

# TEST SPECIFIC CHECKLIST<sup>1</sup>

Prepared: March 2007

## Tests for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil

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Parameter	Specification	Met Specifics		
		Y	N	NA
<b>Sample Handling</b>				
Containers. . . . .	Non-toxic material for transport and storage <b>(Must)</b> . . . . . New or thoroughly cleaned, or lined with high-quality plastic <b>(Must)</b> . . . . .	...	...	...
Labelling. . . . .	Sample containers sealed and labelled or coded immediately after filling for field-collected soils and/or upon receipt in the lab for chemicals <b>(Must)</b> . . . . . 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 <b>(Must)</b> ; name and signature of sampler(s) should also be included. . . . .	...	...	...
Transport. . . . .	Samples to be kept from overheating during transport or storage. . . . . Samples must not freeze or partially freeze (unless they are frozen when collected) or be allowed to dehydrate (unless they are saturated with excess water upon arrival at the lab) during transport or storage <b>(Must)</b> . . . . . 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 <b>(Must)</b> . . . . . 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 <b>(Must)</b> . . . . .	...	...	...
Holding Conditions. . . . .	Samples stored for future use must be held in airtight containers <b>(Must)</b> . . . . . Store samples in dark at 4 ± 2°C; these conditions must be applied if samples contain PAHs, other light-sensitive toxicants, or unstable volatiles <b>(Must)</b> . . . . .	...	...	...
<b>Sample Preparation</b>				
<b>a) Field-Collected</b>				
<b>Test Soil</b>				
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 re-mixed into it if possible <b>(Must)</b> . . . . .	...	...	...
T° & 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. . . . . pH of field-collected soils not adjusted <b>(Must)</b> . . . . .	...	...	...
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 (%), pH and conductivity, as a minimum <b>(Must)</b> . . . . . 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 and determined using a recognized standard procedure <b>(Must)</b> . . . . . Optimal moisture content of test soils (artificial and site) determined and expressed as % WHC <b>(Must)</b> . . . . . For soils with high peat content where WHC cannot be reliably determined using the recommended method, the optimal moisture content can be estimated by eye (appropriate consistency) and moisture content determined thereafter and reported as such (i.e., as moisture content instead of %WHC). . . . . WHC determined gravimetrically by drying subsample for ~24h at 105°C. . . . . Test soil hydrated to optimal % of WHC after preparing test conc.. . . . .	...	...	...

<sup>1</sup>

Checklist based on Environment Canada's "Test for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil". See Endnote for references.

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.

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<b><u>Sample Preparation (continued)</u></b>				
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 multi-concentration test to prepare each treatment/concentration using geometric series; ensure homogeneity (i.e., mix until texture, colour, and moisture are homogeneous) and divide into replicates. . . . .	...	...	...
<b>b) Chemical-Spiked Test Soil</b>				
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 <b>(Must)</b> . . . . .	...	...	...
	Each batch (i.e., treatment) is prepared 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 <b>(Must)</b> . . . . .	...	...	...
	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 <b>(Must)</b> . . . . .	...	...	...
	For any test that includes solvent control soil, test results for that soil are compared statistically with those for negative control soil <b>(Must)</b> . . . . .	...	...	...
Test Soil Equilibrium.	All test soil batches are hydrated, homogenized and placed in replicate test vessels on the day prior to testing (Day -1). . . . .	...	...	...
	Replicate vessels covered (unperforated lids) and incubated overnight under test conditions (i.e., 20 ± 2°C), prior to testing. . . . .	...	...	...
<b><u>Test Conditions</u></b>				
Test Facility. . . . .	Isolated areas with temperature & lighting control (e.g., environ. chambers, or equivalent); 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, type 316 stainless steel) <b>(Must)</b> . . . . .	...	...	...
	Instruments for routine measurements (e.g., pH, temp.) are available <b>(Must)</b> . . . . .	...	...	...
	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 <b>(Must)</b> . . . . .	...	...	...
	Safety apparatus used when preparing mixtures and test soils <b>(Must)</b> . . . . .	...	...	...
	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 <b>(Must)</b> . . . . .	...	...	...

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Parameter	Specification	Met Specifics		
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<b>Test Conditions (continued)</b>				
Initial Tests.....	<p>≥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 &amp; conditions in test method. . . .</p> <p>Conditions and procedures for initial control performance test should follow those described for conducting definitive tests. . . . .</p> <p>Conditions and procedures for initial reference toxicity tests should be identical to those described for routine reference toxicant tests. . . . .</p> <p>Each test should be performed using a different lot (group of age-synchronized organisms) of test organisms of the same species, from the same source. . . . .</p> <p>Data from initial control performance test shows that criteria for test validity can be met (<b>Must</b>). . . . .</p> <p>Data from initial reference toxicity tests should be compared by calculating and appraising the magnitude of the coefficient of variation (CV) of the derived LC50s. . . . .</p>	...	...	...
Negative Control Soil.	<p>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. . . . .</p> <p>Negative control soil included as a treatment in every toxicity test (<b>Must</b>). . . . .</p>	...	...	...
Clean Field-Collected Soil. . . . .	<p>Natural soil collected from a clean (uncontaminated) site; free of pesticide or fertilizer for ≥5 years. . . . .</p> <p>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 (<b>Must</b>). . . . .</p> <p>Soil analysed for recommended physicochemical characteristics (see Section 3.3.1 in EPS 1/RM/47). . . . .</p>	...	...	...
Artificial Soil. . . . .	<p>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. . . . .</p> <p>10% <i>Sphagnum</i> sp. peat, air dried and sieved (2-mm mesh); 20% kaolin clay (with particle size &lt; 40µm); and 70% silica sand (grade 70); mixed dry. . . . .</p> <p>Add reagent-grade calcium carbonate to dry mixture to adjust pH to 6.0 - 7.5 . . . . .</p> <p>Hydrate using test water to ~28% of WHC and adjust pH as necessary with more calcium carbonate. . . . .</p> <p>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. . . . .</p>	...	...	...
Positive Control Soil.	<p>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. . . . .</p>	...	...	...
Reference Soil. . . . .	<p>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. . . . .</p> <p>Physicochemical characteristics including organic carbon, organic matter, particle size distribution, texture, and pH are similar to test soils. . . . .</p> <p>Tests involving samples of reference soil must also include a sample of negative control soil (<b>Must</b>). . . . .</p>	...	...	...
Initial Hydration of Test Soils. . . . .	<p>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. . . . .</p>	...	...	...
Test Water. . . . .	<p>Deionized or distilled water or better, such as reagent-grade water produced by a system of reverse osmosis, carbon and ion exchange cartridges (<b>Must</b>). . . . .</p>	...	...	...

