This checklist is a summary of the requirements and recommendations in the Environment Canada (now Environment and Climate Change 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.

Y= Yes, meets requirements; N= No, does not meet requirements; NA= not applicable.
temp = temperature; conc = concentration(s); WHC = water holding capacity; MC = moisture content; CEC = cation exchange capacity; d = day; SD = standard deviation; # = number (of); wt = weight

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Sample Collection and Handling: Field-Collected Soil							
Sample Collection	Soil collection procedures follow the guidance provided in EPS 1/RM/53 (EC, 2012)						
	Specific procedures for the collection, handling and preparation of soils contaminated with volatile or unstable compounds described in EPS 1/RM/53 are followed						
	Physicochemical properties of reference soil(s) are similar to test soil(s)						
	Samples are collected in separate horizons, where possible; soils without distinct horizons are collected according to depth **(must)**						
	Procedure used for sample collection are appropriate for study objectives and nature of the soil being collected, and the same for all field sites sampled						
	Soil profile is classified when sampling by horizon **(must)**; soils are classified to the subgroup level following CSSC and guidance in EPS 1/RM/53						
	Each horizon is placed and stored in separate containers **(must)**						
	Guidance for compositing subsamples provided in EPS 1/RM/53 is followed						
Containers	Made of non-toxic, inert material for transport and storage **(must)**						
	Clean and sealable **(must)**; plastic is not used if there is a possibility of leaching						
Volumes	Calculated before sampling and is enough to prepare replicates and characterize soil(s)						
Labelling	Sample containers are sealed and labelled or coded immediately after filling **(must)**; air space is minimized						
	Labelling and accompanying records include a code or description that identifies sample type (e.g., point, bulk, composite), sample date and time, sample site, precise location of sampling, sampling conditions, sample identification number (including replicate #, where applicable, and sample volume **(must)**; label also includes name and signature of sampler(s)						
Transport	Samples are shipped with appropriate documentation including chain of custody forms and any regulatory documentation for transport of contaminated material **(must)**						
	Samples are kept cool (e.g., 7 ± 3°C) during transport						

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Transport cont.	Samples do not freeze or partially freeze during transport or storage (unless they are frozen when collected)						
	Samples are not allowed to dehydrate during transport or storage (unless samples are saturated with excess water upon arrival at the lab) **(must)**						
	Samples are kept in the dark (i.e., light-tight or opaque containers)						
Holding Time	Test is initiated within 6 weeks after sampling **(must)** unless soil contaminants are known to be stable; recommend testing within 2 weeks, and preferably 1 week after sampling						
	Date of receipt of the sample(s) at lab is recorded **(must)**						
Holding Conditions	Sample temp and MC is measured and recorded upon receipt at lab **(must)**; other initial observations include colour, texture, presence of water, indigenous invertebrates, fungi or plants, and strong odour						
	Samples stored for future use are held in airtight containers **(must)**; if volatile contaminants are suspected, headspace is purged with nitrogen gas before storing						
	Samples are stored in the dark at 4 ± 2 °C; these storage conditions must be applied if PAHs or other light-sensitive contaminants are present or if the samples are known to contain unstable volatiles **(must)**						
Sample Handling	Have special consideration for collection, handling and preparation of soil from Canada's Ecozones provided in EPS 1/RM/53 been cited in lab's SOP?						
Sample Preparation : Field-Collected Soil							
Soil Manipulation	Samples of field-collected soil are not adjusted or manipulated (e.g., washing, aging/weathering, pH adjustment, conditioning, etc) **(must)**						
	Research-oriented investigations where soils are manipulated or adjusted include adjusted and non-adjusted treatments in side-by-side tests; documentation of soil manipulation procedures are reported **(must)**						
Sieving	Sample is not sieved with water **(must)**; coarse debris and indigenous macro-organisms are removed using forceps, gloved hand, or coarse sieve (e.g., 4 to 10 mm)						
Homogenization	Soil and/or solid particulate waste for testing is homogenized, unless inappropriate (e.g., affects concentration or bioavailability of contaminants) before use						
	Any moisture that separates from a sample during its transport and/or storage is remixed into it if possible **(must)**						
	Care is taken to ensure that methods (and duration) for mixing soil minimizes impact on soil structure						
	For each soil horizon, mixing conditions (i.e., duration, and temp) are as similar as possible **(must)**						

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Subsample Handling	Immediately following sample hydration (or dehydration) and mixing, subsamples for toxicity testing and for physicochemical analyses are removed and placed into labelled test vessels and containers for subsequent physicochemical analysis **(must)**						
	Subsamples to be stored are held in the dark at 4 ± 2 °C until used or analysed **(must)**; in sealed containers with minimal airspace						
	If stored, each subsample is thoroughly remixed to ensure homogeneity, just before it is analysed or used in a toxicity test **(must)**						
Characterization	Each soil horizon (including those for negative control and reference soils) is analysed for particle size distribution (% sand, silt, and clay), total organic carbon content (%), organic matter content (%), pH, conductivity, MC (%), WHC (%), nitrogen (as total, nitrate, nitrite, and ammonium), total or plant-available phosphorous and potassium, C:N ratio, and CEC, as a minimum **(must)**						
	Other analyses listed in Section 5.2 of EPS 1/RM/56 are carried out						
	Optional analyses of contaminants of concern are conducted (e.g., metals, polycyclic aromatic hydrocarbons (PAHs), pesticides)						
Moisture Content	Water Holding Capacity (WHC) of horizons/soils (artificial and site) are known **(must)**						
	Optimal moisture contents of test soils (artificial and site) are determined and expressed as % WHC **(must)**						
	MC, WHC, and optimal % WHC of each soil horizon are determined separately **(must)**						
	WHC is determined gravimetrically by drying subsample for ~24h at 105°C, saturating the subsample with water, and using wet weight and dry weight of soil following formula in Section 5.3						
	Test soil is hydrated or dried out to optimal % WHC during preparation of test concentrations						
Test Concentrations	Test soil is prepared on the day of the start of the test (Day 0); each horizon mixed as a batch is prepared in sufficient quantity for all replicates and physicochemical analyses						
	Each homogenized test soil horizon is mixed with the same horizon of negative control soil or reference soil, if possible, to prepare each treatment/concentration in a geometric series for multi-conc tests; homogeneity is ensured (i.e., mixed until texture, colour, and moisture are homogeneous) and is divided into replicates						
	Test includes a treatment comprised solely of negative control soil **(must)**						
Soil Horizons	Samples of field-collected soils, collected in separate horizons are prepared separately and reassembled in test units in proportions correlated to the depths of each horizon, as collected in the field **(must)**; alternatively, only the relevant soil horizon is tested if contaminants of concern have been confirmed in that horizon only						

