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 and Climate Change 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.

DO = dissolved oxygen; temp = temperature; conc = concentration(s); SD = standard deviation; TOC = total organic carbon content; PAH = polycyclic aromatic hydrocarbons; WHC = water-holding capacity; CEC = cation exchange capacity; OM = organic matter content

TEST SPECIFIC CHECKLIST

Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

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), including procedures for soils contaminated with volatile or unstable compounds						
	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 or any soils exhibiting distinct horizons collected as separate soil horizons where possible **(must)**						
	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)**						
	Soils without distinct horizons are collected by depth						
	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						
Labelling	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						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Labelling cont.	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) or become overheated						
	Samples are not 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, temperature and moisture content measurements are recorded upon receipt of sample(s) at laboratory **(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 under conditions that maintain the characteristics and quality of the soil for its intended use **(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	Water not used during sieving **(must)**						
	Debris and indigenous organisms removed by hand, by passing through coarse sieve (mesh size 4-10 mm) or by using freeze-thaw; grinding is avoided						
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)**						
	For each sample or soil horizon, mixing conditions (e.g., duration and temperature) as similar as possible **(must)**						
	Mixed manually or mechanically until texture and colour are homogeneous						
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						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Temp & pH Adjustment and Soil Equilibrium cont.	Samples of field-collected soil are not adjusted or manipulated (e.g., washing, aging/weathering, pH adjustment, conditioning, etc.) **(must)**						
	When soils are manipulated or adjusted, side-by-side tests with adjusted and non-adjusted samples are used						
	Documentation of soil manipulation procedures are reported **(must)**						
Characterization	Each soil/horizon (including negative control and reference soil) is analyzed for: moisture content (%), WHC (%), pH, conductivity, TOC (%), OM (%), particle sizes (% sand, % silt, % clay), and CEC **(must)**; analyses for forms of nitrogen, phosphorus, potassium, C:N ratio, and major cations and anions recommended						
	Optional analyses of 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 (i.e., soil is a homogenous, crumbly consistency; clumps 1 - 3 mm) after preparing test conc.						
Test Concentrations	Each batch (i.e., treatment) is prepared in sufficient quantity for all replicates and physicochemical analyses						
	If multi-concentration test, mix homogenized test soil with negative control soil or reference soil to prepare each treatment/concentration using geometric series; ensure homogeneity						
	For soil horizons, each horizon prepared and tested separately **(must)**; each horizon of test soil mixed with same horizon of negative control or reference soil						
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 coefficients, 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						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
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 (if artificial soil, to ~ 70% WHC), 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)**						
	Isolated from areas for organism culturing **(must)** and sample preparation/storage; well-ventilated & free of fumes						
	Equipment, apparatus and construction materials made of non-toxic material **(must)** 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)						
	Copper, zinc, brass, galvanized metal, lead, and natural rubber are not used **(must)**						
	Instruments for measuring pH, temp, weights (accurate to 0.1 mg), and a drying oven (capable of 105°C) are available **(must)**						
	Safety apparatus used when preparing mixtures and test soils **(must)**						
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)**						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Equipment Cleaning	All test vessels, equipment, and supplies that might contact site soils, test soils, control 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-grade 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 toxicity tests						
	Each test is performed using a different lot (group) of test organisms 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 IC50s						
Negative Control Soil	Natural clean field-collected soil or artificial soil for which previous tests demonstrated that the test validity criteria could be regularly met **(must)**; artificial soil is used for tests with chemicals or chemical products spiked in soil; uncontaminated natural soil is used for definitive tests with field-collected boreal forest and taiga 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						
	Soil is analyzed for: particle size distribution (% sand, % silt, % clay); total organic carbon content (%); organic matter content (%); pH; conductivity; MC (%); WHC (%); and CEC **(must)**						
	Soil is analyzed for recommended cations and anions, forms of nitrogen, phosphorus, potassium, C:N ratio, and contaminants (see Section 3.3.1 in STB 1/RM/61)						
	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						
	If present, indigenous organisms are recorded and removed or killed						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
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						
	Soil is analyzed for: particle size distribution (% sand, % silt, % clay); total organic carbon content (%); organic matter content (%); pH; conductivity; MC (%); WHC (%); and CEC **(must)**						
	Soil is analyzed for recommended cations and anions, forms of nitrogen, phosphorus, potassium, C:N ratio, and contaminants (see Section 3.3.1 in STB 1/RM/61)						
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)**						
Measurements During Test							
Moisture Content	Soil moisture content in each treatment/concentration at test start and end **(must)**						
	Moisture content determined gravimetrically (see STB 1/RM/61)						
	Moisture content calculated on a dry wt. basis **(must)**						
pH	Soil pH in each treatment/concentration at test start and end **(must)**						
	Soil pH measured using a modified $CaCl_2$ slurry method (see STB 1/RM/61)						
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	Choose between positive control concentration or multi-concentration reference toxicity test **(must)**						
	Use age-synchronized mites derived from the same population (i.e., culture) of mites used to produce age-synchronized organisms for the definitive tests **(must)**						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Reference Toxicity Tests cont.	Reference toxicity test invalid if mean survival of adult mites in negative control soil is <70% at test end, or if mean reproduction for adult mites in negative control soil is <30 progeny/vessel **(must)**						
Reference Toxicity Test: Positive Control	Include with every definitive test **(must)**						
	≥5 replicates of one concentration of a toxicant previously shown to elicit a consistent partial response, plus negative control **(must)**, using artificial soil; use boric acid						
	Conditions the same as those for definitive tests **(must)**						
	Calculate % reduction of progeny production in positive control relative to negative control at test end (Day 28) **(must)**						
	Positive control response and acceptability limits defined **(must)**						
	Identified outliers or extreme variability trigger investigations **(must)**						
Reference Toxicity Test: Multi-concentration Test	Perform at least twice/year **(must)**						
	Prepare and test ≥5 concentrations plus a negative control **(must)**, using artificial soil; use boric acid						
	Conditions the same as those for a multi-concentration definitive test, except for # of test concentrations **(must)**						
	Determine 28-day IC50 for inhibition of number of progeny (including 95% confidence limits) **(must)**; express as mg boric acid/kg soil dry wt.						
Warning Chart	Prepared and updated with all comparable endpoints (i.e., IC50s derived from multi-concentration reference toxicity tests, or % reduction of progeny production relative to control for a single concentration of reference toxicant tested as positive controls) plotted successively on a warning chart **(must)**						
	Separate warning chart prepared and updated for each dissimilar procedure (e.g., different reference toxicant) and endpoint **(must)**						
	For multi-concentration reference toxicity test, log concentration is used in all calculations **(must)**						
	If a particular data point is outside warning limits, quality checks are performed **(must)**						
	Recommendations and options for warning charts are in Section 4.9 of test method						
Test Type	Static; whole soil **(must)**						
Test Duration	28 days **(must)**						
Test Temp	Air temperature: 20 ± 2°C daily average **(must)**; 20 ± 3°C instantaneous **(must)**						
Light Quality	Incandescent, fluorescent, or LED						
Light Intensity	≥400 lux **(must)**; preferably 400 – 800 lux						
Photoperiod	Fixed daily photoperiod **(must)** (i.e., 16 h light:8 h dark or 12 h light:12 h dark)						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Vessel Size & Type	Test vessels (e.g., 30 mL glass shell vials, ~2.6 cm internal diameter) are covered with a plastic or metal lid, with a small hole to allow for gas exchange						
	Test vessels are inert to test and reference substances or contaminant mixtures **(must)**						
	All test units are cleaned thoroughly and rinsed with test water before use **(must)**						
Soil Volume	≥3 cm soil depth and identical volume in each test vessel **(must)**; ~20 mL for 30-mL glass shell vials or equivalent; smoothed but not compressed						
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.	≥5 replicates/treatment **(must)**; ≥8 for higher statistical power						
	Extra replicates for each treatment prepared for physicochemical analysis						
	For site soils, use replicate samples (i.e., field replicates) collected individually from a given sample location (see Section 5.1 in STB 1/RM/61)						
# Test Conc.	1, plus controls for single-concentration test						
	≥7, plus controls for multi-concentration test **(must)**; more recommended (≥10, plus controls); geometric series						
	If a range-finding test is conducted prior to the definitive multi-concentration test, the number of concentrations may be reduced to ≥5 (plus control) in the definitive test						
# Mites/Vessel	15 age-synchronized organisms/vessel **(must)**						
Organism Selection	Mites are transferred to test vessels on the day after the soil equilibration period (Day 0)						
	Excess number of mites than those required for testing are available from age-synchronized culture vessels						
	Individuals that appear damaged, undersized, or coloured differently (e.g., opaque or “milky”) are not used in the test **(must)**						
	For each replicate, mites 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 mites to each vessel are randomly allocated with respect to treatment						
Feeding Regime	Granulated dry yeast on Days 0, 7, 14, and 21 **(must)**; ~0.5 - 1 mg/vessel each feeding; if yeast not consumed from previous feeding, a reduced amount of fresh yeast is added; yeast not removed if unconsumed						
Test Soil Hydration	Soil moistened with test water weekly during aeration, as necessary (i.e., if > 2% loss)						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Test Soil Aeration	Lids of each test vessel removed ≥ once/week for aeration **(must)**						
Test Validity Criteria	Test invalid if mean survival of adult mites in negative control soil is <70% at test end, or if mean reproduction for adult mites in negative control soil is <30 progeny/vessel at test end **(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 mites placed in each test vessel on Day 0 **(must)**						
	Weekly observations of any excessive growth of bacteria or fungi, and the presence and quantity of any uneaten food						
	# surviving adult mites and # surviving progeny in each test vessel on Day 28 **(must)**						
	Test vessels processed in random manner						
	Mites extracted using heat-extraction **(must)**						
	Heat-extraction efficiency verified to recover ≥ 95% of test organisms **(must)**; if efficiency is not acceptable, all treatments are processed in a similar matter (i.e., using flotation following heat-extraction) **(must)**						
	All adult mites and progeny observed in the heat extraction collection vessel, regardless of whether they are alive or dead, are included in the counts for adult survival and surviving progeny **(must);** dead adults and progeny are noted; see Section 4.7 and footnote 45 for details						
	Mites enumerated directly (i.e., manually), or with image analysis software						
	Missing adults are counted as dead **(must)**						
Biological Endpoint	# surviving progeny in each test vessel at test end **(must)**						
Statistical Endpoint	Mean (± SD) % survival of adults in each treatment (including reference and negative control soils) on Day 28 **(must)**						
	Mean (± SD) # surviving progeny in each treatment (including reference and negative control soils) on Day 28 **(must)**						
	For multi-concentration test, the 28-day ICp for reproductive inhibition based on number of surviving progeny produced in each concentration **(must)**						
Calculation of ICp	Calculation of endpoints by entering concentrations as logarithms **(must)**						
	Linear and/or non-linear regression procedures used for calculation of ICps and 95% confidence limits **(must)**						
	Initial plot of raw data against log concentration						
	All requirements for regression analysis outlined in Section 4.8.1 of STB 1/RM/61 are met **(must)**						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Calculation of ICp cont.	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 *Oppia nitens* **(must)**						
	Species identification confirmed and documented by qualified personnel upon establishment of a new culture, and/or with each new batch of *O. nitens* introduced to the laboratory culture **(must)**						
	Cultures held in a testing laboratory are identified to species every 2 years, as a minimum						
Source	Test organisms are cultured in testing laboratory **(must)**						
	All organisms used in a test are derived from the same population **(must)**						
Source of Breeding Culture	Mixed-age cultures from government or private laboratories						
Life Stage at Test Start	Adult mites from age-synchronized culture, aged 8-10 days post-ecdysis (moulting) **(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 Vessels	Plastic trays or breeding boxes; transparent or translucent sides and/or lid; minimum substrate depth of 1 cm (2 cm if combination substrate used); solid or perforated lids; wood is not recommended						
Culture Substrate	Soil rich in organic matter, a mixture of plaster of Paris and charcoal, or a combination (i.e., ≥ 1 cm plaster of Paris covered with ≥ 1 cm soil)						
Hydration	Hydrate with test water; re-hydrate 1-2 times/week to maintain moisture (i.e., water just begins to remain on surface) during aeration						
Aeration	Vessels aerated (i.e., remove lid for ≥ 1 min.) once/week **(must)**; twice/week recommended, or more if history of fungal growth						
Air Temp	Daily average, 20 ± 2°C; instantaneous, 20 ± 3°C; monitored weekly						
pH	6.0 - 7.5; verified for each new batch of substrate using $CaCl_2$ slurry method for soil, or pH paper on wet substrate surface for plaster of Paris						
Lighting	Incandescent, fluorescent, or LED; 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						

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Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Acclimation	Gradually (recommend ≤3°C/day) for temperature differences upon arrival						
	During age-synchronizing period, organisms are acclimated in the lab to food **(must)** and temperature to be used in test						
	Transported to the lab using a portion of soil/substrate to which they are adapted						
Culture Maintenance	Examine contents of culture vessels twice/week; record condition of culture (organisms and substrate); if health of culture deteriorates, increase monitoring frequency to daily; maintain loading density of ~5 to 15 adults/cm^2 for plaster of Paris substrate						
Substrate Renewal	As necessary, and at least once every 4 - 6 months, regardless of test organism density						
	Prepare new culture vessels and transfer mites into new vessels following Section 2.3.7 of the test method; “milky” mites discarded; mix organisms between independent culture vessels to avoid inbreeding; maintain cultures on ≥ 2 types of substrate to protect against culture loss						
Feeding	Granulated dry yeast or baker’s yeast (e.g., Fleischmann’s™); quantity based on previous food consumption; twice/week at time of aeration and re-hydration; place food in several piles or sprinkle over moist (i.e., to activate yeast) substrate surface after removing excess (uneaten) food						
	Avoid excessive fungal and bacterial growth						
Indices of Culture Health	Cultures have low mortalities, appear healthy, and behave and feed normally **(must)**						
	Considered healthy if: (1) mites of differing ages are moving actively over the substrate surface or are clustered around yeast following feeding, (2) there is a low incidence (i.e., ≤ 10%) of non-pigmented (“milky”) mites, and (3) results for reference toxicity tests or positive controls using age-synchronized mites derived from the same population (i.e., source) as the age-synchronized mites used to start a definitive test are acceptable						
Age-Synchronized Cultures to be used in Tests	Lab follows age-synchronization procedures described in STB 1/RM/61						
	Newly emerged (moulted) adult mites are collected over a ≤ 3 day period based on the colour of their integument, and aged for 8-10 days before test start **(must)**						
	Age-synchronization procedures produce the required number of healthy test organisms of the required life stage and age (i.e., adults aged 8-10 days post-ecdysis) **(must)**						
	Age-synchronized cultures meet specific health and performance-related indices **(must)**						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

Parameter	Specification	Document Review			Implementation		
		Y	N	NA	Y	N	NA
Handling of Mites	Handling minimized; options for transferring include using a fine-tipped paintbrush or by gently tapping one vessel over another						
	Mites 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)**						
	Brief description of soil sampling, storage, and preparation (i.e., pretreatment) procedures **(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)**						
	Type of negative control soil (natural or artificial) and, if applicable, reference soil **(must)**						
	Date of sample collection **(must)**						
	Date and time sample(s) received at test facility **(must)**						
	Sample temperature and moisture content upon receipt at the test facility **(must)**						
Test Organisms	Species and source of breeding stock and test organisms **(must)**						
	Age range of 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) and verifying results **(must)**						
Test Method	Citation of biological test method used (i.e., as per STB 1/RM/61) **(must)**						
	Design and description if specialized procedure(s) (e.g., soil manipulation; 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 procedures and conditions specified in STB 1/RM/61 **(must)**						
	Number of discrete samples per treatment **(must)**						
	Number of replicate test vessels for each treatment **(must)**						

TEST SPECIFIC CHECKLIST
Tests for measuring survival and reproduction of Mites exposed to contaminants in soil

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) **(must)**						
	Volume and depth of soil in each test vessel **(must)**						
	Number of organisms per test vessel and treatment **(must)**						
	Dates and times when test and control soils were prepared, and when test was started and ended **(must)**						
	Feeding regime and ration during test **(must)**						
	Indication of test vessel aeration and assessment of soil moisture during the test						
	For each soil sample: any measurements of soil particle size, moisture content, water-holding capacity, pH, TOC (%), OM (%), CEC, and conductivity **(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)**						
Test Results	Mean (± SD) percent survival of adult mites in each treatment, including control(s) and reference soils, on Day 28 **(must)**						
	Mean (± SD) number of surviving progeny in each treatment, including control(s) and reference soils, on Day 28 **(must)**						
	Any ICp (with its 95% confidence limits) determined for the data on reproductive inhibition (i.e., number of surviving progeny 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 and degree of difference from nominal strength **(must)**						
	If using multi-concentration reference toxicity test, any 28-day IC50 (including its 95% confidence limits) performed with the reference toxicant in conjunction with the definitive soil toxicity test **(must)**						
	If using positive control concentration, any % reduction in progeny relative to the negative control for positive controls performed with the reference toxicant in conjunction with the definitive soil toxicity test **(must)**						

TEST SPECIFIC CHECKLIST Tests for measuring survival and reproduction of Mites exposed to contaminants in soil							
Parameter	**Specification**	**Document Review**			**Implementation**		
		Y	**N**	**NA**	**Y**	**N**	**NA**
Test Results cont.	Mean value (± 2 SD) for the same reference toxicant, as derived at the test facility in previous tests with the reference toxicant using the procedures and conditions for testing with a reference toxicant described in STB 1/RM/61 **(must)**						
	Anything unusual about the test, any problems encountered, and any remedial measures taken **(must)**						
Original Data Sheets	Original data sheets must be signed or initialled, and dated by the laboratory personnel conducting the tests **(must)**						
Information to be Kept On-file							
	Do lab SOPs indicate that the information on Section 7.2 of the STB 1/RM/61 method must be kept on file for ≥ 5 years? **(must)**						
	For details of this information, see Section 7.2 of STB 1/RM/61						

Notes: