TEST SPECIFIC CHECKLIST [1]

Prepared: March 2005

Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil

Parameter	Specification	Met Specifics Y	N	NA
Sample Handling				
Containers	Non-toxic material for transport and storage **(Must)**	☐	☐	☐
	New or thoroughly cleaned, or lined with high-quality plastic **(Must)**	☐	☐	☐
Labelling	Sample containers sealed and labelled or coded immediately after filling **(Must)**	☐	☐	☐
	Labelling and accompanying records include a code or description that identifies sample type (e.g., grab, core, composite), source, precise location, land use information, replicate number, and date of collection **(Must)**; name and signature of sampler(s) should also be included	☐	☐	☐
Transport	Samples to be kept from overheating during transport or storage, especially if samples are thought to or known to contain volatile substances	☐	☐	☐
	Samples must not freeze or partially freeze during transport or storage (unless they are frozen when collected) **(Must)**	☐	☐	☐
	Samples must not be allowed to dehydrate during transport or storage (unless samples are saturated with excess water upon arrival at the lab)**(Must)**	☐	☐	☐
	Samples should be kept in the dark (i.e., light-tight or opaque containers)	☐	☐	☐
	Samples should remain cool (e.g., 7 ± 3°C) during transit	☐	☐	☐
	Date sample(s) received at the laboratory recorded **(Must)**	☐	☐	☐
	Sample temperature upon receipt at laboratory measured and recorded	☐	☐	☐
Holding Time	Test should start within 2 weeks, and must start within 6 weeks unless soil contaminants are known to be stable **(Must)**	☐	☐	☐
Holding Conditions	Samples to be stored for future use must be held in airtight containers **(Must)**	☐	☐	☐
	Store samples in darkness at 4 ± 2°C	☐	☐	☐
	These storage conditions must be applied in instances where PAHs or other light-sensitive contaminants are present or if the samples are known to contain unstable volatiles **(Must)**	☐	☐	☐
Sample Preparation				
a) Field-Collected Test Soil				
Sieving	Sample sieved (e.g. 4 - 6 mm mesh) without water to remove oversize material, if necessary (e.g., debris and indigenous macro-organisms)	☐	☐	☐
Homogenization	Soil and/or solid particulate waste for testing should be homogenized, unless inappropriate (e.g., affects concentration or bioavailability of contaminants)	☐	☐	☐
	Any moisture that separates from a sample during its transport and/or storage must be remixed into it if possible **(Must)**	☐	☐	☐
Characterization	Each soil (including negative control and reference soil) is analysed for particle size distribution (%sand, silt, and clay), total organic carbon content (%), organic matter content (%), moisture content (%), WHC (%), total nitrogen, total phosphorous, pH and conductivity, as a minimum **(Must)**	☐	☐	☐
	Optional analyses of contaminants of concern (e.g., metals, polycyclic aromatic hydrocarbons (PAHs), pesticides)	☐	☐	☐
Moisture Content	Water Holding Capacity (WHC) of soils (artificial and site) are known **(Must)**	☐	☐	☐
	Optimal moisture content of test soils (artificial and site) determined and expressed as % WHC **(Must)**	☐	☐	☐
	WHC determined gravimetrically by drying subsample for ~24h at 105°C	☐	☐	☐
	Test soil hydrated to optimal % of WHC during preparation of test conc.	☐	☐	☐

[1] Checklist based on Environment Canada's "Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil". See Endnote for references.

Parameter	Specification	Met Specifics Y	N	NA
Sample Preparation (continued)				
Test Concentrations. . .	Each batch (i.e., treatment) is prepared on the day of the start of the test (Day 0) in sufficient quantity for all replicates and physicochemical analyses	☐	☐	☐
	Mix homogenized test soil with negative control soil or reference soil to prepare each treatment/concentration in a geometric series for multi-concentration tests; ensure homogeneity (i.e., mix until texture, colour, and moisture are homogeneous) and divide into replicates.	☐	☐	☐
b) Chemical-Spiked Test Soil				
Chemical Characterization.	Information on chemical or chemical product(s) obtained before test starts, includes: stability, water solubility, vapour pressure, purity, estimated toxicity to test species and humans and biodegradability.	☐	☐	☐
	Concentration of test chemical in soil measured at beginning and end of test, in high, medium and low concentrations, as a minimum.	☐	☐	☐
Preparation of Mixtures.	Procedure depends on nature of test substance(s), test design, and objectives; test substance(s) may be prepared manually or by mechanical agitation; test substance(s) may be added as measured quantities in solution (i.e., in water or an organic solvent) or as a solid material comprised partly or completely of the test substance(s); ensure homogeneity.	☐	☐	☐
	For each treatment, mixing conditions (solution:soil ratio, mixing and holding time and T°) must be standardized **(Must)**.	☐	☐	☐
	Each batch (i.e., treatment) is prepared on the day of the start of the test (Day 0) in sufficient quantity for all replicates and physicochemical analyses.	☐	☐	☐
Solvent.	Solvent control included in test (in addition to negative control) if organic solvent used for test substance(s) that are not soluble in water **(Must)**	☐	☐	☐
	Solvent control from same batch used to make the stock solution of test substance and contains the same concentration of solubilizing agent that is present in the highest concentration of test chemical **(Must)**	☐	☐	☐
Test Conditions				
Test Facility.	Environmental chamber or equivalent with acceptable temperature & lighting control **(Must)**.	☐	☐	☐
	Facility well ventilated & free of fumes; isolated from any contaminants that might affect test organisms, and areas for sample preparation/storage.	☐	☐	☐
	Equipment, apparatus and construction materials made of non toxic material (e.g., borosilicate glass, nylon, Teflon™, high-density polyethylene, high density polystyrene, polypropylene, polycarbonate, fluorocarbon plastics, Nalgene™, type 316 stainless steel, fibre glass) **(Must)**.	☐	☐	☐
	Use of toxic materials including copper, zinc, brass, galvanized metal, lead, and natural rubber is avoided **(Must)**.	☐	☐	☐
	Instruments for routine measurements (e.g., pH, temp.) are available **(Must)**. . . .	☐	☐	☐
	Laboratory equipped for analysis of soil moisture content.	☐	☐	☐
	Other equipment includes: drying oven (capable of 90°C & 105°C), a weighing balance (accurate to 0.1 mg), and a pH meter **(Must)**.	☐	☐	☐
	Safety apparatus used when preparing mixtures and test soils **(Must)**.	☐	☐	☐
	All test vessels, equipment, and supplies that might contact site soils, test soils, test (hydration) water, stock solutions, or test solutions, are clean and rinsed with test water before being used **(Must)**	☐	☐	☐

Parameter	Specification	Met Specifics Y	N	NA
Test Conditions (continued)				
Initial Tests	≥5 control performance tests and ≥5 reference toxicity tests with candidate samples of artificial or natural negative control soil intended for routine use, should be undertaken by laboratory personnel to confirm acceptable performance of test species using procedures & conditions in test method.	☐	☐	☐
	Conditions and procedures for initial control performance test should follow those for the definitive test.	☐	☐	☐
	Conditions and procedures for initial reference toxicity tests should be identical to those described for routine reference toxicant tests.	☐	☐	☐
	Each set of initial tests should be performed using each plant species intended for use in future definitive toxicity tests.	☐	☐	☐
	Data from initial control performance test shows that criteria for test validity can be met **(Must)**.	☐	☐	☐
	Data from initial reference toxicity tests should be compared by calculating and appraising the magnitude of the coefficient of variation (CV) of the derived ICps.	☐	☐	☐
Negative Control Soil	Natural clean field-collected soil or artificial soil for which previous tests with the chosen test species demonstrated that the test validity criteria could be regularly met; recommend artificial soil for tests with chemicals or chemical products spiked in soil.	☐	☐	☐
	Negative control soil included as a treatment in every toxicity test **(Must)**.	☐	☐	☐
Clean Field-Collected Soil	Natural soil collected from a clean (uncontaminated) site; free of pesticide or fertilizer for ≥5 years.	☐	☐	☐
	Laboratory demonstrates experimental evidence that natural soil from a given source has met test validity criteria before being used as negative control soil in a definitive test **(Must)**.	☐	☐	☐
	Soil analysed for recommended physicochemical characteristics (see Section 3.4.1 in EPS 1/RM/45).	☐	☐	☐
	Seeds that germinate from a natural seedbank in samples of natural soil (i.e., either during storage or testing) are removed **(Must)**.	☐	☐	☐
	Natural soil can be air-dried (10 - 20% moisture content), coarse-screened (4 - 6 mm), transferred to clean plastic pails, and stored in darkness at 4 ± 2°C.	☐	☐	☐
Artificial Soil	10% *Sphagnum* sp. peat, air dried and sieved (2-mm mesh); 20% kaolin clay (with particle size < 40 µm); and 70% silica sand (grade 70); mixed dry.	☐	☐	☐
	Add reagent-grade calcium carbonate to dry mixture to adjust pH to 6.0 - 7.5	☐	☐	☐
	Hydrate using test water to ~28% of WHC and adjust pH as necessary with more calcium carbonate.	☐	☐	☐
	Artificial soil stored in the dark at 20 ± 2°C for ≥3 days before use in toxicity test; thereafter soil can be stored at 4 ± 2°C.	☐	☐	☐
Positive Control Soil	Should be included in each series of soil toxicity tests; may be a negative control soil spiked with a reference toxicant or with one or more toxic chemicals of concern; or a highly contaminated sample of field-collected soil.	☐	☐	☐
Reference Soil	One or more samples for tests with field-collected soil, ideally taken from site(s) presumed to be clean but near sites of test soil collection.	☐	☐	☐
	Characteristics including percent organic matter, particle size distribution, texture, and pH are similar to test soils.	☐	☐	☐
	Tests involving samples of reference soil must also include a sample of negative control soil **(Must)**.	☐	☐	☐
Initial Hydration of Test Soils	Field-collected soils are hydrated with test water to the optimal percentage of its WHC (i.e., soil is a homogenous, crumbly consistency; clumps 3 - 5 mm); artificial soils are hydrated to ~70% of WHC; once seeds have been added to test vessels, their contents (i.e., test soils) are hydrated to "near saturation" using a fine-mist spray bottle, and vessels are covered.	☐	☐	☐

Parameter	Specification	Met Specifics Y	N	NA
Test Conditions (continued)				
Test Water.	Deionized or distilled water or better, such as reagent-grade water produced by a system of reverse osmosis, carbon and ion exchange cartridges **(Must)**.	☐	☐	☐
Hydration Water.	Water used to hydrate test soils; test water, de-chlorinated tap water, or nutrient solution, where applicable.	☐	☐	☐
Glassware Cleaning. . .	Soak; detergent wash; 2 tap water rinses; acid wash (e.g., 10% nitric or hydrochloric acid, metal-free grade) to remove scale, metals and bases; 2 rinses with test water; pesticide free acetone wash to remove organic compounds and HPLC-grade hexane wash for oily residues; allow organic solvent to volatilize and rewash with detergent if necessary; 3 rinses with test water..	☐	☐	☐
Measurements During Test				
Moisture Content.	Soil moisture content in each treatment/concentration at test start **(Must)**.	☐	☐	☐
	Moisture content determined gravimetrically (see EPS 1/RM/45).	☐	☐	☐
	Moisture content calculated on a dry wt. basis **(Must)**..	☐	☐	☐
pH..	Soil pH in each treatment/concentration at test start and end **(Must)**.	☐	☐	☐
	Soil pH measured using a modified $CaCl_2$ Slurry Method (see EPS 1/RM/45). . . .	☐	☐	☐
Temperature..	Air temperature in test facility, daily or continuously **(Must)**..	☐	☐	☐
Humidity.	Humidity in test facility, periodically	☐	☐	☐
Conductivity.	Conductivity measured at test start and end when test soil is suspected of having a high salt content.	☐	☐	☐
Light Intensity.	Light fluence rate at least once during test **(Must)**..	☐	☐	☐
Chemical Analyses. . . .	Normally measure at beginning and end of test, in high, medium, and low strengths as a minimum..	☐	☐	☐
Reference Toxicity Test.	Static 7- or 10-day (i.e., species-specific) multi-concentration test **(Must)**.	☐	☐	☐
	Test duration 7 days for alfalfa, barley, cucumber, durum wheat, lettuce, radish, red clover, or tomato; and 10 days for blue grama grass, carrot, northern wheatgrass, or red fescue **(Must)**.	☐	☐	☐
	5 seeds per vessel for barley, cucumber, durum wheat, lettuce, radish, red clover, red fescue, and tomato; 10 seeds per vessel for alfalfa, blue grama grass, carrot, or northern wheatgrass **(Must)**.	☐	☐	☐
	All other test conditions the same as those for a multi-concentration**(Must)**.	☐	☐	☐
	Use seed taken from the same lot as that being used in definitive tests.	☐	☐	☐
	Perform once every two months, or in conjunction with definitive test(s) with soil samples **(Must)**; use boric acid.	☐	☐	☐
	Prepare and test ≥5 concentrations plus a negative control **(Must)**, using artificial soil..	☐	☐	☐
	Prepare ≥3 replicates per concentration **(Must)**.	☐	☐	☐
	Calculate mean (± SD) % emergence and mean (± SD) length of longest shoots in each treatment at test end (i.e., Day 7 or Day 10) **(Must)**.	☐	☐	☐
	Determine 7-day or 10-day ICp for shoot length and 95% confidence limits **(Must)**; express as mg boric acid/kg dry wt..	☐	☐	☐
	Reference test invalid if <u>any</u> of the following occurs in negative control soil at test end:			
	• mean % emergence is <60% for tomato; <70% for blue grama grass, carrot, lettuce, northern wheatgrass, red clover, or red fescue; <80% for alfalfa, barley, cucumber, or durum wheat; and <90% for radish **(Must)**.	☐	☐	☐
	• mean % survival of emerged seedlings in negative control soil at test end is <90% **(Must)**.	☐	☐	☐
	• mean shoot length is <10 mm for lettuce or red clover; <20 mm for alfalfa, blue grama grass, or tomato; <40 mm for carrot, cucumber, radish or red fescue; < 50 mm for northern wheatgrass; <100 mm for barley; and <120 mm for durum wheat **(Must)**.	☐	☐	☐

Parameter	Specification	Met Specifics Y	N	NA
Test Conditions (continued)				
Warning Chart.	Prepared and updated with all comparable ICps based on shoot length, for each species and reference toxicant (i.e., all comparable ICps plotted successively on a warning chart) **(Must)**.	☐	☐	☐
	Separate warning chart prepared and updated for each dissimilar procedure (e.g., species of test organisms, reference toxicant) **(Must)**.	☐	☐	☐
Test Type.	Static; whole soil **(Must)**.	☐	☐	☐
Test Duration.	14 days for barley, cucumber, durum wheat, lettuce, radish, red clover, and tomato; 21 days for alfalfa, blue grama grass, carrot, northern wheatgrass, and red fescue **(Must)**.	☐	☐	☐
Test T°.	Air temperature, 24 ± 3 °C daily average **(Must)**; alternatively, day: 24 ± 3 °C, night: 15 ± 3 °C.	☐	☐	☐
Light Quality.	Full spectrum fluorescent or equivalent (i.e., mimic natural spectrum).	☐	☐	☐
Light Intensity.	300 ± 100 µmol/(m^2 · s) (equivalent to 18,750 ± 6,250 lux) **(Must)**.	☐	☐	☐
	Light fluence rate should not vary by more than ± 15% of the selected light fluence rate.	☐	☐	☐
Photoperiod.	16 h light: 8 h dark **(Must)**.	☐	☐	☐
Humidity.	Relative humidity of test facility ≥50%.	☐	☐	☐
Test Vessel Size & Type.	Inert to test and reference substances or contaminant mixtures **(Must)**.	☐	☐	☐
	Recommend 1-L clear polypropylene container with clear polypropylene lid.	☐	☐	☐
	Test vessels are covered for the first 7-days of the test or until plants reach the top of the container, whichever comes first; thereafter lids are removed **(Must)**. . .	☐	☐	☐
Soil Mass.	Identical wet weight of test soil equivalent to a volume of ~500 mL;~350 g dry weight if artificial soil	☐	☐	☐
Test Vessel Labelling. .	Clearly labelled/coded: test substance, concentration, and replicate # **(Must)**. . . .	☐	☐	☐
	Date and time of test initiation on labels or data sheets (**Must**).	☐	☐	☐
Test Vessel Position. . .	Test containers are positioned randomly within test facility; vessels are rotated and moved randomly within test facility following hydration	☐	☐	☐
# Replicates/Conc. . . .	For single concentration test: ≥5 replicates/treatment **(Must)**.	☐	☐	☐
	For multi-concentration test with equal replication among treatments: ≥4 replicates/treatment **(Must)**.	☐	☐	☐
	For multi-concentration test with unequal replication among treatments: ≥6 replicates for negative control soul, ≥4 replicates per lowest 4 to 6 concentrations, and ≥3 replicates per highest 5 concentrations.	☐	☐	☐
# Test Conc.	1, plus controls for single-concentration test.	☐	☐	☐
	≥9, plus controls for multi-concentration test **(Must)**; more recommended (≥11, plus controls); geometric series.	☐	☐	☐
# Seeds/Vessel.	5 seeds per test vessel for barley, cucumber, durum wheat, lettuce, northern wheatgrass, radish, red clover, red fescue, and tomato; and 10 seeds per test vessel for alfalfa, blue grama grass, and carrot **(Must)**.	☐	☐	☐
Test Soil Hydration. . . .	Soil moistened with hydration water, as necessary throughout test (e.g., every 48 h while lids are on, and every 24 h once lids are removed).	☐	☐	☐

Parameter	Specification	Met Specifics Y	N	NA
Test Conditions (continued)				
Test Validity Criteria. . .	Test invalid if any of the following occurs in negative control soil at test end:			
	• mean % emergence is <60% for carrot, cucumber, or tomato; <70% for alfalfa, barley, blue grama grass, lettuce, northern wheatgrass, red clover, or red fescue; <80% for durum wheat; or <90% for radish **(Must)**.	☐	☐	☐
	• mean % survival of emerged seedlings in negative control soil at test end is <90% **(Must)**.	☐	☐	☐
	• mean percentage of control seedlings exhibiting phytotoxicity or developmental anomalies is >10% **(Must)**.	☐	☐	☐
	• mean root length is <40 mm for tomato; <70 mm for blue grama grass, red clover, or red fescue; <80 mm for carrot; <100 mm for lettuce; <110 mm for northern wheatgrass or radish; <120 mm for alfalfa or cucumber; or <170 mm for barley; or <200 mm for durum wheat **(Must)**.	☐	☐	☐
	• mean shoot length is <20 mm for lettuce; <30 mm for red clover; <40 mm for alfalfa; <45 mm for carrot; < 50 mm for blue grama grass, radish, or tomato; < 60 mm for cucumber; <80 mm for red fescue; <100 mm for northern wheatgrass; <150 mm for barley; or <160 mm for durum wheat **(Must)**.	☐	☐	☐
	Results from negative control soil must be used to judge the validity and acceptability of the test **(Must)**.	☐	☐	☐
Biological Observations.	Each test vessel processed separately to keep seedlings within each replicate isolated from those in other replicate vessels **(Must)**.	☐	☐	☐
	Plants are carefully separated from the test soil and from the roots of other plants **(Must)**.	☐	☐	☐
	# emerged seedlings (i.e., 3 mm above soil surface) at test end in each test vessel **(Must)**.	☐	☐	☐
	Shoot/root length and shoot/root dry mass at test end **(Must)**.	☐	☐	☐
	Recommendations for shoot and root dry mass at test end include:			
	• mean shoot dry mass per surviving plant is ≥1.0 mg for red fescue; ≥1.5 mg for blue grama grass; ≥2.0 mg for carrot; ≥2.5 mg for lettuce; ≥4.0 mg for red clover; ≥5.0 mg for tomato; ≥7.0 mg for northern wheatgrass; ≥8.0 mg for alfalfa; ≥20 mg for radish; ≥25 mg for durum wheat; ≥35 mg for barley; and ≥40 mg for cucumber.	☐	☐	☐
	• mean root dry mass per surviving plant is ≥0.2 mg for tomato; ≥0.5 mg for blue grama grass, carrot, and red fescue; ≥1.0 mg for lettuce, and red clover; ≥3.0 mg for northern wheatgrass and radish; ≥4.0 mg for alfalfa; ≥7.0 mg for cucumber; and ≥25.0 mg for barley and durum wheat.	☐	☐	☐
	# surviving plants at test end showing atypical appearance (e.g., chlorosis, lesions etc.) **(Must)**.	☐	☐	☐
	Optionally, Day-7 seedling emergence (%) and shoot/root wet mass at test end.	☐	☐	☐
	% emergence of seedlings during test **(Must)**.	☐	☐	☐
Biological Endpoint. . . .	Length of longest shoot and longest root at test end **(Must)**.	☐	☐	☐
	Dry weight of entire shoot and root structures (oven dried at 90 °C until constant mass)**(Must)**.	☐	☐	☐
	Appearance of surviving plants at test end **(Must)**.	☐	☐	☐
	Optional shoot/root wet mass at test end.	☐	☐	☐

Parameter	Specification	Met Specifics Y	N	NA
Test Conditions (continued)				
Statistical Endpoint. . . .	Mean (± SD) % emergence in each treatment at test end (i.e., Day 14 or Day 21) **(Must)**.	☐	☐	☐
	Mean (± SD) length of longest shoots & roots in each treatment at test end **(Must)**.	☐	☐	☐
	Mean (± SD) dry wt of shoots and roots in each treatment at test end **(Must)**. . . .	☐	☐	☐
	For multi-concentration test: 14- or 21-day EC50 for inhibition of % emergence, data permitting; 14- or 21-day ICp for each of mean shoot length, root length, shoot dry wt, and root dry wt based on individual plants surviving in each treatment at test end **(Must)**.	☐	☐	☐
	Optional: 7-day EC50 for inhibition of % emergence; and 14- or 21-day ICp for each of mean shoot wet wt, and root wet wt based on individual plant surviving in each treatment at test end.	☐	☐	☐
Calculation of ICp.	Linear and/or nonlinear regression procedures used for calculation of ICps and 95% confidence limits **(Must)**.	☐	☐	☐
	ICPIN analyses used only if regression analyses fail to provide meaningful ICps.	☐	☐	☐
Test Organisms				
Species.	12 potential test species: alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), blue grama grass (*Bouteloua gracilis*), carrot (*Daucus carota*), cucumber (*Cucumis sativus*), durum wheat (*Triticum durum*), lettuce (*Lactuca sativa*), northern wheatgrass (*Elymus lanceolatus*; formerly names *Agropyron dasystachyum*), radish (*Raphanus sativus*), red clover (*Trifolium pratense*), red fescue (*Festuca rubra*), or tomato (*Lycopersicon esculentum*) **(Must)**.	☐	☐	☐
	Use certified (i.e., certified for purity and % germination) seed **(Must)**.	☐	☐	☐
	Untreated seed is preferred.	☐	☐	☐
Source.	Commercial seed companies or government seed banks.	☐	☐	☐
	Seed information includes: species (Latin and common names), variety, grade, year of collection, packet size (g or kg), lot #, cultivar, rating for % germination, date of germination rating, date of purchase, shelf life, and name of supplier.	☐	☐	☐
	Date seed package opened at laboratory, recorded.	☐	☐	☐
	Plant seeds used in a test must be from the same lot number for each of the individual plant species **(Must)**.	☐	☐	☐
	Seed should generally be purchased at least annually, however, a given lot of seed may be used as long as the seed can meet the control performance criteria, and that the sensitivity of the seed does not change significantly over time as determined by reference toxicity tests.	☐	☐	☐
Seed Sorting and Preparation.	Seed sorted or screened to ensure uniformity in size, colour and "quality" and to separate broken or damaged seeds, empty hulls and other vegetative debris from the seed.	☐	☐	☐
	Seeds that have evidence of fungal contamination on the seed coat or seed that appear to be damaged are discarded **(Must)**.	☐	☐	☐
Seed Storage.	Seed should be stored in their original paper packages, in the dark, in labelled, sealed containers at 4 ± 2 °C.	☐	☐	☐
	Test seed must remain refrigerated until the day of test initiation (Day 0), at which time the seed must be removed from the refrigerator and brought to room temperature **(Must)**.	☐	☐	☐
	Seed must not be stored in the freezer **(Must)**.	☐	☐	☐
Seed Condition.	The sensitivity of each new lot of seed used in a definitive test must be measured using a 7- or 10-day (i.e., depending on the species) reference toxicity test **(Must)**.	☐	☐	☐

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Parameter	Specification	Met Specifics Y	N	NA
Test Report				
Test Substance.	Sample type or coding as provided to laboratory personnel **(Must)**.	☐	☐	☐
	Information on labelling or coding of each sample **(Must)**.	☐	☐	☐
	Date of sample collection **(Must)**.	☐	☐	☐
	Date and time sample(s) received at test facility **(Must)**.	☐	☐	☐
Test Organisms.	Species and source of test seeds **(Must)**.	☐	☐	☐
	Scientific name, seed variety, and lot # **(Must)**.	☐	☐	☐
	Any unusual appearance or treatment of the seeds before the test **(Must)**.	☐	☐	☐
Test Facilities.	Name and address of test laboratory **(Must)**.	☐	☐	☐
	Name of person(s) performing the test (or each component of the test)**(Must)**. . .	☐	☐	☐
Test Method.	Citation of biological test method used (i.e., as per EPS 1/RM/45) **(Must)**.	☐	☐	☐
	Design and description if specialized procedure(s) (e.g., preparation of mixtures of spiked soil; preparation and use of solvent and, if so, solvent control) or modification(s) of the standard test method **(Must)**.	☐	☐	☐
	Brief description of frequency and type of all measurements and all observations made during test **(Must)**.	☐	☐	☐
	Name and citation of program(s) and methods used for testing assumptions of the models and calculating statistical endpoints **(Must)**.	☐	☐	☐
Test Conditions.	Design and description of any deviation(s) from, or exclusion of, any of the procedure and conditions specified in EPS 1/RM/45 **(Must)**.	☐	☐	☐
	Number of discrete samples per treatment **(Must)**.	☐	☐	☐
	Number of replicate test vessels for each treatment **(Must)**.	☐	☐	☐
	Number and description of treatments in each test including the control(s); test concentrations (if applicable) **(Must)**.	☐	☐	☐
	Volume and/or mass of soil in each test vessel **(Must)**.	☐	☐	☐
	Number of seeds per test vessel and treatment **(Must)**.	☐	☐	☐
	Dates when test was started and ended **(Must)**.	☐	☐	☐
	Measurements of light intensity adjacent to the surface of soil in test vessels **(Must)**.	☐	☐	☐
	For each soil sample: any measurements of soil particle size, moisture content, water holding capacity, pH, and conductivity **(Must)**.	☐	☐	☐
	For each composite sample of subsamples taken at the same time from all replicates of each treatment: all measurements of temperature, pH, moisture content, and water holding capacity **(Must)**.	☐	☐	☐
Test Results.	Number of seedlings and observations on seedling condition in each test vessel, as noted during each observation period over the test duration **(Must)**.	☐	☐	☐
	Mean (± SD) percent emergence in each treatment, including control(s), on Day 7 (if determined) and at test end (Day 14 or Day 21, depending on species of test organisms) **(Must)**.	☐	☐	☐
	Mean (± SD) shoot length of individual plants surviving in each treatment (including the control(s)) at test end **(Must)**.	☐	☐	☐
	Mean (± SD) root length of individual plants surviving in each treatment at test end **(Must)**.	☐	☐	☐
	Mean (± SD) shoot dry wt of individual plants surviving in each treatment at test end **(Must)**.	☐	☐	☐
	Mean (± SD) root dry wt of individual plants surviving in each treatment at test end **(Must)**.	☐	☐	☐
	Mean (± SD) shoot and root wet weight of individual plants surviving in each treatment (including the control(s)) at test end, if determined **(Must)**.	☐	☐	☐
	Any EC50 (including the associated 95% confidence limits and, if calculated, the slope) determined **(Must)**.	☐	☐	☐
	Any additional ECx (e.g., EC20) calculated **(Must)**.	☐	☐	☐

This checklist is a summary of the requirements and recommendations in the Environment Canada test method. As a summary, it will not contain all supplementary information. If there is a discrepancy between the checklist and the Environment Canada test method, the test method is taken as the definitive source.

Parameter	Specification	Met Specifics Y	N	NA
Test Report (continued)				
Test Results (continued).	Any ICp (with its 95% confidence limits) determined for the data on growth (i.e., shoot and root lengths and shoot and root wet and dry weights of individual plants surviving at test end) **(Must)**.	☐	☐	☐
	Details regarding any transformation of data, and indication of quantitative statistical method used or procedures applied to the data **(Must)**.	☐	☐	☐
	For a multi-concentration test with chemical-spiked soil, indication as to whether results are based on nominal or measured concentrations of chemical(s) or chemical product(s)**(Must)**.	☐	☐	☐
	All values for measured concentrations **(Must)**.	☐	☐	☐
	Results for any 7- or 10-day (depending on test species ICp (including its 95% confidence limits) performed with the reference toxicant in conjunction with the definitive soil toxicity test, using the same lot of test seeds**(Must)**.	☐	☐	☐
	Geometric mean value (± 2 SD) for the same reference toxicant and test species, as derived at the test facility in previous 7- or 10-day ICp tests using the procedures and conditions for reference toxicity tests described in EPS 1/RM/45 **(Must)**.	☐	☐	☐
	Anything unusual about the test, any problems encountered, and any remedial measures taken **(Must)**.	☐	☐	☐
Original Data Sheets. .	Original data sheets must be signed or initialled, and dated by the laboratory personnel conducting the tests **(Must)**.	☐	☐	☐
Info. Kept on-File	Do lab SOPs indicate that the information on Section 7.2 of the EPS 1/RM/45 method must be kept on file for ≥5 years? **(Must)**.	☐	☐	☐
	For details of this information, see Section 7.2 of EPS 1/RM/45.			

Environment Canada, "Biological Test Method: Test for Measuring Emergence and Growth of Terrestrial Plants Exposed to Contaminants in Soil", Method Development and Applications Section, Environment Canada, Ottawa, ON, Report EPS 1/RM/45 (2005).