This checklist is a summary of the requirements and recommendations in the 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. Green shaded text reflect changes in the 2nd edition (published in 2014).

Y= Yes, meets requirements; N= No, does not meet requirements; NA= not applicable.

DO = dissolved oxygen; temp = temperature; conc = concentration(s); SD = standard deviation;
PAH = polycyclic aromatic hydrocarbons; WHC = water holding capacity

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Springtails exposed to contaminants in soil – Second edition

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Sample Collection and Handling							
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						
	Reference soil from sites with similar geochemical properties (especially: particle size distribution, total organic carbon content (%), organic matter content (%), pH, and conductivity but also: CEC, total inorganic carbon, redox potential, and water-holding capacity) to the test soil collected during each field collection						
	Collected soils classified to the subgroup level according to the Canadian System of Soil Classification						
	Soils from boreal or taiga ecozones collected as separate soil horizons where possible **(Must)**						
	Soils exhibiting distinct horizons collected sequentially as separate horizons if collected via pit excavation **(Must)**; soils without distinct horizons are collected by depth						
	If collecting by horizon, soil profiled first as described in EPS 1/RM/53 **(Must)**						
	If collecting by horizon, care taken not to dilute the potential soil contamination						
	If collecting by horizon, each horizon stored in separate containers **(Must)**						
	Required volume of soil per sample calculated before commencing a sampling program						
	Guidance provided in EPS 1/RM/53 regarding compositing subsamples is followed						
Containers	Non-toxic, inert, material for transport and storage **(Must)**						
	Clean and sealable **(Must)**; plastic not used if there is a possibility of leaching						

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Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Labeling	Sample containers sealed and labelled or coded immediately after filling for field-collected soils and/or upon receipt in the lab for chemicals **(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, sample condition, sample identification number (including replicate number, where applicable), and sample volume **(Must)**; name and signature of sampler(s)						
Transport	Samples do not freeze or partially freeze (unless they are frozen when collected)						
	Samples are not be allowed to dehydrate (unless they are saturated with excess water upon arrival at the lab) during transport or storage **(Must)**						
	Samples are kept in the dark (i.e., light-tight or opaque containers)						
	Samples remain cool (e.g., 7 ± 3°C) during transit						
	Date sample(s) received at the laboratory recorded **(Must)**						
	Sample temperature and moisture content upon receipt at laboratory are measured and recorded **(must)**						
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						
Holding Conditions	Samples stored for future use must be held in airtight containers **(Must)**						
	Samples stored in the dark at 4 ± 2°C if they contain PAHs, unstable volatiles, or other light-sensitive toxicants **(Must)**						
	Sample brought to room temperature and thoroughly re-mixed just before analysis or testing **(Must)**						
Sample Preparation: Field-collected Soil							
Sieving	Sample sieved (e.g. 4 - 10 mm mesh) to remove oversize material, if necessary (e.g., debris and indigenous macro-organisms). Water not used during sieving **(Must)**. Grinding should be avoided if possible. Double freeze/thaw cycle for horizons with high organic content						
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)**						

Temp & pH Adjustment and Soil Equilibrium	Test soil prepared on day preceding test (Day -1) and held under test conditions (i.e., 20 ± 2°C) overnight, prior to testing						
	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)**						
Characterization	Each soil/horizon (including negative control and reference soil) is analysed for: moisture content (%), WHC, pH, conductivity, TOC (%), OM (%), particle sizes (% sand, % silt, % clay) **(Must)**, nitrogen, phosphorus, potassium, C:N ratio, and CEC as a minimum						
	Optional analyses of major cations and anions, and contaminants of concern (e.g., metals, PAHs, pesticides)						
Moisture Content	WHC of soils (artificial and site) are known and determined using a recognized standard procedure for each horizon **(Must)**						
	Optimal moisture content of test soils (artificial and site) determined and expressed as % WHC **(Must)**						
	High peat content soils: optimal moisture content can be estimated by eye (appropriate consistency) instead of as %WHC						
	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 hydrated to optimal % of WHC after preparing test conc.						
Test Concentrations	Each batch (i.e., treatment) is prepared in sufficient quantity for all replicates and physicochemical analyses						
	Mix homogenized test soil with negative control soil or reference soil, if multiconcentration test to prepare each treatment/ concentration using geometric series; ensure homogeneity						
Sample Preparation: Chemical-Spiked Soil							
Chemical Characterization	Information on chemical or chemical product(s) obtained before test starts includes: stability, water solubility, vapour pressure, purity, dissociation constants, adsorption co-efficients, estimated toxicity to test species and humans and biodegradability						
	Chemicals are reagent-grade						
	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 temp) must be standardized **(Must**)						
	Each batch (i.e., treatment) is prepared in sufficient quantity for all replicates and physicochemical analyses						
	Soils are hydrated, homogenized and placed in replicate test vessels on the day prior to testing (Day -1); test vessels covered and held overnight under test conditions						
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, contains the same concentration of solubilizing agent that is present in the highest concentration of test chemical, and is prepared using the same procedure **(Must)**						
	For any test that includes solvent control soil, test results for that soil are compared statistically with those for negative control soil **(Must)**						
Test Conditions							
Test Facility	Isolated areas with temperature & lighting control (e.g., environ. chambers, or equivalent) **(Must)**						
	Well ventilated & free of fumes; isolated from areas for organism culturing, and for sample preparation/storage						
	Equipment, apparatus and construction materials made of non-toxic material and minimize sorption of chemicals (e.g., borosilicate glass, nylon, high-density polyethylene, high density polystyrene, polycarbonate, fluorocarbon plastics, Teflon™, Nalgene™, porcelain, fibreglass, type 316 stainless steel) **(Must)**						
	Copper, zinc, brass, galvanized metal, lead, and natural rubber must not be used **(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 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)**						
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)**						
	Soak; detergent wash; 2 tap water rinses; acid wash (e.g., 10% nitric or hydrochloric acid, metal-free grade); 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; 3 rinses with test water						

Initial Tests	≥5 control performance tests and ≥5 reference toxicity tests using artificial or natural negative control soil intended for routine use are performed to confirm acceptable performance						
	Conditions and procedures for initial control performance test follow those described for conducting definitive tests						
	Conditions and procedures for initial reference toxicity tests be identical to those described for routine reference toxicant tests						
	Each test is performed using a different group of test organisms of the same species, from the same source						
	Data from initial control performance test shows that criteria for test validity can be met **(Must)**						
	Data from initial reference toxicity tests is evaluated using the magnitude of the coefficient of variation (CV) of the derived LC50s						
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; artificial soil for tests with chemicals or chemical products spiked in soil; uncontaminated natural soil is used l for definitive tests with field-collected boreal forest soils						
	Negative control soil included as a treatment in every toxicity test **(Must)**						
Negative Control Soil: Natural Soil	Natural soil collected from a clean (uncontaminated) site; free of pesticide or fertilizer for ≥5 years						
	Natural soil from a given source meets 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 (%);and CEC **(Must)**						
	Soil is analysed for recommended cations and anions, forms of nitrogen, phosphorus, potassium, C:N ratio, and contaminants (see Section 3.4.1 in EPS 1/RM/47)						
	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 < 40µm); 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 to 6.0 – 7.5 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	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						
	Physicochemical characteristics including organic carbon, organic matter, particle size distribution, texture, pH and conductivity are similar to test soils						
	Soils collected as separate horizons tested individually (i.e. each horizon treated as a separate soil sample) **(Must)**						
	Tests using reference soil 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						
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)**						
Measurements During Test							
Moisture Content	Soil moisture content in each treatment/concentration at test start and end **(Must)**						
	Moisture content determined gravimetrically (see EPS 1/RM/47 2nd ed.)						
	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 CaCl2 Slurry Method (see EPS 1/RM/47 2nd ed.)						
Temperature	Air temperature in test facility, daily or continuously **(Must)**						
Conductivity	Conductivity measured at test start and end when test soil is suspected of having a high salt content						
Chemical Analyses	Normally measure at beginning and end of test, in high, medium, and low strengths as a minimum						
Reference Toxicity Tests	Static, 7-day (for *O. folsomi*, and *F. fimetaria)* or 14-day (for *F. candida*, and *P. minuta*) multi-concentration acute lethality test **(Must)**						
	Conditions the same as those for a multi-concentration definitive test except for test duration, # of test concentrations, # of organisms per vessel for *O. folsomi*, and *F. fimetaria*, and test validity criteria **(Must)**						
	Prepare and test ≥5 concentrations plus a negative control **(Must)**, using artificial soil						
	Use age-synchronized springtails derived from the same population (i.e., culture) of springtails used to produce age-synchronized organisms for the definitive tests **(Must)**						
	Perform once every 2 months, or in conjunction with definitive test(s) with soil samples **(Must)**; use boric acid						
	Calculate mean (± SD) % survival in each treatment at test end (Day 7 or Day 14) **(Must)**						

Reference Toxicity Tests (cont)	Reference toxicity test invalid if mean survival of adults in negative control soil is <80% at test end for *F. candida*, *F. fimetaria* and *O. folsomi* and <70% at test end for *P. minuta* **(Must)**						
	Determine 7- or 14-day LC50 and 95% confidence limits **(Must)**; express as mg boric acid/kg dry wt.						
	Tests with lethality and reproduction endpoints (i.e., 21-day or 28-day, depending on species) with boric acid be performed according to Section 4 of EPS 1/RM/47 2nd ed., at least twice a year or in conjunction with definitive test						
Warning Chart	Prepared and updated with all comparable LC50s for each species and reference toxicant (i.e., all comparable LC50s 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, test duration) **(Must)**						
Test Type	Static; whole soil **(Must)**						
Test Duration	21 days for *F. fimetaria* and *P. minuta*; 28 days for *O. folsomi* and *F. candida* **(Must)**						
Test Temp	Air temperature: 20 ± 2°C daily average **(Must)**; 20 ± 3°C instantaneous **(Must)**						
Light Quality	Incandescent or fluorescent						
Light Intensity	400 to 800 lux; ≥400 lux **(Must)**						
Photoperiod	Fixed daily photoperiod **(Must)** (i.e., 16 h light: 8 h dark or 12 h light:12 h dark)						
Vessel Size & Type	100- to 125-mL wide-mouthed glass jars (~ 5 to 8 cm diam) **(Must)**; *P. minuta* vessels covered with parafilm **(Must)**, other species covered (plastic or metal lid)						
	Test vessels are inert to test and reference substances or contaminant mixtures **(Must)**						
Vessel Size & Type cont.	All test units are cleaned thoroughly and rinsed with test water before use **(Must)**						
Soil Mass	30 g wet weight of test soil per replicate; smoothed but not compressed						
	For boreal forest soils, variable (10-35 g), approximately equal in volume to 30 g of artificial soil						
Vessel Labelling	Clearly labelled/coded: test substance, concentration, and replicate # **(Must)**						
	Date and time of test initiation on labels or data sheets (**Must)**						
Vessel Position	Test containers are positioned randomly within test facility and moved during test						
# Replicates/Conc.	For site soils, use replicate samples (i.e., field replicates) collected individually from a given sample location (see Section 5.1).						
	≥3 replicates/treatment; ≥5 replicates/control for multi-concentration test; ≥5 replicates/treatment and control soil for single-concentration test **(Must)**						
	≥2 additional replicates for each treatment for conducting physicochemical analyses on Day 0 and at test end						
# Test Conc.	1, plus controls for single-concentration test						
	≥7, plus controls for multi-concentration test **(Must)**; more recommended (≥10, plus controls); geometric series						

# Springtails/Vessel	For *O. folsomi*: 15 organisms/vessel (10 females and 5 males) **(Must)**						
	For *F. candida*: 10 organisms/vessel **(Must)**						
	For *F. fimetaria*: 20 organisms/vessel (10 females and 10 males) **(Must)**						
	For *P. minuta*: 10 organisms/vessel (5 females and 5 males) **(Must)**						
Organism Selection	Springtails are transferred to test vessels on the day after the soil equilibration period (Day 0)						
	Excess number of springtails than those required for testing are available from age-synchronized culture vessels						
	For *O. folsomi*, *P. minuta* and *F. fimetaria*, the appropriate number of females (larger with round abdomens) and males (smaller, more slender) are transferred to each vessel						
	For *F. candida*, the required number of individuals (asexual) are transferred to each vessel						
	For each replicate, springtails are selected and moved to a transfer container, given a final observation to confirm number, sex, and health, and gently transferred as a group to the soil surface of the test vessel; for *P. minuta*, use a low-suction vacuum aspiration system in conjunction with a dissecting microscope						
	The order of adding springtails to each vessel are randomly allocated with respect to treatment						
Feeding Regime	Granulated dry yeast; for *O. folsomi*: ~5 mg/vessel on Days 0, 7, 14, and 21; for *F. candida*: ~10 mg/vessel on Day 0 and ~20 mg/vessel on Day 14; for *F. fimetaria*: ~10 mg/vessel on Days 0 and 14; and for *P. minuta*: ~10 mg/vessel on Days 0, 7 and 14; if yeast not consumed from previous feeding, no further yeast is added **(Must)**; yeast not removed if unconsumed						
Test Soil Hydration	Soil moistened with de-ionized water weekly during aeration, as necessary						
Test Soil Aeration	Lids of each test vessel removed ≥ once/week for aeration **(Must)**						
Test Validity Criteria	Test invalid if mean survival of adults in negative control soil at test end is: *F. fimetaria* <70% in natural or artificial soil, *O. folsomi* <70% in natural or artificial soil, *F. candida* <70% in natural soil and <80% in artificial soil, *P. minuta* <60% in natural soil and <70% in artificial soil; or reproduction rate for adult springtails in negative control soil is <100 live progeny per control vessel at test end for all four species **(Must)**						
	Negative control soil used to judge validity of test regardless of whether the reference or negative control soils are used for statistical comparisons **(Must)**						
Biological Observations	Condition, appearance, and # live springtails placed in each test vessel on Day 0 **(Must)**						
	Weekly observations of any excessive growth of bacteria or fungi, any feeding activity, and the presence and quantity of any uneaten food						
	# live adult springtails and # of live progeny in each test vessel on Day 21 for *F. fimetaria* and *P. minuta* or Day 28 for *O. folsomi* and *F. candida* **(Must)**						

Biological Observations (cont)	Test vessels processed in random manner						
	Springtails extracted using floatation or heat extraction						
	Heat extraction efficiency verified to recover ≥ 95% of test organisms **(Must)**						
	Springtails enumerated directly (i.e., manually), through digital analysis, or with image analysis software						
	Image analysis and automated counting method verified with manual count **(Must)**						
	Missing adults are counted as dead **(Must)**						
Biological Endpoint	# live springtail progeny in each test vessel at test end **(Must)**						
	# live adult springtails in each replicate at test end **(Must)**						
Statistical Endpoint	Mean (± SD) % survival of adults in each treatment on Day 21 for *F. fimetaria* and *P. minuta*; Day 28 for *O. folsomi* and *F. candida* **(Must)**						
	Mean (± SD) # live progeny in each treatment on Day 21 for *F. fimetaria* and *P. minuta*; Day 28 for *O. folsomi* and *F. candida* **(Must)**						
	For multi-concentration test: 21- or 28-day LC50 for adult springtails and 21- or 28-day ICp for reproductive inhibition based on numbers of live progeny produced in each concentration during 21- or 28-day test **(Must)**						
Calculation of ICp	Calculation of endpoints by entering concentrations as logarithms **(Must)**						
	Linear and/or nonlinear regression procedures used for calculation of ICps and 95% confidence limits **(Must)**						
Calculation of ICp cont.	Initial plot of raw data against log concentration						
	All requirements for regression analysis outlined in Section 4.8.2 of EPS 1/RM/47 2nd ed. are met **(Must)**						
	Endpoints generated by regression analysis are bracketed by test concentrations (i.e., extrapolation of endpoints beyond the highest test concentration is not acceptable) **(Must)**						
	ICPIN analyses used only if regression analyses fail to provide meaningful ICps						
Test Organisms							
Species	Laboratory cultured *Orthonychiurus folsomi*, *Folsomia candida*, *Proisotoma minuta*, or *Folsomia fimetaria* **(Must); *P. minuta*** only for use in soils collected from boreal/taiga ecozones						
	Species identification confirmed and documented by qualified personnel **(Must)**						
	Cultures held in a testing laboratory are identified to species every 2 years, as a minimum						
	Test organisms are cultured in testing laboratory **(Must)**						
Source	All organisms used in a test are derived from the same population **(Must)**						
Source of Brood Stock for Culture	Mixed-age cultures from government, private, or commercial culture						

Age at test start	*O. folsomi*: 28 to 31 days old **(Must)**						
	F. candida: 10 to 12 days old **(Must)**						
	P. minuta: 13 to 14 days old **(Must)**						
	F. fimetaria: 23 to 26 days old **(Must)**						
Culture Conditions							
Facilities	Controlled-temperature laboratory facility						
	Culture area isolated from testing, sample storage, or sample-preparation areas; designed and constructed to prevent culture contamination **(Must)**						
	Culture practices such that each culture is not cross-contaminated with another Collembola species **(Must)**						
	For *P. minuta*, efforts made to minimize vibration						
Culture Vessels	Polystyrene containers of 1-6-L capacity for *O. folsomi*, *P. minuta*, and *F. candida*; transparent or translucent sides and/or lid for *F. candida*; 10 cm polystyrene Petri dishes for *F. fimetaria*; minimum substrate depth of 1 cm; solid or perforated lids; wood is not recommended						
Culture Substrate	Plaster of Paris and charcoal; fertilizer-free potting soil may be used for maintaining mass or back-up cultures of *O.folsomi*, *F. fimetaria* and *P. minuta*						
Hydration	Hydrate with distilled or de-ionized water; re-hydrate 1-2 times/week to maintain moisture (i.e., water just begins to remain on surface)						
Aeration	Vessels aerated (i.e., remove lid for ≥ 1 min.) once/week **(Must)**; twice/week recommended for *F. fimetaria* and other species if history of fungal growth						
Air Temp	Daily average, 20 ± 2°C; instantaneous, 20 ± 3°C						
pH	6.0 - 7.0; verified for each new batch of substrate with pH paper on wet substrate surface						
Lighting	Incandescent or fluorescent; 400 to 800 lux at substrate surface; fixed daily photoperiod (e.g., 16 h: 8 h or 12 h:12 h; light: dark); avoid overheating cultures; *O. folsomi*, *P. minuta*, and *F. fimetaria* can be cultured in complete darkness						
Acclimation	Gradually (recommend ≤3°C/day) for temperature differences upon arrival						
	During age-synchronizing period, organisms are acclimated in the lab to temperature and food to be used in test **(Must)**						
	Transported to the lab using a portion of soil/substrate to which they are adapted						
Culture Maintenance	Examine contents of culture vessels at least once/week; record condition of culture (organisms and substrate); maintain loading density of ~2 to 3 adults/cm^3 for *O. folsomi* and *F. candida*, ~ 5 to 6 adults/cm^3 for *F. fimetaria*, and ~ 6 to 8 adults/cm^3 for *P. minuta*						