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<b>Test Conditions</b> <b>(continued)</b>				
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; 3 rinses with test water. . . . .	...	...	...
<b>Measurements</b>				
<b>During Test</b>				
Moisture Content. . . . .	Soil moisture content in each treatment/concentration at test start and end <b>(Must)</b> . . . . .	...	...	...
	Moisture content determined gravimetrically (see EPS 1/RM/47). . . . .	...	...	...
	Moisture content calculated on a dry wt. basis <b>(Must)</b> . . . . .	...	...	...
pH. . . . .	Soil pH in each treatment/concentration at test start and end <b>(Must)</b> . . . . .	...	...	...
	Soil pH measured using a modified CaCl <sub>2</sub> Slurry Method (see EPS 1/RM/47). . . . .	...	...	...
Temperature. . . . .	Air temperature in test facility, daily or continuously <b>(Must)</b> . . . . .	...	...	...
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 <i>O. folsomi</i> , and <i>F. fimetaria</i> ) or 14-day (for <i>F. candida</i> ) multi-concentration test <b>(Must)</b> . . . . .	...	...	...
	Conditions the same as those for a multi-concentration definitive test except for test duration, # of test concentrations, # of organisms per vessel for <i>O. folsomi</i> , and <i>F. fimetaria</i> , and test validity criteria <b>(Must)</b> . . . . .	...	...	...
	Use age-synchronized springtails derived from the same population (i.e., culture) of springtails used to produce age-synchronized organisms for the definitive tests <b>(Must)</b> . . . . .	...	...	...
	Recommend using a portion of the same age-synchronized culture used to start the definitive test, if possible. . . . .	...	...	...
	Perform once every 2 months, or in conjunction with definitive test(s) with soil samples <b>(Must)</b> ; use boric acid. . . . .	...	...	...
	Prepare and test ≥5 concentrations plus a negative control <b>(Must)</b> , using artificial soil. . . . .	...	...	...
	Calculate mean (± SD) % survival in each treatment at test end (Day 7 or Day 14) <b>(Must)</b> . . . . .	...	...	...
	Reference toxicity test invalid if mean survival of adults in negative control soil is <80% at test end <b>(Must)</b> . . . . .	...	...	...
	Determine 7- or 14-day LC50 and 95% confidence limits <b>(Must)</b> ; express as mg boric acid/kg dry wt. . . . .	...	...	...
	Recommend 21-day (for <i>F. fimetaria</i> ) or 28-day (for <i>O. folsomi</i> & <i>F. candida</i> ) tests with boric acid be performed according to Section 4 of EPS 1/RM/47, 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) <b>(Must)</b> . . . . .	...	...	...
	Separate warning chart prepared and updated for each dissimilar procedure (e.g., species of test organisms, reference toxicant, test duration) <b>(Must)</b> . . . . .	...	...	...
Test Type. . . . .	Static; whole soil <b>(Must)</b> . . . . .	...	...	...
Test Duration. . . . .	21 days for <i>F. fimetaria</i> ; 28 days for <i>O. folsomi</i> and <i>F. candida</i> <b>(Must)</b> . . . . .	...	...	...
Test T°. . . . .	Air temperature: 20 ± 2°C daily average <b>(Must)</b> ; 20 ± 3°C instantaneous <b>(Must)</b> . . . . .	...	...	...
Light Quality. . . . .	Incandescent or fluorescent <b>(Must)</b> . . . . .	...	...	...
Light Intensity. . . . .	400 to 800 lux; ≥400 lux <b>(Must)</b> . . . . .	...	...	...

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Parameter	Specification	Met Specifics		
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<b>Test Conditions (continued)</b>				
Photoperiod. . . . .	Fixed daily photoperiod <b>(Must)</b> (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) <b>(Must)</b> ; covered (plastic or metal lid). . . . . Test vessels are inert to test and reference substances or contaminant mixtures <b>(Must)</b> . . . . . All test units are cleaned thoroughly and rinsed with test water before use <b>(Must)</b> . . . . .	...	...	...
Soil Mass. . . . .	30 g wet weight of test soil . . . . .	...	...	...
Vessel Labelling. . . . .	Clearly labelled/coded: test substance, concentration, and replicate # <b>(Must)</b> . . . . . Date and time of test initiation on labels or data sheets <b>(Must)</b> . . . . .	...	...	...
Vessel Position. . . . .	Test containers are positioned randomly within test facility and moved during test. . . . .	...	...	...
# Replicates/Conc. . . . .	≥3 replicates/treatment; ≥5 replicates/control for multi-concentration test; ≥5 replicates/treatment and control soil for single-concentration test <b>(Must)</b> . . . . . ≥2 additional replicate for each treatment is recommended 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 <b>(Must)</b> ; more recommended (≥10, plus controls); geometric series. . . . .	...	...	...
# Springtails/Vessel.	For <i>O. folsomi</i> : 15 organisms/vessel (10 females and 5 males) <b>(Must)</b> . . . . . For <i>F. candida</i> : 10 organisms/vessel <b>(Must)</b> . . . . . For <i>F. fimetaria</i> : 20 organisms/vessel (10 females and 10 males) <b>(Must)</b> . . . . .	...	...	...
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 <i>O. folsomi</i> and <i>F. fimetaria</i> , the appropriate number of females (larger with round abdomens) and males (smaller, more slender) are transferred to each vessel. . . . . For <i>F. candida</i> , 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. . . . . The order of adding springtails to each vessel are randomly allocated with respect to treatment. . . . .	...	...	...
Feeding Regime. . . . .	Granulated dry yeast; for <i>O. folsomi</i> : ~5mg/vessel on Days 0, 7, 14, and 21; for <i>F. candida</i> : ~10 mg/vessel on Day 0 and ~20 mg/vessel on Day 14; and for <i>F. fimetaria</i> : ~10 mg/vessel on Days 0 and 14; if yeast not consumed from previous feeding, no further yeast is added <b>(Must)</b> ; 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 <b>(Must)</b> . . . . .	...	...	...
Test Validity Criteria.	Test invalid if mean survival of adults in negative control soil at test end is <70% for <i>F. fimetaria</i> and <i>O. folsomi</i> ; and for <i>F. candida</i> <70% in natural soil and <80% 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 three species <b>(Must)</b> . . . . . Negative control soil used to judge validity of test regardless of whether the reference or negative control soils are used for statistical comparisons <b>(Must)</b> . . . . .	...	...	...

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<b>Test Conditions</b> <b>(continued)</b>				
Biological				
Observations. . . . .	Condition, appearance, and # live springtails placed in each test vessel on Day 0 <b>(Must)</b> . . . . .	...	...	...
	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 <i>F. fimetaria</i> or Day 28 for <i>O. folsomi</i> and <i>F. candida</i> <b>(Must)</b> . . . . .	...	...	...
	Springtails extracted using floatation or heat extraction. . . . .	...	...	...
	Springtails enumerated directly (i.e., manually), through digital analysis, or with image analysis software. . . . .	...	...	...
	Missing adults are counted as dead <b>(Must)</b> . . . . .	...	...	...
Biological Endpoint. . .	# live springtail progeny in each test vessel at test end <b>(Must)</b> . . . . .	...	...	...
	# live adult springtails in each replicate at test end <b>(Must)</b> . . . . .	...	...	...
Statistical Endpoint. . .	Mean ( $\pm$ SD) % survival of adults in each treatment on Day 21 for <i>F. fimetaria</i> and Day 28 for <i>O. folsomi</i> and <i>F. candida</i> <b>(Must)</b> . . . . .	...	...	...
	Mean ( $\pm$ SD) # live progeny in each treatment on Day 21 for <i>F. fimetaria</i> and Day 28 for <i>O. folsomi</i> and <i>F. candida</i> <b>(Must)</b> . . . . .	...	...	...
	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 <b>(Must)</b> . . . . .	...	...	...
Calculation of ICp. . . .	Calculation of endpoints by entering concentrations as logarithms <b>(Must)</b> . . . . .	...	...	...
	Linear and/or nonlinear regression procedures used for calculation of ICps and 95% confidence limits <b>(Must)</b> . . . . .	...	...	...
	Initial plot of raw data against log concentration. . . . .	...	...	...
	All requirements for regression analysis outlined in Section 4.8.2 of EPS 1/RM/47 are met <b>(Must)</b> . . . . .	...	...	...
	Endpoints generated by regression analysis are bracketed by test concentrations (i.e., extrapolation of endpoints beyond the highest test concentration is not acceptable) <b>(Must)</b> . . . . .	...	...	...
	ICPIN analyses used only if regression analyses fail to provide meaningful ICps. . . . .	...	...	...
<b>Test Organisms</b>				
Species. . . . .	Laboratory cultured <i>Orthonychiurus folsomi</i> , <i>Folsomia candida</i> , or <i>Folsomia fimetaria</i> <b>(Must)</b> . . . . .	...	...	...
	Species identification confirmed and documented by qualified personnel <b>(Must)</b> . . . . .	...	...	...
	Cultures held in a testing laboratory should be identified to species every 2 years, as a minimum. . . . .	...	...	...
Source. . . . .	Test organisms must be cultured in testing laboratory <b>(Must)</b> . . . . .	...	...	...
	All organisms used in a test are derived from the same population <b>(Must)</b> . . . . .	...	...	...
Source of Brood Stock for Culture. . . .	Mixed-age cultures from government, private, or commercial culture. . . . .	...	...	...
Age at test start. . . . .	<i>O. folsomi</i> : 28 to 31 days old <b>(Must)</b> . . . . .	...	...	...
	<i>F. candida</i> : 10 to 12 days old <b>(Must)</b> . . . . .	...	...	...
	<i>F. fimetaria</i> : 23 to 26 days old <b>(Must)</b> . . . . .	...	...	...
<b>Culture Conditions</b>				
Facilities. . . . .	Controlled-temperature laboratory facility. . . . .	...	...	...
	Culture area isolated from testing, sample storage, or sample-preparation areas; designed and constructed to prevent culture contamination <b>(Must)</b> . . . . .	...	...	...
	Culture practices such that each culture is not cross-contaminated with another Collembola species <b>(Must)</b> . . . . .	...	...	...

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<b><u>Culture Conditions (continued)</u></b>				
Apparatus.....	All equipment, vessels, and accessories that might contact organisms, test water or substrate is clean, rinsed and made of non-toxic material (e.g., glass, Teflon™, type 316 stainless steel, nylon, Nalgen™, porcelain, polyethylene, polypropylene)( <b>Must</b> ) . . . . .	...	...	...
	Culture vessels (e.g., plastic trays or breeding boxes) of 1-6-L capacity are recommended for <i>O. folsomi</i> , and <i>F. candida</i> ; transparent or translucent sides and/or lid for <i>F. candida</i> ; 10 cm polystyrene Petri dishes are recommended for <i>F. fimetaria</i> ; minimum substrate depth of 1 cm; solid or perforated lids; wood is not recommended. . . . .	...	...	...
	Copper, zinc, brass, galvanized metal, lead, and natural rubber must not be used ( <b>Must</b> ). . . . .	...	...	...
Culture Substrate. . . . .	Plaster of Paris and charcoal recommended; fertilizer-free potting soil may be used for maintaining mass or back-up cultures. . . . .	...	...	...
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 ( <b>Must</b> ); twice/week recommended for <i>F. fimetaria</i> and other species if history of fungal growth. . . . .	...	...	...
Temperature of Substrate. . . . .	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 light: 8 h dark or 12 h light:12 h dark); avoid overheating cultures; <i>O. folsomi</i> and <i>F. fimetaria</i> 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 ( <b>Must</b> ).. . . .	...	...	...
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 <sup>3</sup> for <i>O. folsomi</i> and <i>F. candida</i> , & ~ 5 to 6 adults/cm <sup>3</sup> for <i>F. fimetaria</i> . . . . .	...	...	...
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 <i>O. folsomi</i> and <i>F. fimetaria</i> new cultures should contain a mixture of males and females. . . . .	...	...	...
Substrate Monitoring.	Air temp.of culture facility measured once per week; 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; recommend ~100 mg for <i>O. folsomi</i> and <i>F. candida</i> (in a 15 x 23 x 8 cm vessel) and ~10 mg for <i>F. fimetaria</i> (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 <i>F. fimetaria</i> ).....	...	...	...

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**TEST SPECIFIC CHECKLIST<sup>1</sup>**  
**Tests for Measuring Survival and Reproduction of**  
**Springtails Exposed to Contaminants in Soil**

Prepared: March 2007

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Parameter	Specification	Met Specifics		
		Y	N	NA
<b><u>Culture Conditions</u></b> <b><u>(continued)</u></b>				
Indices of Culture Health.....	Cultures have low mortalities, appear healthy, and behave and feed normally <b>(Must)</b> ..... 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 <b>(Must)</b> .....	...	...	...
Age-Synchronized Cultures.....	Lab follows age-synchronization procedures described in EPS 1/RM/47..... Age-synchronization procedures produce the required number of healthy test organisms of the required age (i.e., 28-31 days old for <i>O. folsomi</i> , 10-12 days old for <i>F. candida</i> , and 23-26 days old for <i>F. fimetaria</i> ) and of similar size <b>(Must)</b> ..... Age-synchronized cultures meet specific health and performance-related indices <b>(Must)</b> .....	...	...	...
Handling.....	Handling minimized; springtails are transferred using a moist, fine-tipped paintbrush; a low suction exhaustor; or by gently tapping one vessel over another..... Springtails that are injured or appear stressed are discarded and must not be used in a test <b>(Must)</b> .....	...	...	...
<b><u>Test Report</u></b>				
Test Substance.....	Sample type or coding as provided to laboratory personnel <b>(Must)</b> ..... Information on labelling or coding of each sample <b>(Must)</b> ..... Date of sample collection <b>(Must)</b> ..... Date and time sample(s) received at test facility <b>(Must)</b> .....	...	...	...
Test Organisms.....	Species and source of brood stock <b>(Must)</b> ..... Age-range of test organisms, at start of test <b>(Must)</b> ..... Any unusual appearance, behaviour, or treatment of the organisms before the test <b>(Must)</b> .....	...	...	...
Test Facilities.....	Name and address of test laboratory <b>(Must)</b> ..... Name of person(s) performing the test (or each component of the test) <b>(Must)</b> .....	...	...	...
Test Method.....	Citation of biological test method used (i.e., as per EPS 1/RM/47) <b>(Must)</b> ..... 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 <b>(Must)</b> ..... Brief description of frequency and type of all measurements and all observations made during test <b>(Must)</b> ..... Name and citation of program(s) and methods used for calculating statistical endpoints <b>(Must)</b> .....	...	...	...
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 <b>(Must)</b> ..... Number of discrete samples per treatment <b>(Must)</b> ..... Number of replicate test vessels for each treatment <b>(Must)</b> ..... Number and description of treatments in each test including the control(s); test concentrations (if applicable) <b>(Must)</b> ..... Volume and/or mass of soil in each test vessel <b>(Must)</b> ..... Number of organisms per test vessel and treatment <b>(Must)</b> ..... Dates when test was started and ended <b>(Must)</b> .....	...	...	...

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# TEST SPECIFIC CHECKLIST<sup>1</sup>

Tests for Measuring Survival and Reproduction of  
Springtails Exposed to Contaminants in Soil

Prepared: March 2007

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Parameter	Specification	Met Specifics		
		Y	N	NA
Test Conditions. . . . . (continued)	Feeding regime and ration during test <b>(Must)</b> . . . . . For each soil sample: any measurements of soil particle size, moisture content, water holding capacity, pH and conductivity (if done) <b>(Must)</b> . . . . . 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 <b>(Must)</b> . . . . . Method used for extracting the Collembola from the soil (i.e., flotation or heat extraction) at test end <b>(Must)</b> . . . . . Method used for enumerating the Collembola (i.e., manual, digital analysis, or image analysis) at test end <b>(Must)</b> . . . . .	...	...	...
Test Results. . . . .	Mean ( $\pm$ SD) percent survival of adult Collembola in each treatment, including controls on Day 21 for <i>F. fimetaria</i> , and Day 28 for <i>F. candida</i> and <i>O. folsomi</i> <b>(Must)</b> . . . . . Mean ( $\pm$ SD) number of surviving juveniles in each treatment, including controls on Day 21 for <i>F. fimetaria</i> , and Day 28 for <i>F. candida</i> and <i>O. folsomi</i> <b>(Must)</b> . . . . . Any LC50 (including the associated 95% confidence limits and, if calculated, the slope) determined <b>(Must)</b> . . . . . Any additional LCx (e.g., LC20) calculated <b>(Must)</b> . . . . . 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) <b>(Must)</b> . . . . . Details regarding any transformation of data, and indication of quantitative statistical method used or procedures applied to the data <b>(Must)</b> . . . . . 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) <b>(Must)</b> . . . . . All values for measured concentrations <b>(Must)</b> . . . . . Results for any 7-day LC50 (including its 95% confidence limits) for <i>O. folsomi</i> , or <i>F. fimetaria</i> or 14-day LC50 (including its 95% confidence limits) for <i>F. candida</i> performed with the reference toxicant in conjunction with the definitive soil toxicity test, using the same lot (group) of test organisms <b>(Must)</b> . . . . . Geometric mean value ( $\pm$ 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 <b>(Must)</b> . . . . .	...	...	...
Original Data Sheets.	Anything unusual about the test, any problems encountered, and any remedial measures taken <b>(Must)</b> . . . . . Original data sheets must be signed or initialled, and dated by the laboratory personnel conducting the tests <b>(Must)</b> . . . . .	...	...	...
<u>Info. Kept on-File</u>	Do lab SOPs indicate that the information on Section 7.2 of the EPS 1/RM/47 method must be kept on file for $\geq$ 5 years? <b>(Must)</b> . . . . .  For details of this information, see Section 7.2 of EPS 1/RM/47.	...	...	...

Environment Canada, "Biological Test Method: Test for Measuring Survival and Reproduction of Springtails Exposed to Contaminants in Soil", Method Development and Applications Section, Environment Canada, Ottawa, ON, Report EPS 1/RM/47 (2007).

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