DRAFT DOCUMENT
September 2003

OECD GUIDELINE FOR THE TESTING OF CHEMICALS

PROPOSAL FOR UPDATING GUIDELINE 208

Terrestrial Plant Test:
208: Seedling Emergence and Seedling Growth Test

INTRODUCTION

1. OECD Guidelines for the Testing of Chemicals are periodically reviewed in the light of scientific progress and current regulatory procedures. This updated guideline is designed to assess potential effects of substances on seedling emergence and growth. As such it does not cover chronic effects or effects on reproduction (i.e. seed set, flower formation, fruit maturation). 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 (e.g. when testing metals/metal compounds the effects of pH and associated counter ions should be considered)(1). This guideline does not address plants exposed to vapours of chemicals. The 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 (2) (3) (4) (5) (6) (7) (8). Other references pertinent to plant testing were also considered (9) (10) (11). Definitions used are given in Annex 1.

PRINCIPLE OF THE TEST

2. The test assesses effects on seedling emergence and early growth of higher plants following exposure to the test substance in the soil (or other suitable matrix). Seeds are placed in contact with soil treated with the test substance and evaluated for effects following 14 to 21 days after 50% emergence of the seedlings in the control group. Endpoints measured are visual assessment of seedling emergence, biomass (fresh or dry shoot weight, or shoot height) and visual detrimental effects (chlorosis, mortality, plant development abnormalities, etc.). Measurements are made at least weekly or more often when recording the emergence of the seeds and compared to those of untreated control plants.

3. The seedling emergence and growth test is intended to meet testing requirements for both general chemicals and crop protection products. Depending on the expected route of exposure, the test substance is either incorporated into the soil (or artificial soil matrix) or applied to the soil surface, which properly represents the potential route of exposure. In the case of soil incorporation, the soil is transferred to pots after treatment and seeds of the given plant species are then planted. Surface applications are made to potted soil in which the seeds have already been planted. The test units (controls and treated soils plus seeds) are then placed under appropriate conditions to support germination/growth of plants. Optionally, the test can be extended to combine both soil exposure and further foliar exposure as in the test guideline 227 (12).

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 seedling emergence should be at least 80% for crop and 65 % for non-crop species;
- the mean control seedling growth does not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformation);
- the mean control survival is at least 90% for the duration of the study;
- 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 performance of the test and the response of the test plants has not changed significantly over time. Suitable reference substances for example for certain species can be found in Annex 5 or 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. Natural soil could complicate interpretation of results and increase variability due to varying physical/chemical properties and microbial populations. These variables in turn alter moisture-holding capacity, chemical-binding capacity, aeration, and nutrient and trace element content. In addition to the variations in these physical factors, there will also be variation in chemical properties such as pH and redox potential, which may affect the bioavailability of the test substance (15) (16) (17).

11. Glass beads, mineral wool, and 100 percent acid washed sand (with nutrient solution added) are usually not recommended for testing of crop protection products, however they may be of use for the testing of general chemicals or where it is desired to minimize the variability of the natural soils. Growth support media or substrates used should be composed of inert materials that minimize interaction with the test substance, the solvent carrier, or both. Quartz sand and glass beads (e.g., 0.35 to 0.85 mm in diameter) have been found to be suitable inert materials that minimally absorb the test substance (18), ensuring that the substance will be maximally available to the seedling via root uptake. Unsuitable substrates would include vermiculite, perlite or other highly absorptive materials. Nutrients for plant growth should be provided to ensure that plants are not stressed through nutrient deficiencies, and where possible this should be assessed via chemical analysis or by visual assessment of control plants.

Selection and number of test species

12. The selection of species should be based on the ecological relevance of species, species specific life-cycle characteristics, region of natural occurrence etc. (9) (19) (20) (21) (22) (23). 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.

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

Application of the test substance

Incorporation into soil/artificial substrate

14. Substances which are water soluble or suspended in water, can be added to water and the test solution then mixed with soil. This type of test may be appropriate if exposure to the chemical is through soil or soil pore-water and that there is concern for root uptake. The water-holding capacity of the soil should not be exceeded by the addition of the test substance. The volume of water added should be the same for each test concentration, but should be limited to prevent soil agglomerate clumping.

15. Substances with low water solubility should be dissolved in a suitable volatile solvent (e.g. acetone, ethanol) and mixed with sand. The solvent can then be removed from the sand using a stream of air while continuously mixing the sand. The treated sand is mixed with the experimental soil. Equal amounts of sand and solvent are added to all treatment levels. A second control is established which receives only sand and solvent. For solid, insoluble test substances, dry soil and the chemical are mixed in a suitable mixing device (e.g. end- over-end shaker). Hereafter, the soil is added to the pots and seeds are sown immediately.

16. When an artificial substrate is used, chemicals that are soluble in water can be dissolved in the nutrient solution just prior to the beginning of the test. Chemicals that are insoluble in water, but which can be suspended in water by using a solvent carrier, should be added with the carrier, to the nutrient solution. Water-insoluble chemicals for which there is no non-toxic water-soluble carrier available, should be dissolved in an appropriate volatile solvent. The solution is mixed with the sand or glass beads, placed in a rotary vacuum apparatus, and evaporated, leaving a uniform coating of chemical on the sand or beads. A weighed portion of beads should be extracted with the same organic solvent and the chemical assayed before the potting containers are filled.

Surface application

17. For crop protection products, spraying the soil surface with the test solution is often used for application of the test substance. 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 soil surface simulating typical spray tank applications. Generally, spray volumes should be in the range of normal agricultural practice and the volumes (amount of water etc. should be reported). Nozzle type should be selected to provide uniform coverage of the soil surface. If solvents other than water are applied, a second group of control plants should be established receiving only the solvent/carrier.

18. Optionally for certain purposes, the test can be modified and extended to include further foliar exposure starting for example at the 2- to 4- true leaf stage of the plants as in the test guideline 227 (12).

Verification of test substance concentration

19. The rates of application and concentration of the test substances in soil should be confirmed by analytical verification. When the test is designed to determine an EC_x, the analytical verification should be performed at least at the lowest and at the highest test concentration. If the test substance is applied on the soil surface, the calibration of application equipment should also be checked.

PERFORMANCE OF THE TEST

Test groups and controls

20. 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 see (20). 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 (24). 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.

21. 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. . Within a given test, all test plants including the controls should be from the same source and identification. To prevent bias, random assignment of test and control pots is required.

22. 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

23. The test conditions should approximate those conditions necessary for normal growth for the species and varieties tested (see Annex 4). The emerging plants should be maintained under good horticultural practices in controlled environment chambers, phytotrons, or greenhouses. 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 of the selected species. 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.

24. Soil nutrients may be supplemented as needed 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 when possible. However, initial top watering can be used to stimulate seed germination and, for soil surface application it facilitates movement of the chemical into the soil.

25. 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 among different treatments and of the controls to the test substance is avoided.

Testing at a single concentration/rate

26. 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 labeling 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 1000 mg/kg dry soil and for crop protection products three times the recommended field application rate.

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

28. 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 germination or variable growth habits may be needed to increase the statistical power of the test.

Range-finding test

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

Testing at multiple concentrations/rates

30. 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 emergence, biomass and/or visual effects compared to un-exposed controls.

31. 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. A minimum of 20 plants per concentration divided into a minimum of four replicates is required. If a larger number of test concentrations are used, the number of replicates may be reduced. The variability of emergence and 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 number of replicates is to be increased.

Observations

32. During the observation period, 14 to 21 days after 50% of the control plants (also possible solvent controls) have emerged, the plants are observed frequently (at least weekly) for visual phytotoxicity and mortality. At the end of the test, measurement of % emergence and biomass should be recorded as well as visual phytotoxicity (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^{o} C to a constant weight) or complementary height of the shoot. A uniform scoring

system for visual injury should be used to evaluate the observable toxic responses. Examples for performing qualitative and quantitative visual ratings are provided in references (25)(26).

DATA ANALYSIS AND REPORTING

Statistical analysis

Single rate test

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

Multiple rate test

32. 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 emergence as quantal data, logit, probit, Weibull, Spearman-Karber, trimmed Spearman-Karber 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 (27). 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 (24) (28).

Test report

33. 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: soil particle distribution and classification, physical and chemical properties including % organic matter, % organic carbon, and 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;
- for the seedling emergence and growth test, the number and percent emergence as compared to controls;
- biomass measurements, i.e. shoot weight (fresh or dry) or shoot height of the plants as percentage of the controls;
- percent visual injury and qualitative 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. EC_{50}/ER_{50} or EC_{25}/ER_{25}) values and related confidence limits 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

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- 850.4225: Seedling Emergence, Tier II
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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 seedling emergence, shoot weight, final number of plants present, or increase in visual injury would constitute an EC_{25}/ER_{25} or EC_{50}/ER_{50} 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
DICOTYLEDONAE		
Chenopodiaceae	*Beta vulgaris*	Sugar beet
Compositae (Asteraceae)	*Lactuca sativa*	Lettuce
Cruciferae (Brassicaceae)	*Sinapis alba*	Mustard
Cruciferae (Brassicaceae)	*Brassicachinensis*	Chinese cabbage
Cruciferae (Brassicaceae)	*Brassica napus*	Oilseed rape
Cruciferae (Brassicaceae)	*Brassica oleracea var. capitata*	Cabbage
Cruciferae (Brassicaceae)	*Brassica rapa*	Turnip
Cruciferae (Brassicaceae)	*Lepidium sativum*	Garden cress
Cruciferae (Brassicaceae)	*Raphanus sativus*	Radish
Cucurbitaceae	*Cucumis sativa*	Cucumber
Leguminosae (Fabaceae)	*Glycine max (G. soja)*	Soybean
Leguminosae (Fabaceae)	*Phaseolus aureus*	Mung bean
Leguminosae (Fabaceae)	*Pisum sativum*	Pea
Leguminosae (Fabaceae)	*Trigonella foenum-graecum*	Fenugreek
Leguminosae (Fabaceae)	*Lotus corniculatus*	Birdsfoot trefoil
Leguminosae (Fabaceae)	*Trifolium pratense*	Red Clover
Leguminosae (Fabaceae)	*Vicia sativa*	Vetch
Solanaceae	*Lycopersicon esculentum*	Tomato
Umbelliferae (Apiaceae)	*Daucus carota*	Carrot
MONOCOTYLEDONAE		
Gramineae (Poaceae)	*Avena sativa*	Oats
Gramineae (Poaceae)	*Hordeum vulgare*	Barley
Gramineae (Poaceae)	*Lolium perenne*	Perennial ryegrass
Gramineae (Poaceae)	*Oryza sativa*	Rice
Gramineae (Poaceae)	*Secale cereale*	Rye
Gramineae (Poaceae)	*Sorghum vulgare*	Shattercane, grain sorghum
Gramineae (Poaceae)	*Triticum aestivum*	Wheat
Gramineae (Poaceae)	*Zea mays*	Corn
Liliaceae (Amarylladaceae)	*Allium cepa*	Onion

ANNEX 3

List of potential non-crop species

The list below has been compiled from several published sources, and it is not and 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	*Amaranthus retroflexus*	Pigweed	Fletcher et al, 1990.
Apiaceae	*Anthriscus sylvestrius*	Wild Chervil	Marshall & Birnie, 1985
	Conium maculatum	Poison Hemlock	Marshall & Birnie, 1985; Breeze et al 1999
	Daucus carota	Queen Anne`s Lace	Cole et al, 1993; U.S. EPA
	Pimpinella saxifraga	Burnet-saxifrage	Marrs et al, 1993
	Torilis japonica	Japanese Hedge-parsley	Breeze et al, 1992; Breeze et al 1999
Apocynaceae	*Apocynum cannabinum*	Dogbane	Fletcher et al, 1990
Asteraceae	*Achillea millefolium*	Common Yarrow	Marshall & Birnie, 1985; Breeze et al 1999
	Bellis perrenis	English Daisy	Boutin et al, in press
	Bidens cernua	Bur-marigold	Boutin et al, 2000
	Bidens pilosa	Beggar-ticks	Cole et al, 1993
	Carduus acanthoides	Plumeless Thistle	Marshall & Birnie, 1985
	Centaurea cyanus	Cornflower	Boutin et al, in press; Blackburn & Boutin 2003
	Centaurea nigra	Black Knapweed	Breeze et al, 1992; Marrs et al, 1989
	Centaurea scabiosa	Greater Knapweed	Breeze et al. 1999
	Cirsium arvense	Canada Thistle	Marshall & Birnie, 1985; Breeze et al 1999
	Cirsium vulvare	Spear Thistle	Breeze et al 1999
	Galium aparine	Goosegrass	Brown & Farmer, 1991
	Inula helenium	Elecampane	Boutin et al, in press
	Leontodon automnalis	Automnal Hawkbit	Breeze et al 1999
	Leontodon hispidus	Big Hawkbit	Breeze et al, 1992; Marrs et al, 1989; Marrs et al, 1991
	Leucanthemum vulgare	Ox-eye Daisy	Breeze et al 1999
	Rudbeckia hirta	Black-eyed Susan	Boutin et al, in press
	Solidago canadensis	Canada Goldenrod	Boutin et al, in press
	Sonchus oleraceous	Sow Thistle	Cole et al, 1993
	Tragopogon pratensis	Goeat's Beard	Breeze et al 1999
	Xanthium pensylvanicum	Common Cocklebur	U.S. EPA
	Xanthium spinosum	Spiny Cocklebur	Brown & Farmer, 1991
	Xanthium strumarium	Italian Cocklebur	Brown & Farmer, 1991; Cole et al, 1993; Clay & Griffin, 20
Betulaceae	*Betula papyrifera Marsh.*	White Birch	Stasiak et al, 1992
Brassicaceae	*Alliaria petiolata*	Garlic Mustard	Marrs et al, 1989
	Cardamine pratensis	Cuckoo Flower	Breeze et al, 1992; Marrs et al, 1989
	Sinapsis arvensis	Wild Mustard	Boutin et al, in press; Boutin et al, 2000
	Thlaspi arvense	Pennycress	Blackburn & Boutin 2003
Caryophyllaceae	*Lychnis flos-cuculi*	Ragged Robin	Breeze et al, 1992; Marrs et al, 1989; Marrs et al, 1991
	Silene alba	White Campion	Marshall & Birnie, 1985; Marrs et al, 1993; Breeze et al 199
	Silene dioica	Red Campion	Marrs et al, 1989
	Chenopodium album	Lamb`s Quarters	Zwerger & Pestemer, 2000; U.S. EPA

Clusiaceae	*Hypericum hirsutum*	St. John`s Wort	Marrs et al, 1989
	Hypericum perforatum	Common St. John`s Wort	Marshall & Birnie, 1985; Breeze et al, 1992; Marrs et al, 19
Convolvulaceae	*Convolvulus arvensis*	Field Bindweed	Marshall & Birnie, 1985; Breeze et al 1999
	Ipomea hederacea	Purple Morning Glory	Brown & Farmer, 1991
Cyperaceae	*Cyperus rotundus*	Purple Nutsedge	Brown & Farmer, 1991; U.S. EPA
Dipsaceae	*Dipsacus fullonum*	Teasel	Marrs et al, 1989
Euphorbiaceae	*Mercurialis perennis*	Dog's Mercury	Breeze et al 1999
Fabaceae	*Cassia obtusifolia*	Sicklepod	Brown & Farmer, 1991
	Lotus corniculatus	Bird`s-foot Trefoil	Breeze et al, 1992; Marrs et al, 1991; Marrs et al, 1993;
	Medicago lupulina	Black Medick	Marrs et al, 1989; Blackburn & Boutin 2003
	Senna obtusifolia	Sicklepod	Clay & Griffin, 2000
	Sesbania exaltata	Hemp	Clay & Griffin, 2000; U.S. EPA
	Trifolium pratense	Red Clover	Breeze et al, 1992
	Trifolium repens	White Clover	Marshall & Birnie, 1985
	Vicia cracca	Vetch	OECD in Wang, 1991; Breeze et al 1999
	Vicia tetrasperma	Four-seed Vetch	Marshall & Birnie, 1985
Lamiaceae	*Ballota nigra*	Black Horehound	Breeze et al 1999
	Betonica officinalis	Betony	Marrs et al, 1993
	Lamiastrum galeobdolon	Yellow Archangel	Marrs et al, 1989
	Lamium album	White Dead Nettle	Marshall & Birnie, 1985
	Leonorus cardiaca	Motherwort	Boutin et al, in press
	Lycopus europaeus	European Water-horehound	Marrs et al, 1993
	Mentha spicata	Spearmint	Boutin et al, in press
	Nepeta cataria	Catnip	Boutin et al, in press
	Prunella vulgaris	Self-heal	Boutin et al, in press; Marrs et al, 1989
	Stachys officinalis	Hedge-nettle	Breeze et al, 1992; Marrs et al, 1989
	Teucrium scorodonia	Wood-sage	Marrs et al, 1989; Marrs et al, 1993
Limnanthaceae	*Geranium pusillum*	Small Field Geranium	Marshall & Birnie, 1985
Malvaceae	*Abutilon theophrasti*	Velvetleaf	Brown & Farmer, 1991; Cole et al, 1993; U.S. EPA
	Malva sylvestris	Common mallow	Breeze et al 1999
	Sida spinosa	Prickly Sida	U.S. EPA
Papaveraceae	*Papaver rhoeas*	Poppy	Boutin et al, in press
Pinaceae	*Pinus resinosa*	Red Pine	Fletcher et al, 1990
Plantaginaceae	*Plantago lanceolata*	Ribwort	Breeze et al 1999
	Plantago media	Plantain	Marrs et al, 1989; Marrs et al, 1993
Poaceae	*Agropyrons repens*	Quackgrass	Fletcher et al, 1990.
	Agrositus tenuis	Common Bent-grass	Cole et al, 1993
	Agrostis stolonifera	Bent-grass	Breeze et al 1999
	Alopecurus myosuroides	Foxtail	Zwerger & Pestemer, 2000.
	Arrhenatherum elatius	Oat-grass	Breeze et al 1999
	Avena fatua	Wild Oat	Fletcher et al, 1990; Brown & Farmer, 1991; Cole et al, 199
	Brachypodium sylvaticum	Slender False-brome	Breeze et al 1999
	Bromus erectus	Upright Brome	Breeze et al 1999
	Bromus sterilis	Barren Brome	Breeze et al 1999
	Bromus tectorum	Downy Brome	U.S. EPA
	Cynosurus cristatus	Dog`s-tail grass	Breeze et al, 1992
	Dactylis glomerata	Orchardgrass	Cole et al, 1993; Breeze et al 1999
	Digitaria sanguinalis	Crabgrass	Fletcher et al, 1990; U.S. EPA; Breeze et al 1999
	Echinochola crusgalli	Barnyardgrass	Boutin et al, 2000; U.S. EPA