Substrate Renewal	As required, and at least once every 1 - 2 months, regardless of loading densities						
	Prepare new culture vessels and transfer springtails into new vessels by tapping the old vessel over the new one; transfer only a portion to reduce the population; mix organisms between independent culture vessels to avoid inbreeding						
	For *O. folsomi*, *P. minuta*, and *F. fimetaria* new cultures contain a mixture of males and females						
Substrate Monitoring	Air temp of culture facility measured weekly; moisture level observed at time of weekly aeration; adjust as necessary						
Feeding	Activated dry yeast (e.g., Fleischmann's™); quantity based on previous food consumption; ~100 mg for *O. folsomi* and *F. candida* (in a 15 x 23 x 8 cm vessel), ~200 mg for *P. minuta* (in a 15 x 23 x 8 cm vessel), and ~10 mg for *F. fimetaria* (in a 10 cm Petri dish); twice/week at time of aeration and re-hydration; place food in 2-3 piles or sprinkle over moist (i.e., to activate yeast) substrate surface after removing excess (uneaten) food						
	Avoid excessive fungal and bacterial growth (especially for *F. fimetaria* and *P. minuta*)						
Indices of Culture Health	Cultures have low mortalities, appear healthy, and behave and feed normally **(Must)**						
	Considered healthy if: (1) springtails are moving actively over the substrate surface, and (2) results for reference toxicity tests using age-synchronized springtails derived from the same population (i.e., source) as the age-synchronized springtails used to start a definitive test fall within historic warning limits **(Must**)						
Age-Synchronized Cultures	Lab follows age-synchronization procedures described in EPS 1/RM/47 2nd ed.						
	Age-synchronization procedures produce the required number of healthy test organisms of the required age (i.e., 28-31 days old for *O. folsomi*, 10-12 days old for *F. candida*, 13-14 days old for *P. minuta*, and 23-26 days old for *F. fimetaria*) and of similar size **(Must)**; for *P. minuta*, 14 days old recommended						
	Age-synchronized cultures meet specific health and performance-related indices **(Must)**						
Handling of Springtails	Handling minimized; options for transferring include using a moist, fine-tipped paintbrush; a low suction exhaustor; Pasteur pipette, fitted with a suction bulb; a water-based aspiration system or by gently tapping one vessel over another						
	Springtails that are injured or appear stressed are not used in a test **(Must)**						
Test Report							
Test Substance	Brief description of sample type or coding as provided to laboratory personnel **(Must)**						
	Information on labelling or coding of each sample **(Must)**						
	Date of sample collection **(Must)**						
	Information on sample horizons as they were collected (i.e., number, relative depth of each soil horizon), for test, reference, and negative control soils, if applicable **(Must)**						
	Date and time sample(s) received at test facility **(Must)**						

Test Organisms	Species and source of brood stock **(Must)**						
	Age-range of test organisms, at start of test **(Must)**						
	Any unusual appearance, behaviour, or treatment of the organisms 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/47 2nd ed.) **(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 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/47 2nd ed. **(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 organisms per test vessel and treatment **(Must)**						
	Dates when test was started and ended **(Must)**						
	Feeding regime and ration during test **(Must)**						
	For each soil sample: any measurements of soil particle size, moisture content, water holding capacity, pH and conductivity (if done) **(Must)**						
	For each composite sample of subsamples taken at the same time from all replicates of each treatment: all measurements of temperature (air and soil), pH, moisture content, and water holding capacity **(Must)**						
	Method used for extracting the Collembola from the soil (i.e., flotation or heat extraction) at test end **(Must)**						
	Method used for enumerating the Collembola (i.e., manual, digital analysis, or image analysis) at test end **(Must)**						
Test Results	Mean (± SD) percent survival of adult Collembola in each treatment, including controls on Day 21 for *F. fimetaria* and *P. minuta*, and Day 28 for *F. candida* and *O. folsomi* **(Must)**						
	Mean (± SD) number of surviving juveniles in each treatment, including controls on Day 21 for *F. fimetaria* and *P. minuta*, and Day 28 for *F. candida* and *O. folsomi* **(Must)**						

Test Results (cont)	Any LC50 (including the associated 95% confidence limits and, if calculated, the slope) determined **(Must)**						
	Any additional LCx (e.g., LC20) calculated **(Must)**						
	Any ICp (with its 95% confidence limits) determined for the data on reproductive success (i.e., number of surviving juvenile Collembola in each treatment 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-day LC50 (including its 95% confidence limits) for *O. folsomi*, or *F. fimetaria* or 14-day LC50 (including its 95% confidence limits) for *F. candida* or *P. minuta* performed with the reference toxicant in conjunction with the definitive soil toxicity test, using the same lot (group) of test organisms **(Must)**						
	Geometric mean value (± 2 SD) for the same reference toxicant and test species, as derived at the test facility in previous 7- or 14-day LC50 tests using the procedures and conditions for reference toxicity tests described in EPS 1/RM/47 2nd ed. **(Must)**						
Original Data Sheets	Anything unusual about the test, any problems encountered, and any remedial measures taken **(Must)**						
	Original data sheets must be signed or initialed, and dated by the laboratory personnel conducting the tests **(Must)**						
Information to be Kept On-file							
	Do lab SOPs indicate that the information on Section 7.2 of the EPS 1/RM/47 2nd ed. method must be kept on file for ≥ 5 years? **(Must)**						
	For details of this information, see Section 7.2 of EPS 1/RM/47 2nd ed.						

Notes: