

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

PROPOSAL FOR A NEW GUIDELINE 227

Terrestrial Plant Test: Vegetative Vigour Test

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress and current regulatory procedures. The existing updated guideline 208 is designed to assess potential effects of substances on seedling emergence and growth, and this new guideline is to assess effects on vegetative vigour of terrestrial plants. Conditions of exposure and properties of the substance to be tested must be considered to ensure that appropriate test methods and test substance levels are used. As such it does not cover all chronic effects or effects on reproduction (i.e. seed set, flower formation, fruit maturation). This guideline is applicable to the testing of both general chemicals and crop protection products (also known as plant protection products or pesticides). It is based upon existing methods (1) (2) (3) (4) (5) (6) (7) (8). Other references pertinent to plant testing were also considered (9) (10) (11) (12). Definitions used are given in Annex 1.

PRINCIPLE OF THE TEST

2. The test assesses the potential effects on plants following deposition of the test substance on the leaves and above-ground portions of plants. Plants are grown from seed to the 2- to 4- true leaf stage. Test substance is sprayed on the plant and leaf surfaces at appropriate rate(s). The plants are then evaluated against untreated control plants for effects on vigour and growth at various time intervals through 28 days from treatment. Endpoints measured are biomass (fresh or dry shoot weight, or shoot height) and visual detrimental effects (chlorosis, mortality, plant development abnormalities, etc.).

3. The vegetative vigour test is intended to meet testing requirements for both general chemicals and crop protection products. Optionally, for specific purposes the foliar exposure and soil exposure can be combined by extending updated test guideline 208 (2) to cover also foliar exposure.

4. The test can be conducted at a single concentration/rate as a limit test according to the aim of the study or to determine if further testing (i.e. a dose-response test) was warranted. If results from the single rate test exceed a certain phytotoxicity level (e.g. whether effects greater than x% are observed), multiple rate testing preceded by range-finding test(s) is conducted to generate a dose-response curve using appropriate statistical analysis to obtain an EC_x for the most sensitive parameter(s), where x is the % effect level.

INFORMATION ON THE TEST SUBSTANCE

5. The following information is useful in designing the test: structural formula, purity, water solubility, solubility in organic solvents, n-octanol/water partition coefficient, soil sorption behaviour, vapour pressure, chemical stability in water and light, and biodegradability. This guideline may need to be modified to accommodate highly volatile substances, to eliminate possible cross contamination e.g. by using separate growing chambers or other adequate means.

6. The substance must be applied in an appropriate carrier (e.g. water, acetone, ethanol, polyethylene glycol, gum arabic, sand). Crop protection products are tested as final preparations intended for registration or in certain cases as representative formulation.

VALIDITY OF THE TEST

7. In order for the test to be considered valid, the following performance criteria must be met in the controls:

- the plant growth does not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformation);
- the mean plant survival is at least 90% at the end of the test.
- for any species, all organisms in a test must be from the same source;
- all test chambers or rooms used for a particular species should be identical and should have same conditions and contain same amount of soil matrix, support media, or substrate from the same source.

REFERENCE SUBSTANCE

8. A reference substance could be tested either at regular intervals or possibly included in each test to verify that the performance of the test and the response of the test plants has not changed significantly over time. A suitable reference substance for example for certain species can be found in (13).

DESCRIPTION OF THE METHOD

Soil - Artificial substrate

9. Plants may be grown in pots using a sandy loam, loamy sand, or sandy clay loam soil that contains up to 1.5 percent organic carbon. Commercial potting soil or synthetic soil mixes may be used as the "soil medium". Clay soils should not be used if the test substance is known to have a high affinity for clays. Field soil should be sieved to remove coarse particles greater than those which will pass through a 2 mm screen. Soil type and texture, % organic carbon and pH should be reported. The soil should be classified according to a standard classification scheme (14). Pasteurized or heat treated soil could be used to reduce the effect of soil pathogens.

10. Glass beads, mineral wool, and 100 percent acid washed sand are not recommended for testing of crop protection products, however they may be of use for the testing of general chemicals. Nutrients for plant growth should be provided as appropriate (see paragraph 11 in test guideline 208 (2)).

Selection and number of test species

11. The selection of species should be based on the ecological relevance of species, species specific life-cycle characteristics, region of natural occurrence etc. (15) (16) (17) (18) (19). The following characteristics of the possible test species should be considered in the selection:

- accessibility to characterized test species,
- plant is amenable to testing in the laboratory, and reproducibility within and across testing facilities,
- plant uniformity,
- their distribution, abundance and taxonomic representation suggest broad coverage of the plant kingdom,
- they are sensitive to many toxic compounds and have been used to some extent in previous bioassays (their use in herbicide bioassays, heavy metal screening, salinity and mineral stress tests and allelopathy studies indicates sensitivity to a wide variety of stressors), and
- they are compatible with the environmental growth conditions and time constraints of the test method;
- they meet the performance criteria of the test

Some of the historically most used test species are listed in Annex 2 and potential non-crop species in Annex 3.

12. The number of species for use in this guideline should comply with the relevant regulatory requirements.

Application of the test substance

13. All equipment used in conducting the test, including equipment used to prepare and administer the test substance should be of such design and capacity that tests involving this equipment can be conducted in an accurate way and it will give a reproducible coverage. The test substance is sprayed onto the plant surface simulating typical spray tank applications. Generally, spray volumes should be in the range of normal agricultural practice, and not to exceed plant runoff. If solvents other than water are applied, a second group of control plants should be established receiving only the solvent/carrier. This is not necessary for crop protection products tested as formulations. In the case of test substance under the form of dusts, the test would require further modification. Optionally, the foliar exposure and soil exposure can be combined (see test guideline 208 (2)).

Verification of test substance concentration

14. The rates of application of the test substances will be confirmed by analytical verification of the highest concentration of test solution and calibration of the application equipment.

PERFORMANCE OF THE TEST

Test groups and controls

15. The number of seeds planted per pot will depend upon the species, pot size and test duration. As an example, one to two corn, soybean, tomato, cucumber, or sugar beet plants per 15cm container; three rape or pea plants per 15cm container; and 5 to 10 onion, wheat, or other small seeds per 15 cm container are recommended (for details see (18)). The number of seeds and replicate pots (the pot is defined as the replicate, hence plants within the same pot do not constitute a replicate) should be adequate for optimal

statistical analysis (20). It should be noted that for some test species that variability will be greater when using fewer large seeds per pot (replicate), when compared to test species where it is possible to use greater numbers of small seeds per pot. So by planting equal seed numbers in each pot this variability between species may be minimized.

16. Control groups are used to assure that effects observed are associated with or attributed only to the test substance exposure. The appropriate control group should be identical in every respect to the test group except for exposure to the test substance. For these tests, the pot is defined as the replicate (plants within the same pot do not constitute a replicate). Within a given test, all test plants including the controls should be from the same source. To prevent bias, random assignment of test and control pots is required.

17. The seeds should not be imbibed with water. Seeds coated with an insecticide or fungicide (i.e. “dressed” seeds) should be avoided when possible. If seed-borne pathogens are a concern, the seeds may be soaked briefly in a weak hypochlorite solution, then rinsed extensively in running water and dried.

Test conditions

18. The test conditions should approximate those conditions necessary for normal growth for the species and varieties tested (see Annex 4). They should avoid crowding of the plants that could affect growth and, in the emerged plant test, overlapping of leaves that could affect exposure to test substance.

19. The emerging plants and the emerged plants should be maintained under good horticultural practices. These practices include usually control and recording of temperature, humidity, carbon dioxide concentration, light (intensity, wave length) and light period, amount and timing of watering, etc., to assure good plant growth as judged by the control plants. The plants should be grown in non-porous plastic or glazed pots with a tray or saucer under the pot. The pots must be large enough to allow normal growth and, limit overlap of leaves among plants.

20. Soil nutrients may be supplemented to maintain good plant health. The need and timing of additional nutrients can be judged by observation of the control plants. Bottom watering of test containers with de-ionized water is recommended if possible. However, initial top watering can be used to stimulate seed germination before the test.

21. The specific growing conditions should be appropriate for the species tested and the test substance under investigation. Control and treated plants must be kept under the same environmental conditions, however, separated as necessary so that cross exposure of the controls to the test substance is avoided in controls. The plants may be grown and tested in controlled environment chambers, phytotrons, or greenhouses.

Testing at a single concentration/rate

22. In order to determine the appropriate concentration of a substance for conducting a single-concentration or rate (challenge/limit) test, a number of factors must be considered. For general chemicals, these include the physical-chemical properties of the substance and the purpose for conducting the test (e.g. hazard labelling requirements, etc.). For crop protection products, the physical-chemical properties and use pattern of the test substance, its maximum application rate, the number of applications per season and/or the persistence of the test compound need to be considered. To determine whether a general chemical possesses phytotoxic properties, it may be appropriate to test at a maximum level of application and for crop protection products three times the recommended field application rate.

23. For crop protection products such as herbicides or other substances with known or expected phytotoxicity, single rate testing may not be appropriate for some species and testing should directly progress to generate dose – response data.

24. The treatment and control groups should be replicated a minimum of four times with an appropriate number of plants per pot. More replicates of certain plants with low variable growth habits may be needed to increase the statistical power of the test.

Range-finding test

25. When necessary a range-finding test could be performed to provide guidance on application rate or concentrations to be tested in definitive dose-response study. For the range-finding test, the test concentrations/rates should be widely spaced (e.g. 0.1, 1.0, 10, 100 and 1000 units of application). For crop protection products concentrations could be based on the recommended application rate, e.g. 1/10, 1/2, 1, 5, 10 times of the recommended application rate.

Testing at multiple concentrations/rates

26. The purpose of the multiple rate test is to establish a dose-response relationship for the test species exhibiting greater than a prescribed level of effect in a single rate test. It is intended to determine an effective concentration EC_x or effective application rate ER_x for biomass and/or visual effects compared to un-exposed controls.

27. The number and spacing of the concentrations or rates should be sufficient to generate a reliable dose-response relationship and regression equation and give an estimate of the EC_x . Ideally, the selected rates should encompass the EC_{50} or ER_{50} and optionally NOEC. For example, if an EC_{50} is required it would be desirable to test at rates that produce a 20 to 80 % effect. The recommended number of test concentrations to achieve this is at least five in a geometric series plus untreated control, and spaced by a factor not exceeding 3. If five test rate/concentrations are used, a minimum of four replicates per treatment of 10 plants each is recommended. If a larger number of test rate/concentrations are used, the number of plants per replicate may be reduced. The variability of plant growth will influence the number of plants per replicate and number of pot replicates required in order to obtain the statistical power desired. Therefore, this increased variability requires that both the number of plants per replicate and the number of replicates is to be increased.

Observations

28. During the observation period, 14 to 28 days after treatment, the plants are observed frequently (at least weekly) for visual phytotoxicity and mortality. At the end of the test, measurement of biomass should be recorded as well as visual phytotoxicity assessment (chlorosis, necrosis, wilting, leaf and stem deformation). Biomass can be measured using final shoot weight (preferably dry weight by harvesting the shoot at the soil surface and dry them at 60° C to a constant weight), or complementary shoot height. A uniform scoring for visual injury should be used to evaluate the observable toxic responses. Examples for performing qualitative and quantitative visual ratings are provided in references (11) (21).

DATA ANALYSIS AND REPORTING

Statistical analysis

Single rate test

29. Data for each plant species should be analyzed using an appropriate statistical method. The level of effect at the test rate/concentration should be reported, or the lack of reaching a given effect at the test rate/concentration (e.g., $<x$ % effect observed at y rate or concentration).

Multiple rate test

30. A rate-response relationship is established in terms of a regression equation. Different models can be used, for example for estimating EC_x/ER_x (e.g. EC_{25}/ER_{25} or EC_{50}/ER_{50}) and its confidence limits for mortality as quantal data, logit, probit, Weibull, Spearman-Kärber, trimmed Spearman-Kärber methods, etc. could be appropriate. For the growth of the seedlings (weight and height) as continuous endpoints EC_x/ER_x (e.g. EC_{25}/ER_{25} or EC_{50}/ER_{50}) and its confidence limits can be estimated by using appropriate regression analysis (e.g. Bruce-Versteeg non-linear regression analysis (22)). Wherever possible, the R^2 should be 0.7 or higher and the test concentrations used encompass 20% to 80% effects. If the NOEC is to be estimated application of powerful statistical tests should be preferred and these should be selected on the basis of data distribution (20) (23).

Test report

31. The test report should present results of the studies as well as a detailed description of test conditions, a thorough discussion of results, analysis of the data, and the conclusions drawn from the analysis. A tabular summary and abstract of results should be provided. The report should include the following:

Test substance:

- chemical identification data, relevant properties of the substance tested, such as physical state and stability;
- details on preparation of the test solution.

Test species:

- details of the test organism: species/variety, plant families, scientific and common names, source and history of the seed (i.e. name of the supplier, percentage germination, seed size class, batch or lot number, seed year or growing season collected, date of germination rating), viability, etc.;
- number of mono- and di-cotyledon species tested;
- description of seed storage, treatment and maintenance.

Test conditions:

- testing facility i.e. growth chamber, phytotron, greenhouse;
- description of test system (e.g., pot dimensions, pot material, and amounts of soil);

- soil characteristics (texture or type of soil: e.g. soil particle distribution and classification, physical and chemical properties including % organic matter, % organic carbon, pH,);
- soil/substrate (i.e. soil, artificial soil, sand, others) preparation prior to test;
- description of nutrient medium if used;
- application of the test substance: description of method of application, description of equipment, exposure rates and volumes including chemical verification, description of calibration method, description of environmental conditions during application;
- growth conditions: light intensity, photoperiod, day/night temperatures, watering schedule and method, fertilization;
- number of seeds per pot; number of plants per dose; number of replicates (pots) per exposure rate;
- type and number of controls (negative and/or positive controls, solvent control if used)
- duration of the test.

Results:

- table of all endpoints for each replicate, test concentration and species;
- the percent inhibition for each species as compared to controls;
- biomass measurements, i.e. shoot weight (preferably dry weight) or shoot height of the plants as a percentage of the controls;
- percent visual injury and qualitative and quantitative description of visual injury (chlorosis, necrosis, wilting, leaf and stem deformation, as well as, any lack of effects) by the test substance as compared to control plants;
- a description of the rating scale used to judge visual injury, if visual rating is provided;
- for single rate studies, the % injury should be reported;
- EC_x/ER_x (e.g. EC₅₀/ER₅₀ and EC₂₅/ER₂₅) values and related confidence intervals for the endpoints and equation, calculated from the appropriate dose-response data and using the appropriate statistical procedures;
- description of the statistical procedures and assumptions used;
- graphical display of data and dose-response relationship.

Deviations from the procedures described in this guideline and any unusual occurrences during the test.

LITERATURE

- (1) OECD Guideline for Testing of Chemicals 208. (1984). Terrestrial Plants, Growth Test.
- (2) OECD Guideline for Testing Chemicals 208. (2004) Terrestrial Plant Test: Seedling emergence and Seedling Growth Test.
- (3) ISO 11269-1. (1993). Determination of the Effects of Pollutants on Soil Flora - Method for the Measurement Growth.
- (4) ISO 11269-2. (1995). Determination of the Effects of Pollutants on Soil Flora - Effects of Chemicals on the Emergence and Growth of Higher Plants.
- (5) ASTM. Method E 1963. (2002). Standard Guide for Conducting Terrestrial Plant Toxicity Tests.
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- (7) US EPA, OPPTS Ecological Effect Guideline, 850 Series. (1996).
 - 850.4000: Background - Non-target Plant Testing.
 - 850.4025: Target Area Phytotoxicity
 - 850.4100: Terrestrial Plant Toxicity, Tier I (Seedling Emergence)
 - 850.4200: Seed Germination/Root Elongation Toxicity Test
 - 850.4225: Seedling Emergence, Tier II
 - 850.4230: Early Seedling Growth Toxicity Test
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- (13) Aquaterra Environmental (1998) Development of plant toxicity tests for assessment of contaminated soils. Report prepared for Method Development and Application Section, Environmental Technology Centre, Environment Canada.
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- (16) Boutin, C.; N. Elmegaard and C. Kjær (in press) Toxicity testing of fifteen non-crop plant species with six herbicides in a greenhouse experiment: implications for risk assessment. Ecotoxicology 58:1161-1174
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- (18) Boutin, C. and Harper, J.L. (1991). A comparative study of the population dynamics of five species of *Veronica* in natural habitats. J. Ecol. 9:155-271.
- (19) Boutin, C., H.-B. Lee, T.E. Peart, S.P. Batchelor and R.J. Maguire. 2000. Effects of the sulfonylurea herbicide metsulfuron methyl on growth and reproduction of five wetland and terrestrial plant species. Envir. Toxicol. Chem. 19 (10): 2532-2541.
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- (21) Frans, R.E. and R.E. Talbert (1992) Design of field experiments and the measurement and analysis of plant response. In: B. Truelove (Ed.) Research Methods in Weed Science, 2nd ed. Southern weed Science Society, Auburn, 15-23.
- (22) R. D. Bruce R.D. and D. J. Versteeg, A Statistical Procedure for Modelling Continuous Toxicity Data, Environmental Toxicology and Chemistry 11, 1485-1494.
- (23) OECD Guideline for Testing Chemicals 208. (2003 final draft) Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*)

ANNEX 1

Definitions

Active ingredient (a.i.) or active substance (a.s.). A material designed to provide a specific biological effect (e.g., insect control, plant disease control, weed control in the treatment area). Also known as technical grade active ingredient, active substance.

Crop Protection Products (CPPs) or plant protection product (PPPs) or pesticides. A material with a specific biological activity used intentionally to protect crops from pests (e.g., fungal diseases, insects, competitive plants).

EC_x, x% Effect Concentration or ER_x, x% Effect Rate. The concentration or the rate that results in an undesirable change or alteration of x% in the test endpoint being measured relative to the control (e.g., 25% or 50% reduction in shoot weight, final number of plants present, or increase in visual injury would constitute an EC₂₅/ER₂₅ or EC₅₀/ER₅₀ respectively).

Emergence. The appearance of the coleoptile or cotyledon above the soil surface.

Final Preparation. The formulated product containing the active substance (active ingredient) sold in commerce.

Formulation. The commercial formulated product containing the active substance (active ingredient). Also known as final preparation or typical end-use product (TEP).

NOEC (No Observed Effect Concentration) is the highest test substance concentration immediately below the LOEC at which no effect is observed. In this test, the concentration corresponding to the NOEC, has no statistically significant effect ($p < 0.05$) within a given exposure period when compared with the control.

Non-target plants. Those plants that are outside the target plant area, for crop protection products, this usually refers to plants outside the treatment area.

Phytotoxicity. Detrimental deviations (by measured and visual assessments) from the normal pattern of appearance and growth of plants in response to a given substance.

Replicate. The experimental unit which represents the control group and/or treatment group. In these studies, the pot is defined as the replicate.

Visual assessment. Rating of visual damage based on observations of plant stand, vigour, malformation, chlorosis, necrosis, and overall appearance compared with a control.

ANNEX 2

List of Species used in existing plant tests guidelines

Family	Species	Common names
<i>DICOTYLEDONAE</i>		
Chenopodiaceae	<i>Beta vulgaris</i>	Sugar beet
Compositae (Asteraceae)	<i>Lactuca sativa</i>	Lettuce
Cruciferae (Brassicaceae)	<i>Sinapis alba</i>	Mustard
Cruciferae (Brassicaceae)	<i>Brassica chinensis</i>	Chinese cabbage
Cruciferae (Brassicaceae)	<i>Brassica napus</i>	Oilseed rape
Cruciferae (Brassicaceae)	<i>Brassica oleracea var. capitata</i>	Cabbage
Cruciferae (Brassicaceae)	<i>Brassica rapa</i>	Turnip
Cruciferae (Brassicaceae)	<i>Lepidium sativum</i>	Garden cress
Cruciferae (Brassicaceae)	<i>Raphanus sativus</i>	Radish
Cucurbitaceae	<i>Cucumis sativa</i>	Cucumber
Leguminosae (Fabaceae)	<i>Glycine max (G. soja)</i>	Soybean
Leguminosae (Fabaceae)	<i>Phaseolus aureus</i>	Mung bean
Leguminosae (Fabaceae)	<i>Pisum sativum</i>	Pea
Leguminosae (Fabaceae)	<i>Trigonella foenum-graecum</i>	Fenugreek
Leguminosae (Fabaceae)	<i>Lotus corniculatus</i>	Birdsfoot trefoil
Leguminosae (Fabaceae)	<i>Trifolium pratense</i>	Red Clover
Leguminosae (Fabaceae)	<i>Vicia sativa</i>	Vetch
Solanaceae	<i>Lycopersicon esculentum</i>	Tomato
Umbelliferae (Apiaceae)	<i>Daucus carota</i>	Carrot
<i>MONOCOTYLEDONAE</i>		
Gramineae (Poaceae)	<i>Avena sativa</i>	Oats
Gramineae (Poaceae)	<i>Hordeum vulgare</i>	Barley
Gramineae (Poaceae)	<i>Lolium perenne</i>	Perennial ryegrass
Gramineae (Poaceae)	<i>Oryza sativa</i>	Rice
Gramineae (Poaceae)	<i>Secale cereale</i>	Rye
Gramineae (Poaceae)	<i>Sorghum vulgare</i>	Shattercane, Grain sorghum
Gramineae (Poaceae)	<i>Triticum aestivum</i>	Wheat
Gramineae (Poaceae)	<i>Zea mays</i>	Corn
Liliaceae (Amarylladaceae)	<i>Allium cepa</i>	Onion

ANNEX 3

List of potential non-crop species

The list below has been compiled from several published sources, and it is not an exhaustive list by any means. All proposed plant species have been studied in experimental work involving herbicides, some are weed species extensively tested in efficacy tests. Many of the listed species, weed or non-weed, have been recognized as important to wildlife as food sources (Freemark and Boutin 1994).

Family	Species name	English Common Name	References
Amaranthaceae	<i>Amaranthus retroflexus</i>	Pigweed	Fletcher et al, 1990.
Apiaceae	<i>Anthriscus sylvestrius</i>	Wild Chervil	Marshall & Birnie, 1985
	<i>Conium maculatum</i>	Poison Hemlock	Marshall & Birnie, 1985; Breeze et al 1999
	<i>Daucus carota</i>	Queen Anne`s Lace	Cole et al, 1993; U.S. EPA
	<i>Pimpinella saxifraga</i>	Burnet-saxifrage	Marrs et al, 1993
	<i>Torilis japonica</i>	Japanese Hedge-parsley	Breeze et al, 1992; Breeze et al 1999
Apocynaceae	<i>Apocynum cannabinum</i>	Dogbane	Fletcher et al, 1990
Asteraceae	<i>Achillea millefolium</i>	Common Yarrow	Marshall & Birnie, 1985; Breeze et al 1999
	<i>Bellis perrenis</i>	English Daisy	Boutin et al, in press
	<i>Bidens cernua</i>	Bur-marigold	Boutin et al, 2000
	<i>Bidens pilosa</i>	Beggar-ticks	Cole et al, 1993
	<i>Carduus acanthoides</i>	Plumeless Thistle	Marshall & Birnie, 1985
	<i>Centaurea cyanus</i>	Cornflower	Boutin et al, in press; Blackburn & Boutin 2003
	<i>Centaurea nigra</i>	Black Knapweed	Breeze et al, 1992; Marrs et al, 1989
	<i>Centaurea scabiosa</i>	Greater Knapweed	Breeze et al. 1999
	<i>Cirsium arvense</i>	Canada Thistle	Marshall & Birnie, 1985; Breeze et al 1999
	<i>Cirsium vulgare</i>	Spear Thistle	Breeze et al 1999
	<i>Galium aparine</i>	Goosegrass	Brown & Farmer, 1991
	<i>Inula helenium</i>	Elecampane	Boutin et al, in press
	<i>Leontodon automnalis</i>	Automnal Hawkbit	Breeze et al 1999
	<i>Leontodon hispidus</i>	Big Hawkbit	Breeze et al, 1992; Marrs et al, 1989; Marrs et al, 1991
	<i>Leucanthemum vulgare</i>	Ox-eye Daisy	Breeze et al 1999
	<i>Rudbeckia hirta</i>	Black-eyed Susan	Boutin et al, in press
	<i>Solidago canadensis</i>	Canada Goldenrod	Boutin et al, in press
	<i>Sonchus oleraceous</i>	Sow Thistle	Cole et al, 1993
	<i>Tragopogon pratensis</i>	Goat's Beard	Breeze et al 1999
	<i>Xanthium pensylvanicum</i>	Common Cocklebur	U.S. EPA
	<i>Xanthium spinosum</i>	Spiny Cocklebur	Brown & Farmer, 1991
	<i>Xanthium strumarium</i>	Italian Cocklebur	Brown & Farmer, 1991; Cole et al, 1993; Clay & Griffin, 2000
Betulaceae	<i>Betula papyrifera Marsh.</i>	White Birch	Stasiak et al, 1992
Brassicaceae	<i>Alliaria petiolata</i>	Garlic Mustard	Marrs et al, 1989
	<i>Cardamine pratensis</i>	Cuckoo Flower	Breeze et al, 1992; Marrs et al, 1989
	<i>Sinapsis arvensis</i>	Wild Mustard	Boutin et al, in press; Boutin et al, 2000
	<i>Thlaspi arvense</i>	Pennycress	Blackburn & Boutin 2003
Caryophyllaceae	<i>Lychnis flos-cuculi</i>	Ragged Robin	Breeze et al, 1992; Marrs et al, 1989; Marrs et al, 1991
	<i>Silene alba</i>	White Champion	Marshall & Birnie, 1985; Marrs et al, 1993; Breeze et al 1999
	<i>Silene dioica</i>	Red Champion	Marrs et al, 1989
	<i>Chenopodium album</i>	Lamb`s Quarters	Zwerger & Pestemer, 2000; U.S. EPA

Clusiaceae	<i>Hypericum hirsutum</i>	St. John`s Wort	Marrs et al, 1989
	<i>Hypericum perforatum</i>	Common St. John`s Wort	Marshall & Birnie, 1985; Breeze et al, 1992; Marrs et al, 1999
Convolvulaceae	<i>Convolvulus arvensis</i>	Field Bindweed	Marshall & Birnie, 1985; Breeze et al 1999
	<i>Ipomea hederacea</i>	Purple Morning Glory	Brown & Farmer, 1991
Cyperaceae	<i>Cyperus rotundus</i>	Purple Nutsedge	Brown & Farmer, 1991; U.S. EPA
Dipsacaceae	<i>Dipsacus fullonum</i>	Teasel	Marrs et al, 1989
Euphorbiaceae	<i>Mercurialis perennis</i>	Dog's Mercury	Breeze et al 1999
Fabaceae	<i>Cassia obtusifolia</i>	Sicklepod	Brown & Farmer, 1991
	<i>Lotus corniculatus</i>	Bird`s-foot Trefoil	Breeze et al, 1992; Marrs et al, 1991; Marrs et al, 1993;
	<i>Medicago lupulina</i>	Black Medick	Marrs et al, 1989; Blackburn & Boutin 2003
	<i>Senna obtusifolia</i>	Sicklepod	Clay & Griffin, 2000
	<i>Sesbania exaltata</i>	Hemp	Clay & Griffin, 2000; U.S. EPA
	<i>Trifolium pratense</i>	Red Clover	Breeze et al, 1992
	<i>Trifolium repens</i>	White Clover	Marshall & Birnie, 1985
	<i>Vicia cracca</i>	Vetch	OECD in Wang, 1991; Breeze et al 1999
	<i>Vicia tetrasperma</i>	Four-seed Vetch	Marshall & Birnie, 1985
Lamiaceae	<i>Ballota nigra</i>	Black Horehound	Breeze et al 1999
	<i>Betonica officinalis</i>	Betony	Marrs et al, 1993
	<i>Lamiaeum galeobdolon</i>	Yellow Archangel	Marrs et al, 1989
	<i>Lamium album</i>	White Dead Nettle	Marshall & Birnie, 1985
	<i>Leonorus cardiaca</i>	Motherwort	Boutin et al, in press
	<i>Lycopus europaeus</i>	European Water-horehound	Marrs et al, 1993
	<i>Mentha spicata</i>	Spearmint	Boutin et al, in press
	<i>Nepeta cataria</i>	Catnip	Boutin et al, in press
	<i>Prunella vulgaris</i>	Self-heal	Boutin et al, in press; Marrs et al, 1989
	<i>Stachys officinalis</i>	Hedge-nettle	Breeze et al, 1992; Marrs et al, 1989
	<i>Teucrium scorodonia</i>	Wood-sage	Marrs et al, 1989; Marrs et al, 1993
Limnanthaceae	<i>Geranium pusillum</i>	Small Field Geranium	Marshall & Birnie, 1985
Malvaceae	<i>Abutilon theophrasti</i>	Velvetleaf	Brown & Farmer, 1991; Cole et al, 1993; U.S. EPA
	<i>Malva sylvestris</i>	Common mallow	Breeze et al 1999
	<i>Sida spinosa</i>	Prickly Sida	U.S. EPA
Papaveraceae	<i>Papaver rhoeas</i>	Poppy	Boutin et al, in press
Pinaceae	<i>Pinus resinosa</i>	Red Pine	Fletcher et al, 1990
Plantaginaceae	<i>Plantago lanceolata</i>	Ribwort	Breeze et al 1999
	<i>Plantago media</i>	Plantain	Marrs et al, 1989; Marrs et al, 1993
Poaceae	<i>Agropyron repens</i>	Quackgrass	Fletcher et al, 1990.
	<i>Agrostis tenuis</i>	Common Bent-grass	Cole et al, 1993
	<i>Agrostis stolonifera</i>	Bent-grass	Breeze et al 1999
	<i>Alopecurus myosuroides</i>	Foxtail	Zwerger & Pestemer, 2000.
	<i>Arrhenatherum elatius</i>	Oat-grass	Breeze et al 1999
	<i>Avena fatua</i>	Wild Oat	Fletcher et al, 1990; Brown & Farmer, 1991; Cole et al, 1999
	<i>Brachypodium sylvaticum</i>	Slender False-brome	Breeze et al 1999
	<i>Bromus erectus</i>	Upright Brome	Breeze et al 1999
	<i>Bromus sterilis</i>	Barren Brome	Breeze et al 1999
	<i>Bromus tectorum</i>	Downy Brome	U.S. EPA
	<i>Cynosurus cristatus</i>	Dog`s-tail grass	Breeze et al, 1992
	<i>Dactylis glomerata</i>	Orchardgrass	Cole et al, 1993; Breeze et al 1999
	<i>Digitaria sanguinalis</i>	Crabgrass	Fletcher et al, 1990; U.S. EPA; Breeze et al 1999
	<i>Echinochola crusgalli</i>	Barnyardgrass	Boutin et al, 2000; U.S. EPA

Poaceae (cont.)	<i>Eleusine indica</i>	Goosegrass	Fletcher et al, 1990.
	<i>Elymus canadensis</i>	Canada Wild Rye	Blackburn & Boutin 2003
	<i>Elymus repens</i>	Couch-grass	Breeze et al 1999
	<i>Festuca arundinacea</i>	Tall Fescue	Fletcher et al, 1990.
	<i>Festuca elatior</i>	Meadow Fescue	U.S. EPA
	<i>Festuca pratensis</i>	Fescue	Cole et al, 1993
	<i>Hordeum murinum</i>	Wall Barley	Breeze et al 1999
	<i>Phleum pratense</i>	Timothy	Cole et al, 1993
	<i>Poa annua</i>	Bluegrass	Fletcher et al, 1990
	<i>Sorghum halepense</i>	Johnson Grass	Fletcher et al, 1990; Ferrell et al, 2003
Polygonaceae	<i>Polygonum lapathifolium</i>	Pale Persicaria	Brown & Farmer, 1991
	<i>Polygonum convolvulus</i>	Wild Buckwheat	Boutin et al, in press; U.S. EPA; Breeze et al 1999; Kjaer 1
	<i>Polygonum pensylvanicum</i>	Pennsylvania Smartweed	U.S. EPA
	<i>Polygonum persicaria</i>	Smartweed	Fletcher et al, 1996
	<i>Rumex crispus</i>	Curled dock	Boutin et al, in press
	<i>Rumex obtusifolius</i>	Bitter Dock	Marshall & Birnie, 1985; Breeze et al 1999
	<i>Rumex sanguineus</i>	Slender Dock	Marshall & Birnie, 1985; Breeze et al 1999
Primulaceae	<i>Anagallis arvensis</i>	Scarlett Pimpernel	Boutin et al, in press; Blackburn & Boutin 2003
	<i>Primula elatior</i>	Primrose	Marrs et al, 1989; Marrs et al, 1993
	<i>Primula veris</i>	Primrose	Marrs et al, 1989; Marrs et al, 1991
	<i>Primula vulgaris</i>	Primrose	Marrs et al, 1989; Marrs et al, 1993; Breeze et al 1999
Ranunculaceae	<i>Ranunculus acris</i>	Common Buttercup	Breeze et al, 1992; Marrs et al, 1989; Marrs et al, 1993
	<i>Ranunculus repens</i>	Creeping Buttercup	Marshall & Birnie, 1985; Breeze et al 1999
Rosaceae	<i>Filipendula ulmaria</i>	Queen of the Meadow	Marrs et al, 1989
	<i>Geum urbanum</i>	Yellow Avens	Breeze et al, 1992; Marrs et al, 1989; Marrs et al 1993; Bre
	<i>Potentilla reptans</i>	Creeping Cinquefoil	Breeze et al 1999
Rubiaceae	<i>Galium mollugo</i>	Cleavers	Breeze et al, 1992; Marrs et al, 1989
	<i>Galium verum</i>	Yellow Cleavers	Marrs et al, 1993; Breeze et al 1999
	<i>Gallium aparine</i>	Cleavers	Breeze et al 1999
Scrophulariaceae	<i>Digitalis purpurea</i>	Foxglove	Boutin et al, in press; Marrs et al, 1989; Marrs et al, 1991 1999
	<i>Linaria vulgaris</i>	Yellow Toadflax	Breeze et al 1999
	<i>Mimulus ringens</i>	Monkey-flower	Boutin et al, 2000
	<i>Verbascum thapsus</i>	Common Mullein	Marrs et al, 1993
	<i>Veronica persica</i>	Speedwell	Cole et al, 1993; Boutin & Harper 1991
Urticaceae	<i>Urtica dioica</i>	Stinging Nettle	Marshall & Birnie, 1985; Breeze et al 1999

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ANNEX 4

Example for appropriate growth conditions for certain crop species

These test conditions were found to be suitable for 10 crop species:

- tomato (*Lycopersicon esculentum*),
- cucumber (*Cucumis sativus*),
- lettuce (*Lactuca sativa*),
- soybean (*Glycine max*),
- cabbage (*Brassica oleracea*),
- carrot (*Daucus carota*), oat (*Avena sativa*),
- perennial ryegrass (*Lolium perenne*),
- corn (*Zea mays*),
- onion (*Allium cepa*).

For these 10 species, these conditions with suggested ranges are:

- Carbon dioxide concentration: 350+/-50 ppm;
- Relative humidity: 70+/-5% during light periods and 90+/-5% during dark periods;
- Temperature: 25+/-3⁰C during the day, 20+/-3⁰C during the night;
- Photoperiod: 16h light/8h darkness, assuming an average wavelength of 400 to 700 nm;
- Light: luminance of 350+/-50 micromol/m²/s, measured at the top of the canopy.