Poaceae (cont.)	*Eleusine indica*	Goosegrass	Fletcher et al, 1990.
	Elymus canadensis	Canada Wild Rye	Blackburn & Boutin 2003
	Elymus repens	Couch-grass	Breeze et al 1999
	Festuca arundinacea	Tall Fescue	Fletcher et al, 1990.
	Festuca elatior	Meadow Fescue	U.S. EPA
	Festuca pratensis	Fescue	Cole et al, 1993
	Hordeum murinum	Wall Barley	Breeze et al 1999
	Phleum pratense	Timothy	Cole et al, 1993
	Poa annua	Bluegrass	Fletcher et al, 1990
	Sorghum halepense	Johnson Grass	Fletcher et al, 1990; Ferrell et al, 2003
Polygonaceae	*Polugonum lapathifolium*	Pale Persicaria	Brown & Farmer, 1991
	Polygonum convolvulus	Wild Buckwheat	Boutin et al, in press; U.S. EPA; Breeze et al 1999; Kjaer 19
	Polygonum pensylvanicum	Pennsylvania Smartweed	U.S. EPA
	Polygonum persicaria	Smartweed	Fletcher et al, 1996
	Rumex crispus	Curled dock	Boutin et al, in press
	Rumex obtusifolius	Bitter Dock	Marshall & Birnie, 1985; Breeze et al 1999
	Rumex sanguineus	Slender Dock	Marshall & Birnie, 1985; Breeze et al 1999
Primulaceae	*Anagallis arvensis*	Scarlett Pimpernel	Boutin et al, in press; Blackburn & Boutin 2003
	Primula elatior	Primrose	Marrs et al, 1989; Marrs et al, 1993
	Primula veris	Primrose	Marrs et al, 1989; Marrs et al, 1991
	Primula vulgaris	Primrose	Marrs et al, 1989; Marrs et al, 1993; Breeze et al 1999
Ranunculaceae	*Ranunculus acris*	Common Buttercup	Breeze et al, 1992; Marrs et al, 1989; Marrs et al, 1993
	Ranunculus repens	Creeping Buttercup	Marshall & Birnie, 1985; Breeze et al 1999
Rosaceae	*Filipendula ulmaria*	Queen of the Meadow	Marrs et al, 1989
	Geum urbanum	Yellow Avens	Breeze et al, 1992; Marrs et al, 1989; Marrs et al 1993; Bree
	Potentilla reptans	Creeping Cinquefoil	Breeze et al 1999
Rubiaceae	*Galium mollugo*	Cleavers	Breeze et al, 1992; Marrs et al, 1989
	Galium verum	Yellow Cleavers	Marrs et al, 1993; Breeze et al 1999
	Gallium aparine	Cleavers	Breeze et al 1999
Scrophulariaceae	*Digitalis purpurea*	Foxglove	Boutin et al, in press; Marrs et al, 1989; Marrs et al, 1991; M 1999
	Linaria vulgaris	Yellow Toadflax	Breeze et al 1999
	Mimulus ringens	Monkey-flower	Boutin et al, 2000
	Verbascum thapsus	Common Mullein	Marrs et al, 1993
	Veronica persica	Speedwell	Cole et al, 1993; Boutin & Harper 1991
Urticaceae	*Urtica dioica*	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+/-3^{0}C during the day, 20+/-3^{0}C during the night;
- Photoperiod: 16h light/8h darkness, assuming an average wavelength of 400 to 700 nm;
- Light: luminance of 350+/-50 micromol/m^2/s, measured at the top of the canopy.

ANNEX 5

Data on reference substances:

Sodium trichloracetate as reference substance.

Species	EC 50* lowest value (mg/kg dry soil)	EC 50* highest value (mg/kg dry soil)
Winter barley	6.8	13.5
Lettuce	143	237

*: biomass