



"Terrestrial Plants, Growth Test"**1. INTRODUCTORY INFORMATION****• Prerequisites**

- Water solubility
- Vapour pressure

• Guidance information

- Structural formula
- Solubility in organic solvents
- n-Octanol/water partition coefficient
- Absorption behaviour
- Purity of the test substance
- Chemical stability in water and light
- Results of a ready biodegradability test (see Test Guideline 301)

• Qualifying statement

This Test Guideline does not give a separate indication of possible damage resulting from vapour action of the test substance, nor does it measure damage which could result from direct contact with the foliage.

• Standard documents

There are no relevant international standards.

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

This Test Guideline is designed to determine possible toxic effects of soil-incorporated solid or liquid chemical substances on the emergence of seedlings and the early stages of growth of a variety of terrestrial plants after a single application.

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- Definitions

- EC 50 in this Test Guideline is the concentration at which the change in growth is 50 per cent of that of the control.
- Emergence in this Test Guideline refers to the appearance of the seedling above the soil surface.
- LC 50 in this Test Guideline is the concentration at which the change in emergence is 50 per cent of that of the control.
- Growth is expressed in terms of plant weight.

- Reference substances

No reference substances are recommended for this test. However, if a reference substance has been tested, the results should be given.

- Principle of the test method

The test substance is incorporated at various concentrations into soil in which the seeds are sown. The number of seedlings that emerge is recorded. At least two weeks after 50 per cent of the seedlings have emerged in the control, the plants are harvested and weighed.

- Conditions for the validity of the test

A minimum of 80 per cent of the control seeds should produce healthy seedlings. The control seedlings should exhibit normal growth throughout the test.

B. DESCRIPTION OF THE TEST PROCEDURE

- Preparations

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Equipment and materials

Suitable facilities for plant testing are necessary, including phytotrons, glasshouses or plant growth chambers. Planting containers must be non-porous plastic or glazed pots.

It is not necessary to use sterile soil. The soil should be sieved (0.5 cm) to remove coarse fragments. Carbon content should not exceed 1.5 per cent (3 per cent organic matter). Fine particles (under 20 μm .) should make up between 10 and 20 per cent. The pH should be between 5.0 and 7.5.

Any method of soil treatment can be used which results in even dispersion of the test substance throughout the soil. Surfactants should not be used.

• Experimental plants

Selection of species

A minimum of three species should be selected for testing, at least one from each of the categories below:

<u>Category</u>	<u>Tests species</u>	
1	ryegrass	<i>Lolium perenne</i>
	rice	<i>Oryza sativa</i>
	oat	<i>Avena sativa</i>
	wheat	<i>Triticum aestivum</i>
	sorghum	<i>Sorghum bicolor</i>
2	mustard	<i>Brassica alba</i>
	rape	<i>Brassica napus</i>
	radish	<i>Raphanus sativus</i>
	turnip	<i>Brassica rapa</i>
	Chinese cabbage	<i>Brassica campestris var. chinensis</i>
3	vetch	<i>Vicia sativa</i>
	mung bean	<i>Phaseolus aureus</i>
	red clover	<i>Trifolium pratense</i>
	fenugreek	<i>Trifolium ornithopodioides</i>
	lettuce	<i>Lactuca sativa</i>
	cress	<i>Lepidium sativum</i>

Other species may be used if the rationale for their selection is justified in the test report.

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- Test conditions

Temperature, humidity and light conditions should be suitable for maintaining normal growth of each species for the test period.

- Performance of the test

A control and three concentrations should be tested in a randomised block design with a minimum of four replicates per treatment. A minimum of five seeds should be planted in each replicate within 24 hours of incorporation of the test substance. All seeds of each species for each test should be of the same size class. The seed should not be imbibed.

The test substance can be incorporated into the soil as follows:

1. Dissolve the chemical in a volatile solvent.
2. Mix the solution with sand.
3. Sit the sand slurry while the solvent evaporates.
4. Mix the sand with soil.
5. Maintain a constant sand-soil ratio for all treatments including controls.

Application rates should be equivalent to 0.0 (control), 1.0, 10.0 and 100.0 mg substance per kg of oven dried soil. The seeds are then planted.

The size of pots or containers should be adequate to allow un restricted growth of the selected species. Plants should be watered as needed.

The test should be terminated no sooner than 14 days after 50 per cent of the control seedlings have emerged.

3. DATA AND REPORTING

- Treatment of the results

The number of plants that emerge per replicate is recorded and the average weight per replicate determined (wet weight immediately after harvest or dry weight after oven drying at

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approximately 70°C) and expressed on a per plant basis. The effect of the test substance on emergence should be expressed as LC50, and the effect on growth as EC50.

- Test report

The test report should include the following information:

Test substance: chemical identification data

Test organisms: species/varieties tested, seed source, weight and viability

Test conditions:

- pot dimensions and amounts of soil
- method of incorporation of the test substance
- growth conditions (e.g. light intensity, photo period, day/night temperature, watering schedule) and type of testing facility (e.g. phytotron, glasshouse, growth chamber)
- soil characteristics (pH, per cent organic matter, per cent particles smaller than 20 µm, sterilised or not)

Results:

- all data in tabular form
- graphical presentation of concentration-effect relationship
- LC 50 values for emergence
- EC 50 values for growth
- phytotoxic effects noted for each concentration and control
- any photographs of plants, phytotoxic effects, etc.
- any deviation from this Test Guideline

4. LITERATURE

1. A. Nyffeler, H.R. Gerber, K. Hurle, W. Pestemer and R.R. Schmidt, *Weed Research* 22, 213-222 (1982).

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