OECD GUIDELINE FOR TESTING OF CHEMICALS

304A

Adopted:
12 May 1981

"Inherent Biodegradability in Soil"

1. INTRODUCTORY INFORMATION

• Prerequisites

– ^{14}C-labelled material is required.

• Guidance information

– Information on the toxicity of the test compound is useful for the interpretation of the data obtained. The concentration of the test compound can then be adapted to this information.

• Qualifying statements

– The test is applicable to volatile or non-volatile, soluble or insoluble compounds which are not inhibitory to micro-organisms. The mineralisation rate refers to the labelled carbonation only. Therefore, the location of the labelling within the structure and the specificity of the label need careful consideration.

• Recommendations

– The results obtained using the basic mineralisation test may be supported by determination of the evaporation rate of the parent compound and some of possible volatile metabolites and by determination of soil extractable and non-extractable residues. Both optional tests are described in this Test Guideline.

– Sometimes it is recommended that information about chemical degradation under anaerobic conditions be obtained. Therefore, in accordance with the description below, the biometer flask filled with the soil sample (preconditioning is not necessary) is flooded with water (2-3 cm layer) to protect against leakage, then evacuated and flushed with nitrogen several times. Degradation may be evaluated by means of measurements of methane gas and analysis of both water and soil for ^{14}C-content.

• Standard documents

This Test Guideline is based on the method cited in reference 1, Section 4, Literature.

2. METHOD

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

The method described in this Test Guideline is designed for the evaluation of the mineralisation rate of a ^{14}C-labelled compound in soil. The method is applicable to volatile or non-volatile, soluble or insoluble compounds which are not inhibitory to micro-organisms.

- Definitions and units

Soil is a mixture of mineral and organic chemical constituents, the latter containing compounds of high carbon and nitrogen content and of high molecular weights, animated by small (mostly micro-) organisms. Soil may be handled in two states:

a) undisturbed, as it has grown with time, in characteristic layers of a variety of soil types,

b) disturbed, as it is usually sampled by digging and used in the test described here.

Mineralisation (in this context) means extensive degradation of a molecule during which a labelled carbon atom is oxidised quantitatively with release of the appropriate amount of $^{14}CO_2$.

- Reference substances

In some cases when investigating a new substance, reference substances may be useful; however, reference substances cannot yet be recommended. Reference substances need not be employed in all cases when investigating a new substance. They may primarily be used 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

Basic test: A small sample of soil is treated with the ^{14}C-labelled test chemical in a biometer flask apparatus. Release of $^{14}CO_2$ from the test chemical is measured by means of alkali absorption and liquid scintillation counting.

Optional experiments include the following tests.

Evaporation test: When testing chemicals of a vapour pressure higher than 0.0133 Pa, a polyurethane foam plug is placed into the biometer flask apparatus to absorb the labelled volatile part of the parent compound and volatile metabolites for liquid scintillation counting.

Residue test: At the point of 50 per cent mineralisation, the test soil may be extracted. The extractable portion of the compound, and its metabolites remaining in the soil, may be determined by liquid scintillation counting. Furthermore, data on the bound residue part may be obtained by measuring the $^{14}CO_2$ released after combustion of the soil.

- Quality criteria

Reproducibility

Reproducibility is good if standard conditions, especially preconditioning of the soil, are strictly observed.

Sensitivity

The evaluation of sensitivity is not relevant because a moderate amount as 37-185 kBq (≈1-5 µCi) of ^{14}C-labelled test chemicals is used for each experiment.

Specificity

The method is only applicable if ^{14}C-labelled test chemicals are available. The specificity is very good.

Possibility of standardisation

This procedure is standardised to a limited extent. The limitation is related to the difficulty of standardisation of soil samples between laboratories.

Possibility of automation

Not foreseen.

B. DESCRIPTION OF THE TEST PROCEDURE

- Preparations

Equipment

– Liquid scintillation counter

– Oxidiser for combustion of radioactive material

– Ultrasonic bath, 500 ml

– Glassware: 250 ml Erlenmeyer flasks fused to 50 ml round bottom tubes (biometer flasks, see Figure 1); 25 ml syringes (e.g. Luer-lock); syringe needle 15 gauge, 15 cm in length; 100 µl syringes (e.g. Hammilton); 25 ml graduated cylinders with stopper; 1 ml pipettes; soxhlet extraction apparatus; scintillation vials; polyurethane plugs, 30 mm diameter, 30 mm length, density 16 kg/m^3.

Reagents

Test substance: ^{14}C-labelled compounds are dissolved in water or acetone to give radioactivity of 37-185 KBq (≈1-5 µCi)/100 µl. Using unlabelled material, this solution is made up to the required concentration (e.g. 0.5 mg/100 µl ≈ 10 mg/kg soil, or depending on the toxicity of the substance).

Chemicals

KOH, analytical grade, 0.1 N solution
Acetone, analytical grade
Methanol, analytical grade (for optional tests)
n-Hexane, analytical grade (for optional test)
Ascarite*
Scintillation cocktail

Soil

Alfisol: pH between 5.5 and 6.5
organic C content between 1 and 1.5 per cent
clay content (i.e. particles < 0.002 mm in diameter) between 10 and 20 per cent
cation exchange capacity between 10 and 15 mval.

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* A.H. Thomas Co. Philadelphia or equivalent

Spodosol: pH between 4.0 and 5.0
organic C content between 1.5 and 3.5 per cent clay content ≤ 10 per cent
cation exchange capacity < 10 mval.

Entisol: pH between 6.6 and 8.0
organic C content between 1 and 4 per cent
clay content between 11 and 25 per cent
cation exchange capacity > 10 mval.

In special cases it is recommended that two additional soils be used: one with high silt-fraction* content, the other with a high clay content (30 per cent).

Air dried test soil stored at +4°C is remoisturised to 40 per cent maximum water capacity. After incubation for 2 weeks at 22°C + 2°C in the dark, it is ready for the experiments.

• Test conditions

Test temperature: During the whole test period, the flasks are incubated in the dark at 22°C ± 2°C.

Soil characterisation data: For determination of the pH value of the soil for selecting the soil type, 10 g air-dried soil are suspended in 25 ml 0.01 M $CaCl_2$.

After standing overnight, the sample is disturbed once more and measured in a potentiometric apparatus with a 0.1 M KCl electrode. Immediately before the measurement, the instrument must be calibrated with two standard solutions within the measuring range of the sample values expected.

For determination of the organic carbon content of the soil for selecting the soil type, 1.0 g air-dried soil is heated with 15 ml 2M $K_2Cr_2O_7$ and 20 ml H_2SO_4 (analytical reagent, ρ = 1.84 g/cm^3) at 145-155°C for 15 minutes. After cooling to room temperature, sample volume is made up to 150 ml with distilled water. A 20 ml aliquot is measured spectro-photometrically, after centrifuging, in a 1 cm cuvette at 590 nm compared to distilled water. The self-destroying property of the $K_2Cr_2O_7$ reagent must be determined by two blank samples. Calculation is conducted using the following equation:

$$C = \frac{1000 \cdot V \cdot E_2 \, (E_x - \alpha_2 \cdot c)}{e \cdot E_1 \cdot (\alpha_1 - \alpha_2 \cdot F)}$$

* Diameter between 0.002 and 0.063 mm

where

C = carbon content (%)
V = gross volume (ml)
E_1 = equivalent weight of Cr_2O_3 (25.332)
E_2 = equivalent weight of carbon (3.0028)
E_x = extinction at 590 nm and 1 cm layer thickness
F = factor calculating $K_2Cr_2O_7$ from Cr_2O_3
c = concentration of Cr (g) per 100 ml (= 1.9356)
e = sample weight (mg)
α_1 = extinction coefficient of Cr (III) α_1 is an average value from five single determinations for the calibration curve, each obtained by division of E_x by the amounts of Cr_2O_3 (in g)
α_2 = extinction coefficient of Cr (VI) α_2 is an average value from two single estimations, each obtained by division of E_x by the respective amounts of $K_2Cr_2O_7$.

For determination of particle size of the soil for selecting the soil type, 10.0 g air-dried soil are reacted with 100 ml H_2O_2 (15 per cent $^w/_v$) for 15 hours, then heated until CO_2 evolution is complete. Afterwards the suspension is left to stand overnight with 25 ml 0.4 N $Na_4P_2O_7$, then water is added to make it up to 250 ml and the solution is sieved through a mesh of 0.2 mm width. The portion > 0.2 mm is fractionated further by sieving. The smaller particles (silty fractions) are fractionated by homogenous partitioning of the particles in the aqueous medium, which is made up to 1000 ml with water in an elutriating cylinder.

10 ml portions are removed by pipette from various heights of the cylinder after different sedimentation times; measurement of the dry weights of the suspended material in these portions yields the particle composition according to the following scheme:

Particle fraction diameter (mm)	dipping depth (cm)			
	20	15	10	5
< 0.002	-	-	7h 45m	3h 52m
< 0.0063	1h 33m 49s	1h 10m 52s	46m 55s	23m 27s
< 0.02	9m 19s	6m 59s	4m 39s	2m 20s
< 0.063	59s	42s	-	-

For determination of the cation exchange capacity of the soil, in order to select the soil type, a glass column 15 cm in length and 30 mm inner diameter is reduced in diameter at one end like a funnel. This side is stuffed with filter wool. About 1 cm quartz sand is strewn on the wool, followed by 10.0 g air-dried test soil, which is in turn covered by about 1 cm quartz sand. Above these layers comes 40 ml of a mixed solution [consisting of 100 g triethanolamine in 2 l water (adjusted to pH 8.1 with HCl) plus 100 g $BaCl_2.2H_2O$ in 2 litres]. After 1 h the solution is collected in an Erlenmeyer flask of 250 ml. The procedure is then repeated. In addition, 40 ml of a solution of 25 g $BaCl_2.2H_2O$ in 1 l are poured into the column.

After standing overnight, this solution is also collected and the column is washed with 100 ml water. The combined eluates are titrated against HCl (bromocresol green plus methyl red as indicators) to measure H^+, Ca^{2+}, K^+, Na^+. For the determination of Ba^{2+} the column is leached in a similar manner with 200 ml of 20 g $MgCl_2.6H_2O$ in 1 litre water. This cation is determined by flame absorption spectrophotometry. The cation exchange capacity is expressed as the sum of all the cation equivalents sorbed by 100 g soil.

- Performance of test

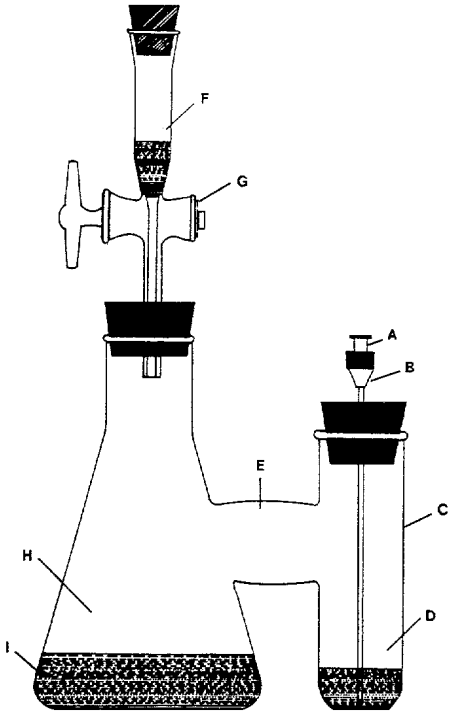
Basic test

Fifty grammes of soil (dry weight basis) are placed into each Erlenmeyer part (H) of the biometer flask (see Figure 1). 100 µl of the radioactive test solution are added in 50 drops over the whole soil surface (I) of each flask. Then, the soil is carefully mixed with a Pasteur pipette (from which the lower part is cut off) and left in the flask.

In addition, an equivalent volume of test solution is placed in a 100 ml volumetric flask for direct determination of the added radioactivity.

The biometer flask is closed with a teflon-coated silicon rubber stopper through which an Ascarite filter (F) is inserted. The filter (F) is provided with a stopper and stopcock (G). The side tube (C) is sealed with a teflon stopper pierced by a 15-gauge needle (B), 15 cm long. The needle (B) is capped by a silicone rubber stopper (A), and its tip at (D) is covered with a short length of silicone tubing that remains in contact with the base of the side tube (C).

Figure 1: Test flask



The unit is charged by injecting 10 ml of alkali solution into the side tube (C) in the following manner: the small stopper (A) is replaced by a calibrated Luer lock syringe containing 0.1 N KOH; then the filter stopper on (F) is removed and the stopcock (G) is opened; the alkali solution is introduced through the needle (B) into the side tube (C); then the stopcock is closed; the syringe is removed; the small stopper (A) and filter stopper on (F) are then returned to their initial positions. The ^{14}C-carbon dioxide produced is adsorbed by the alkali.

To recover the $^{14}CO_2$-loaded alkali for liquid scintillation analysis, the procedure for charging each parallel unit at increasing time intervals after start of the experiment is performed in reverse order. Thereafter the side tube (C) is rinsed with 5 ml alkali. Before recharging the side tube (C) with fresh alkali, 3 volumes of 25 ml air are sucked through the system with the empty syringe to maintain the soil in an aerobic condition. A 1 ml aliquot of the alkali solution is taken for liquid scintillation counting.

Experiment duration times of 1, 2, 4, 8, 16, 32 and - if necessary - 64 days should be chosen for measurement. The test requires parallel determinations. The $^{14}CO_2$ radioactivity recovered is plotted versus time. This graph shows when to terminate the experiment. Incubation time is sufficient when a total of 50 per cent CO_2 expressed as ^{14}C originally applied can be measured. Incubation should be stopped after reaching 64 days, whether or not this value is obtained.

Optional tests

Estimation of evaporation

If the volatility of a chemical is higher than 10^{-5} torr at 20°C, it is recommended that a 3 cm diameter polyurethane foam plug be introduced into the arm E of the biometer flask. This plug absorbs the volatile parent compound as well as volatile organic degradation products but does not absorb $^{14}CO_2$. The plugs are extracted in a soxhlet extraction apparatus with an n-hexane/methanol mixture (1/4), and aliquots are taken for liquid scintillation counting.

Determination of soil-extractable and non-extractable residues

In cases of relatively persistent chemicals (50 per cent mineralisation in > 10 days), further information concerning the soil-extractable radioactivity (parent compound plus degradation products) and soil bound residues is recommended.

For this purpose, two further biometer flasks in addition to the four others must be prepared. At the point of 50 (or x-) percent mineralisation in the basic test, the soil in the two separate biometer flasks is extracted with 100 ml acetone (5 min ultrasonic treatment) followed by an extraction with methanol in the same manner. Aliquots of the combined extracts are taken for liquid scintillation counting. Other extract portions may be used – if necessary – for further identification studies.

Aliquots of the air dried soil are combusted to $^{14}CO_2$ and measured by liquid scintillation counting to determine the soil bound residues.

3. DATA AND REPORTING

- Treatment of results

Basic Test

Radioactivity values for $^{14}CO_2$ (average of 4 parallel experiments) obtained from the alkali solution after 1, 2, 4, 8, 16, 32 and 64 days are expressed as the percentage of test chemical (radioactivity) initially applied and are plotted in a graph versus time. The time at which 50 per cent of the radioactivity is recovered as $^{14}CO_2$ is considered to be the "50% mineralitsation" level. If this level has not been reached by the 64th day, the data at this time are taken and expressed as "x-percent-mineralisation".

Evaporation test

The radioactivity of vaporised (and trapped) original compound plus degradation products at the point of 50 (or x-) per cent mineralisation is extracted, measured and interpreted as the percentage of volatilisation at the point of 50 (or x-) per cent-mineralisation.

Residue test

Radioactivity values for extractable and non-extractable residues of the parent compound plus degradation products obtained after the extraction procedure of the soil at the point of 50 (or x-) per cent mineralisation are given.

- Test report

The report of the degradability of a test chemical must include:

– name of the test chemical, formula
– amount applied, if not standard
– exact characteristic data of the soil used
– dates of the performance of the measurements.

- Interpretation and evaluation of results

The results are artificial because they are obtained with disturbed soil. However, since standardised soils are used, the test data are intercomparable and enable the experimenter to group relatively the chemicals tested within one scale for this property.

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

1. Bartha, R. and Pramer D., Soil Science *100*, 68-70 (1965).

2. *Soil Taxonomy* (Soil Survey Staff) United States Department of Agriculture Handbook N° 436, Washington, D.C., 1975.

3. Butler, B.E. *Soil Classification for Soil Survey*, Oxford, 129 p., 1980.