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Soil Horizons cont.	Care is taken during re-layering to ensure that soil horizons do not mix **(must)**						
	Soils collected as consolidates samples (i.e., cores) remain intact for the duration of the test **(must)**						
Sample Preparation: Chemical-Spiked Test Soil							
Containers	Chemical containers are sealed and coded or labelled upon receipt **(must)**						
Labelling	Required information (chemical name, supplier, date received, etc) is indicated on the label and/or recorded on separate data sheet						
Chemical Characterization	Information on chemical or chemical product(s) is obtained before test starts, and includes: concentration of major or active ingredients and impurities, water solubility, vapour pressure, stability, dissociation constants, adsorption coefficients, toxicity to terrestrial organisms and humans, and biodegradability						
	Chemical analysis for soils spiked with test substances/materials are normally carried out at beginning and end of test, in high, medium, and low strengths as a minimum; analyses are carried out on each separate soil horizon						
Storage	Storage conditions are appropriate for the nature of the chemical						
Preparation of Mixtures	Procedure depends on the 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); homogeneity is ensured						
	Natural control soil is used as the negative control soil to be spiked with chemical and for the corresponding control soil, and includes spiking of separate soil horizons followed by re-layering of the horizons in test vessels prior to testing						
	All horizons are spiked to the same test concentration **(must)**						
	For each soil horizon and treatment, mixing conditions (solution:soil ratio, mixing and holding time, and temp) is standardized **(must)**						
	Each batch (i.e., horizon and 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 is included in the test (in addition to negative control) if an organic solvent is used for test substance(s) that are not soluble in water **(must)**						
	The solvent control is from the 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 SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Conditions							
Facility and Apparatus	Environmental chamber or equivalent with acceptable temp and lighting control **(must)**						
	Facility is well ventilated and free of fumes; isolated from physical disturbances, any contaminants that might affect test organisms, and areas for sample preparation/storage						
	Equipment, apparatus and construction materials are made of non-toxic material (e.g., borosilicate glass, nylon, Teflon™, high-density polyethylene, high density polystyrene, polypropylene, polycarbonate, porcelain, fluorocarbon plastics, Nalgene™, type 316 stainless steel, fibreglass) **(must)**; and minimize sorption and leaching						
	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 is equipped for analysis of soil moisture content						
	Other equipment includes: drying oven (capable of 90°C and 105°C), a weighing balance (accurate to 0.1 mg), and a light meter **(must)**						
	Safety apparatus is used when preparing mixtures and test soils **(must)**						
Equipment Cleaning	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)**						
	Cleaning procedure is followed: 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-grade 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						
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, with test species intended for routine use						
	Conditions and procedures for initial control performance are identical to those described for the definitive test						
	Conditions and procedures for initial reference toxicity tests are identical to those described for routine reference toxicant tests						
	Data from initial control performance test show that criteria for test validity can be met using the natural or artificial soil intended for use **(must)**						
	Data from initial reference toxicity tests are compared by calculating and appraising the magnitude of the coefficient of variation (CV) of the derived ICps						

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
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 a reference toxicant						
	Negative control soil is included as a treatment in every toxicity test **(must)**						
Negative Control Soil: Natural Soil	Natural soil is 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 is analysed for: particle size distribution (% sand, silt, and clay); total organic carbon content (%); organic matter content (%); pH; conductivity; MC (%); WHC (%); nitrogen as total N, nitrate, nitrite, and ammonia; total or plant-available phosphorous and potassium; C:N ratio; and CEC, as a minimum **(must)**						
	Soil is analysed for recommended cations and anions, contaminants (see Section 3.4.1 in EPS 1/RM/56)						
	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 - 10 mm), transferred to clean plastic pails, and stored in darkness at 4 ± 2°C						
Negative Control Soil: 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						
	Reagent-grade calcium carbonate is added to dry mixture to adjust pH to 6 - 7.5						
	Hydrate using test water to ~28% of WHC and adjust pH as necessary with more calcium carbonate						
	Artificial soil is stored in the dark at 20 ± 2°C for ≥3 days before use in toxicity test; thereafter soils are stored at 4 ± 2°C						
Positive Control Soil	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 are included for tests with field-collected soil, ideally taken from site(s) presumed to be clean but near sites of test soil collection; or artificial soil						
	Characteristics, including texture, percent organic matter, percent organic carbon content, pH, conductivity and fertility are similar to test soils						
	Soils are collected as separate soil horizons where possible, and reassembled in test units **(must)**						

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Reference Soil cont.	Tests involving samples of reference soil must also include a sample of negative control soil **(must)**						
Initial Hydration of Test Soils	Each soil horizon from field-collected soils is hydrated separately 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						
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)**						
	Normally used to hydrate soils, although site water may also be used						
Test Type	Static; whole soil **(must)**						
Duration	28 days for trembling aspen or bluejoint reedgrass; 35 days for Canada goldenrod, paper birch, or jack pine; 42 days for white spruce or black spruce **(must)**						
Temperature	Air temp: 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) measured adjacent to soil surface **(must)**						
	Light fluence rate does not vary by more than ± 15% of the selected light fluence rate measured at several points within the test area						
Photoperiod	16 h light: 8 h dark **(must)**						
Humidity	Relative humidity of test facility ≥50%						
Vessel Size, Type, and Labelling	Inert to test and reference substances or contaminant mixtures **(must)**						
	Volume is large enough to accommodate seedling growth for test duration, with sealable lids that do not interfere with light quality; recommend 1-L clear polypropylene container with clear polypropylene lid (with holes to reduce condensation); alternatively 1-L glass jars with transparent lids						
	Plants are covered for the full test duration; i.e., when plants reach top of test vessel, lids are replaced with an inverted test unit taped in place, allowing headspace for further plant growth						
	Vessels are clearly labelled/coded with test substance, concentration, and replicate #, if applicable **(must)**						
	Date and time of test initiation is on labels or data sheets (**must)**						
Randomization	Treatments are positioned randomly within test facility; vessels are rotated and moved randomly within test facility following hydration						

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Soil Mass	Identical wet wt of test soil in each replicate **(must)**; volume of each horizon layered in the test vessel correlates proportionally to the depths of each horizon, as collected in the field; equivalent to a total volume of ~500 mL						
# Seeds/ Vessel	5 seeds per test vessel for trembling aspen, bluejoint reedgrass, paperbirch, and Canada goldenrod; and 10 seeds per test vessel for jackpine, white spruce and black spruce **(must)**; seeds are distributed equally around one seed in the centre; planted to a depth twice the diameter of the seed for jack pine, white spruce and black spruce, and pressed onto soil surface for trembling aspen, bluejoint reedgrass, Canada goldenrod, and paper birch						
# Test conc.	1, plus controls for single-conc test						
	≥9, plus controls for multi-conc test **(must)**; more recommended (≥11, plus controls); geometric series						
# Replicates/Conc	For single-conc test: ≥5 replicates/treatment **(must)**; for site soils, replicate vessels represent replicate samples (i.e., field replicates) collected individually from a given sample location						
	For multi-conc test with equal replication among treatments: ≥4 replicates/treatment **(must)**						
	For multi-conc test with unequal replication among treatments: ≥6 replicates for negative control soil, ≥4 replicates per lowest 4 to 6 concentrations, and ≥3 replicates per highest 5 concentrations						
Test Soil Hydration	Soil is moistened with hydration water (at 24 ± 3°C) to near saturation (i.e., water added to soil surface until ~0.5 cm of water is temporarily visible pooling at bottom of test vessel), as necessary throughout test						
Biological Endpoints	# emerged seedlings in each test vessel at the end of the test **(must)**						
	Length of longest shoot and longest root measured for each surviving plant at test end, and the mean calculated for each replicate **(must)**						
	Dry wt of entire shoot and root structures (oven dried at 90 °C until constant weight) divided by surviving plants to give mean weight of individual shoots (or roots) calculated for each replicate **(must)**						
	Optional total shoot and total root wet wt at test end						
Statistical Endpoints	Mean (± SD) % emergence in each treatment at test end (i.e., Day 28, 35, or 42) **(must)**						
	Mean (± SD) length of longest shoots and roots in each treatment at test end **(must)**						
	Mean (± SD) dry wt of shoots and roots in each treatment at test end **(must)**						

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Statistical Endpoints cont.	For multi-conc test: 28-, 35-, or 42-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)**						
Calculation of ICp	Linear and/or nonlinear regression procedures are used for calculation of ICps and 95% confidence limits **(must)**						
	Initial plot of the raw data is done and general process for statistical analysis and selection of appropriate model is followed						
	For regression analyses, data meets assumption of normality and homogeneity **(must)**						
	Data are assessed for the presence of outliers **(must)**						
	If outliers are present, data are reviewed, analyses are compared with and without the outlier, and a decision is made on removal or inclusion of outlier **(must)**						
	If outliers are absent or not removed from the analysis, the model that demonstrates the smallest residual mean square error is selected as the model of best choice **(must)**						
	Endpoints generated by regression are bracketed by test concentrations (i.e., extrapolation is not acceptable) **(must)**						
	ICPIN analyses is used only if regression analyses fail to provide meaningful ICps; guidance for linear interpolation provided in EPS 1/RM/56 is followed						
Observations and Measurements							
Moisture Content	Soil moisture content is measured in each soil horizon for each treatment/concentration at test start **(must)**						
	Moisture content is determined gravimetrically (see EPS 1/RM/56)						
	Moisture content is calculated on a dry wt. basis **(must)**						
pH	Soil pH is measured in each soil horizon for each treatment/concentration at test start and end **(must)**						
	Soil pH is measured using a modified $CaCl_2$ Slurry Method (see EPS 1/RM/56)						
Temperature	Air temp is measured in test facility, daily or continuously **(must)**						
Humidity	Humidity is measured in test facility, periodically						
Light Intensity	Light fluence rate is measured at least once during test **(must)**						
Biological Observations	Each test vessel is 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, measured from stem at soil surface to tip of longest leaf) is determined at test end in all test vessels **(must)**; also # and condition of emerged plants throughout the test in all vessels						

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
	# surviving plants at test end and any atypical appearance (e.g., chlorosis, lesions etc.)						
Biological Observations cont.	Determined at test end: Longest shoot and longest root length **(must)**; total shoot and total root dry wt **(must)**; total shoot and total root wet wt						
	Length of longest shoot and root for each plant is measured from transition point between hypocotyl and root for trembling aspen, bluejoint reedgrass, Canada goldenrod, and paper birch and from a discernible transition point for the conifers						
	Entire rinsed shoot (and root) biomass from each vessel is blotted and transferred to weighing pan(s)**(must)**; shoot and root structures from each replicate are weighed separately as two groups (i.e., shoots and roots); remaining seed or seed coat is discarded; following drying, mean shoot and root dry wt per surviving plant is calculated for each replicate						
	First weighing pan is returned to desiccator and reweighed at the end of all weighings to ensure that change in weight is not >5%; if it is, all pans are re-dried and re-weighed						
	Recommendations for shoot dry wt per surviving plant at test end in negative control soil are: ≥2.1 mg for trembling aspen; ≥1.5 mg for bluejoint reedgrass; ≥1.0 mg for Canada goldenrod; ≥8.9 mg for paper birch; ≥6.6 mg for jack pine; ≥3.0 mg for white spruce; or ≥2.3 mg for black spruce						
	Recommendations for root dry wt per surviving plant at test end in negative control soil are: ≥0.4 mg for trembling aspen; ≥0.4 mg for bluejoint reedgrass; ≥1.1 mg for Canada goldenrod; ≥1.3 mg for paper birch; ≥2.0 mg for jack pine; ≥0.6 mg for white spruce; or ≥0.3 mg for black spruce						
Solvent	Both solvent and negative control soil meet test validity criteria in order for the test to be considered valid **(must)**						
	If both solvent and negative control soil meet the test validity criteria, the results for the two controls are compared using Student's t-test **(must)**						
	If the results for the two controls are not statistically different from each other, then only the data from the negative control soil are used to calculate the test results						
	If results for two controls are statistically different from each other, further evaluation needed						
Test Organism							
Species	7 potential test species: trembling aspen (*Populus tremuloides*), Canada goldenrod (*Solidago canadensis*), paper birch (*Betula papyrifera*), bluejoint reedgrass (*Calamagrostis canadensis*), white spruce (*Picea glauca*), black spruce (*Picea mariana*), or jack pine *(Pinus banksiana*) **(must)**						

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Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Species cont.	Wild-collected seed is used **(must)**: bluejoint reedgrass seed may be purchased from a suppliers cultivating plants for reclamation purposes						
	Seed is not pretreated (i.e., free of fungicides, insecticides, repellents, etc.)						
Source	Seed is from commercial seed companies or government seed banks						
	Seed information includes: species (Latin and common names), year of collection, geographic location where seed was collected, packet size (g or kg), lot #, rating for % germination, date of germination rating, date of purchase, shelf life, and name of supplier						
	Date seed package opened at the laboratory is recorded						
	Plant seeds used in a test are from the same lot number for each of the individual plant species **(must)**						
	Seed is generally 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 the sensitivity of the seed does not change significantly over time as determined by reference toxicity tests						
Seed Stratification and Selection	Seed of all species except trembling aspen is stratified prior to testing **(must)**; e.g., refrigerate an aliquot of dry seed in moist peat for the species-specific duration						
	Paper birch seed is sorted using an ethanol floatation technique prior to stratification						
	Germination of stratified seed (or non-stratified seed for trembling aspen) is assessed periodically to ensure supply for testing						
	Stratified seed that has evidence of fungal contamination or has ≥10% of seed germinated is discarded **(must)**						
	Seed is sorted under low magnification using a stereomicroscope for selection of seeds that are firm, regular in size, unblemished, undamaged, and showing no signs of germination and to separate seeds from vegetative debris and empty hulls						
Seed Storage	Prior to stratification, seeds are stored in their original paper packages, in the dark, in labelled, sealed containers						
	Aspen seed is frozen immediately after collection and cleaning and stored at -15 to -30°C **(must)**						
	Dry seed of all other species is stored at 4 ± 2°C or at -15 to -30°C until stratification						
	On the day of test initiation (Day 0), the seed is removed from the refrigerator or freezer and brought to room temp (10 to 15 min)**(must)**						
Seed Condition	The sensitivity of each new lot of seed used in a definitive test is measured using a 14-, 21-, 28-, or 35-d (i.e., depending on the species) reference toxicity test **(must)**						

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Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
QA/QC							
Validity Criteria	Results from negative control soil must be used to judge the validity and acceptability of the test **(must)**						
	Invalid test if mean % emergence is <60 % for any species **(must)**						
	Invalid test if mean root length is: <35 mm for trembling aspen; <17 mm for bluejoint reedgrass; <80 mm for Canada goldenrod; <53 mm for paper birch; <62 mm for jack pine; <36 mm for white spruce; or <24 mm for black spruce **(must)**						
	Invalid test if mean shoot length is: <10 mm for trembling aspen; <35 mm for bluejoint reedgrass; <7 mm for Canada goldenrod; <26 mm for paper birch; <44 mm for jack pine; <26 mm for white spruce; or <20 mm for black spruce **(must)**						
Reference Toxicity Test	Static 14-, 21-, 28- or 35-d (i.e., species-specific) multi-conc test **(must)**						
	Test duration is 14 days for bluejoint reedgrass or jackpine; 21 days for Canada goldenrod or trembling aspen; 28 days for paper birch; and 35 days for white spruce or black spruce **(must)**						
	5 seeds per vessel for trembling aspen, paper birch, Canada goldenrod or bluejoint reedgrass; 10 seeds per vessel for black spruce, white spruce, or jack pine **(must)**						
	All other test conditions are the same as those for a multi-conc **(must)**						
	Seed is taken from the same lot as that being used in definitive tests **(must)**						
	Test is performed 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 in control soil and mean (± SD) length of longest roots in each treatment at test end (i.e., Day 14, 21, 28 or 35) **(must)**						
	Determine 14-, 21-, 28-, or 35-d ICp for root length and 95% confidence limits **(must)**; express as mg reference chemical/kg dry wt.						
	Invalid test if mean % emergence in the negative control soil is <60% for any species **(must)**						
	Invalid test if mean root length in the negative control soil is: <30 mm for trembling aspen; <51 mm for bluejoint reedgrass; <47 mm for Canada goldenrod; <48 mm for paper birch; <38 mm for jack pine; <39 mm for white spruce; or <26 mm for black spruce **(must)**						
	Concentration of reference toxicant in stock solutions are measured chemically using appropriate methods; aliquots of soils concentrations from at least negative control soil, and low, medium, and high concentrations are collected for analysis						

