless this feed were replaced by an artificial one. However, since the method is designed to give an indication of the biodegradability potential of a chemical and is not a simulation test, such standardisation is unnecessary.

Possibility of automation

Automation of this method would be possible but would be expensive. As the method is not labour intensive, the exercise would offer few advantages.

B. DESCRIPTION OF THE TEST PROCEDURE

- Preparations

The aeration units are cleaned and fixed in a suitable support. The air inlet tubes are connected to the supply manifold. A small laboratory scale air compressor is used to aerate the units, and the air is presaturated with water to reduce evaporation losses from the units.

A sample of mixed liquor from an activated sludge plant treating predominantly domestic sewage is obtained. Approximately 150 ml of the mixed liquor are required for each aeration unit.

The organic carbon analyser is calibrated using potassium hydrogen phthalate.

Stock solutions of the test compounds are prepared: the concentration normally required is 400 mg/litre as organic carbon which gives a test compound concentration of 20 mg/litre carbon at the start of each aeration cycle if no biodegradation is occurring.

The organic carbon content of the stock solutions is measured.

- Test conditions

A high concentration of aerobic micro-organisms is used, and the effective detention period is 36 hours. The carbonaceous material in the sewage feed is oxidised extensively within 8 hours of the start of each aeration cycle. Thereafter, the sludge respires endogenously for the

remainder of the aeration period, during which time the only available substrate is the test compound unless this is also readily metabolised. These features, combined with daily reinoculation of the test when domestic sewage is used as the medium, provide highly favourable conditions for both acclimatisation and biodegradation.

- Performance of the test

A sample of mixed liquor from a suitable activated sludge plant is obtained and aerated during transportation to the laboratory. Each aeration unit is filled with 150 ml of mixed liquor and the aeration is started. After 23 hours, aeration is stopped, and the sludge is allowed to settle for 45 minutes. The tap is opened and 100 ml of the supernatant liquor withdrawn. A sample of settled domestic sewage is obtained immediately before use, and 100 ml are added to the sludge remaining in each aeration unit. Aeration is started anew. At this stage no test materials are added, and the units are fed daily with domestic sewage only until a clear supernatant liquor is obtained on settling. This usually takes up to two weeks, by which time the dissolved organic carbon in the supernatant liquor at the end of each aeration cycle should be less than 12 mg/litre.

At the end of this period the individual settled sludges are mixed, and 50 ml of the resulting composite sludge are added to each unit.

100 ml of settled sewage are added to the control units and 95 ml plus 5 ml of the appropriate test compound stock solution (400 mg/l) to the test units. Aeration is started again and continued for 23 hours. The sludge is then allowed to settle for 45 minutes and the supernatant drawn off and analysed for dissolved organic carbon content.

The above fill and draw procedure is repeated daily throughout the test.

Before settling it may be necessary to clean the walls of the units to prevent the accumulation of solids above the level of the liquid. A separate scraper or brush is used for each unit to prevent cross contamination.

Ideally, the dissolved organic carbon in the supernatant liquors is determined daily, although less frequent analysis is permissible. Before analysis the liquors are filtered through washed 0.45 µm membrane filters and certifuged. Temperature of the sample must not exceed 40°C while it is in the centrifuge.

The length of the test for compounds showing little or no biodegradation is indeterminate, but experience suggests that this should be at least 12 weeks.

3. DATA AND REPORTING

- Treatment of the results

The dissolved organic carbon results in the supernatant liquors of the test units and the control units are plotted against time. As biodegradation is achieved the level found in the test will approach that found in the control. Once the difference between the two levels is found to be constant over three consecutive measurements, 3 further measurements are made and the percentage biodegradation of the test compound is calculated by the following equation:

$$\% \ biodegradation = \frac{100\ [O_T - (O_t - O_c)]}{O_T}$$

where

O_T = concentration of test compound as organic carbon added to the settled sewage at the start of the aeration period.

O_t = concentration of dissolved organic carbon found in the supernatant liquor of the test at the end of the aeration period.

O_c = concentration of dissolved organic carbon found in the supernatant liquor of the control.

The level of biodegradation is therefore the percentage elimination of organic carbon.

If from the outset there is no difference between the control and the test, or the difference between the two remains constant at a level less than would be expected if no degradation had taken place, further tests are necessary to distinguish between biodegradation and adsorption. This may be done by using the supernatant liquors as a source of inoculum for tests such as the Sturm or the Closed Bottle Tests (OECD Test Guidelines 301).

4. L I T E R A T U R E

1. "A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulphonate and Linear Alkylate Sulphonate", Journal of the American Chemical Society, Vol. *42*, p. 986 (1965).

5. A N N E X

EXAMPLES OF RESULTS OF SCAS TEST ON VARIOUS COMPOUNDS USED IN THE OECD/EEC RING TEST

Test compound	O_T (mg/l)	$O_t - O_c$ (mg/l)	percentage biodegradation/ bioelimination
4-acetyl amino-benzene sulphonate	17.2	2.0	85
Tetra propylene benzene sulphonate	17.3	8.4	51.4
4-nitrophenol	16.9	0.8	95.3
Diethylene glycol	16.5	0.2	98.8
Aniline	16.9	1.7	95.9

Duration of test: 40 days

Results found for cyclopentane tetra carboxylate

O_T (mg/l)	$O_t - O_c$ (ml/l)	percentage biodegradation/ bioelimination
17.9	3.2	81.1

Duration of test: 120 days