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Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Warning Chart	Prepared and updated with all comparable ICps based on root length, for each species and reference toxicant (i.e., all comparable ICps plotted successively on a warning chart) **(must)**						
	Log conc used in all calculations of mean and SD and in all plotting procedures						
	Each new ICp for the reference toxicant is compared with previously established chart limits						
	ICp is acceptable if within warning limits (± 2 SD on log scale)						
	Laboratory observes variation (i.e., reasonable spread of warning limits) for warning chart; and appropriate actions are taken if an ICp falls outside the warning limits						
Test Report (all items here are required, i.e. must be reported)							
Test Substance or Material	Sample type or coding as provided to laboratory personnel						
	Information on labelling or coding of each sample						
	Information on sample horizons as they were collected for test, reference, and negative control soils, if applicable						
	Date of sample collection						
	Date and time sample(s) received at test facility						
Test Organisms	Species and source of test seeds						
	Scientific name and lot #						
	Duration and method of seed stratification and ethanol separation, if used						
	Any unusual appearance or treatment of the seeds before their use in the test						
Test Facilities	Name and address of test laboratory						
	Name of person(s) performing the test (or each component of the test)						
Test Method	Citation of biological test method used (i.e., as per EPS 1/RM/56)						
	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						
	Brief description of soil layering in test vessels (e.g., wet wts and/or depths of each soil), if applicable						
	Brief description of frequency and type of all measurements and all observations made during test						
	Name and citation of program(s) and methods used for testing calculating statistical endpoints						
Test Conditions	Design and description of any deviation(s) from, or exclusion of, any of the procedures and conditions specified in EPS 1/RM/56						
	# discrete samples per treatment						
	# replicate test vessels for each treatment						

TEST SPECIFIC CHECKLIST

Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Conditions cont.	Number and description of treatments in each test including the control(s); test concentrations (if applicable)						
	Volume and/or mass of soil in each test vessel						
	# seeds per test vessel and treatment						
	Dates when test was started and ended						
	Measurements of light intensity adjacent to the surface of soil in test vessels						
	For each soil sample: any measurements of soil particle size, MC, WHC, pH, and conductivity						
	For each composite sample of subsamples taken at the same time from all replicates of each treatment: all measurements of temp, pH, MC, and WHC						
Test Results	Percent emergence of plants in each test vessel at test end (Day 28, 35, or 42, depending on species of test organism)						
	Mean (± SD) percent emergence in control(s) at test end, related to test validity criteria						
	Mean (± SD) shoot length of individual plants surviving in each treatment (including the control(s) and reference soil(s)) at test end						
	Mean (± SD) root length of individual plants surviving in each treatment at test end						
	Mean (± SD) shoot dry wt of individual plants surviving in each treatment at test end						
	Mean (± SD) root dry wt of individual plants surviving in each treatment at test end						
	Mean (± SD) shoot and root wet wt of individual plants surviving in each treatment (including the control(s) and reference soil(s)) at test end, if determined						
	Any ICp (with its 95% confidence limits) determined for the data on growth (i.e., shoot/ root lengths and shoot/root wet and dry wts of individual plants surviving at test end)						
	Details regarding any transformation of data, and indication of quantitative statistical method used or procedures applied to the data						
	For a multi-conc test with chemical-spiked soil, indication as to whether results are based on nominal or measured concentrations of chemical(s) or chemical product(s)						
	All values for measured concentrations						
	Results for any 14-, 21-, 28-, or 35-d (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						
	Geometric mean value (± 2 SD) for the same reference toxicant and test species, as derived at the test facility in previous 14-, 21-, 28-, or 35-d ICp tests using the procedures and conditions for reference toxicity tests described in EPS 1/RM/56						
	Anything unusual about the test, any problems encountered, any remedial measures taken						

TEST SPECIFIC CHECKLIST Test for Growth in Contaminated Soil Using Terrestrial Plants Native to the Boreal Region							
Parameter	**Specification**	**Document Review**			**Implementation**		
		Y	**N**	**NA**	**Y**	**N**	**NA**
Information Kept On-File	Do lab SOPs indicate that the additional reporting requirements in Section 7.2 of the EPS 1/RM/56 method must be kept on file for 5 years? For details of this information, see EPS 1/RM/56, section 7.2.						

EC (Environment Canada). 2012. Guidance Document on the Sampling and Preparation of Contaminated Soil for Use in Biological Testing. Environmental Protection Service, Ottawa, ON. Report EPS 1/RM/53, 222 pp.

Notes: