

Test Method for Anti-House Dust Mite Properties of Textiles under Long-Term Test Conditions

Developed 2006 by AATCC Committee RA49; reaffirmed 2007, 2008, 2013; editorially revised 2010, 2019.

1. Purpose and Scope

1.1 This test method is for the evaluation of the degree of anti-house dust mite activity in a long-term testing environment for textiles treated at the manufacturing level for this purpose.

2. Principle

2.1 Test and control specimens are inoculated with the test organism and nutrients. After six weeks of incubation, sufficient time for mite colonies to flourish under optimal conditions, the dust mites are recovered from the specimens by heat extraction. Results are expressed as per cent reduction on the treated sample versus the untreated control.

3. Terminology

3.1 **activity**, *n.*—*of an anti-dust mite agent*, a measure of the effectiveness of the agent.

3.2 **anti-house dust mite agent**, *n.*—any chemical which kills (acaricide) or repels house dust mites.

4. Safety Precautions

NOTE: These safety precautions are for information purposes only. The precautions are ancillary to the testing procedure and are not intended to be all inclusive. It is the user's responsibility to use safe and proper techniques in handling materials in this test method. Manufacturers MUST be consulted for specific details such as material safety data sheets and other manufacturer's recommendations. All OSHA standards and rules must also be consulted and followed.

4.1 This test should be carried out by persons with training and experience in the use of acarological techniques.

4.2 CAUTION: Although house dust mites are not considered to be a direct hazard to humans, their fecal pellets have been demonstrated to be a potential potent allergen for those susceptible to asthma. Therefore, every necessary and reasonable precaution must be taken to eliminate this risk to the laboratory personnel and to personnel in the associated environment. Where appropriate, wear

protective clothing and respiratory protection that prevents penetration by the matter.

4.3 All work should be conducted using standard safe laboratory practices.

4.4 All chemicals should be handled with care.

4.5 An eyewash/safety shower should be located nearby for emergency use.

4.6 Exposure to chemicals used in this procedure must be controlled at or below levels set by governmental authorities (e.g., Occupational Safety and Health Administration's [OSHA] permissible exposure limits [PEL] as found in 29 CFR 1910.1000; see web site: www.osha.gov for latest version). In addition, the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs) comprised of time weighted averages (TLV-TWA), short term exposure limits (TLV-STEL) and ceiling limits (TLV-C) are recommended as a general guide for air contaminant exposure which should be met (see 13.1).

5. Limitations

5.1 The method can not be used to determine the specific mode of action of a given acaricide treatment.

5.2 The method should not be used to predict the performance of a finished article if the test specimen will go through additional processing steps or represents only a component of the final article.

5.3 While this test method provides some insight into the effect of different textile treatments in controlling the ability of mites to successfully establish breeding colonies, direct conclusions can not be made with regard to a treatment removing or reducing allergens.

5.4 This test method does not allow recovery of house dust mite eggs. However, it does provide a good measurement of the effectiveness of anti-dust mite treatment on a breeding mite colony.

6. Test Organisms

6.1 Test mites: *Dermatophagoides pteronyssinus* or *D. farinae*. Any other species suitable to a given country or region can also be used.

7. Maintenance of House Dust Mite Stock Cultures

7.1 Dust mite colonies should be main-

tained at $25 \pm 1^\circ\text{C}$ ($77 \pm 1^\circ\text{F}$) and 73-76% relative humidity on one part desiccated ox liver powder: one part dried yeast powder (see 13.2). Before use, the mixture should first be ground with a mortar and pestle, then sieved so that the particle size is between 500-750 μm .

7.2 Care must be taken to ensure that stock cultures of mites used for testing have not previously been exposed to chemicals or treatments that might have some interaction with the mites.

8. Preparation of Specimens

8.1 Test Specimens.

8.1.1 Prepare a minimum of three test specimens by cutting them to fit snugly in the bottom of a 10 cm diameter glass or polystyrene Petri dish. This can accurately be done by tracing around the dish on the sample, or by using of a suitably sized die. For loose fibers, sufficient material to cover the bottom of the Petri dish should be used.

8.1.2 Petri dishes or other test chambers of larger or smaller size can be substituted where desired. The size of the test specimen should change accordingly to ensure a snug fit of the sample in the bottom of the dish.

8.2 Control specimens.

8.2.1 A minimum of three specimens of the same fiber type and construction as the test specimen but containing no anti-mite finish (negative control) should also be prepared.

8.2.2 In addition, an internal lab control previously determined to support dust mite colonies should be included for each test. The purpose of this internal control is to provide validation that the mite colony has developed at the expected rate on a known sample over the course of the six weeks test.

8.3 Sterilization of Specimens.

8.3.1 Specimens may be sterilized when there is potential for fungal growth on the specimen over the test period due to the presence of spores. The method of sterilization used will depend on the sample composition and finish, as well as the particular anti-house dust mite treatment. Report method of sterilization, if used.

9. Procedure

9.1 Test Setup.

9.1.1 Distribute 50 mg of the ground/sieved nutrient mixture on each test

specimen. The mixture can be applied through a sieve to enable an even distribution of the material.

9.1.2 To prevent mites from escaping, the sides of the container can be coated with petroleum jelly. A heavy layer of coating should be avoided; as it can melt and interfere with mite recovery during the subsequent heat step. Tangle Trap has also been found to provide an effective barrier (see 13.3). However, final recovery numbers may be negatively influenced due to the numbers of mites becoming irreversibly trapped on the material. A third option is to place a textile sample that has previously been demonstrated to be effective as a mite barrier fabric tightly across the top of the test chamber and fix in place.

9.1.3 Close each test chamber and place the test units at $25 \pm 1^\circ\text{C}$ ($77 \pm 1^\circ\text{F}$) and 73-76% relative humidity for approximately 48 h to acclimatize the specimen microenvironment.

9.1.4 Place 25 male and 25 female mites from a robust colony on each acclimatized test and control specimen.

9.1.5 If possible, use mating pairs to ensure that the females are at a similar stage of oviposition.

9.1.6 Close the test chamber and incubate the samples at $25 \pm 1^\circ\text{C}$ ($77 \pm 1^\circ\text{F}$) and 73-76% relative humidity for six weeks.

9.2 Mite Recovery.

9.2.1 After the six weeks of incubation, remove one test chamber at a time from the incubator. For each plate, pre-cut a sample of nylon mesh material (see 13.4) to fit snugly within the test plate. Cover the mesh with adhesive film so that the sticky side of the tape is in direct contact with the mesh, and the edges of the tape extends beyond the edge of the mesh by approximately 5 mm.

9.2.2 Remove the lid from the test chamber and firmly place the tape/mesh combination directly on top of the test specimen, with the sticky side of the tape facing down. Ensure the outer edges of the tape adhere to the sides of the test plate. The mesh should serve to limit the amount of food particles and dead mites that might adhere to the sticky tape during the subsequent recovery step.

9.2.3 Place the plate, minus the lid, directly on a heating plate (see 13.5) set at

50°C (122°F). Place a weight on top of the tape/mesh combination to ensure intimate contact between the mesh and the test specimen. Use of a pre-cut circle of Styrofoam that will fit in the test plate between the tape/mesh and the weight can aid in evenly distributing the weight, and may also limit moisture buildup on the tape that might otherwise occur.

9.2.4 Leave each test chamber on the hot plate for a minimum of 5 h. This should be sufficient for mite recovery from textiles, as well as thicker samples or those with a heavy backing, such as carpets.

9.2.5 After the heat exposure, remove the weight and recover the tape/mesh. Cover the second side of the mesh with a clear polyethylene film or a second coat of sticky tape, to secure the mites for subsequent counting.

10. Evaluation

10.1 For each specimen, count the total number of mites recovered on the film using a low power stereo-binocular microscope.

10.2 For each set of test and control specimens, calculate the mean and standard deviation.

11. Report

11.1 Express results as percent reduction versus the control, using the following formula:

$$R = \frac{A - B}{A} \times 100$$

where:

R = percent reduction of the test specimen versus the control.

A = the mean number of dust mites found on the control specimen.

B = the mean number of dust mites found on the test specimen.

11.2 For a valid test: If a healthy mite colony has been established on the negative control specimens during the six week test period, mites on the internal lab control do not need to be recovered and counted. However, if the mite numbers on the negative controls are lower than expected for a control in this time period,

mites from the internal lab control must be recovered as outlined in 9.2. Recovery numbers outside of the normal range of mites previously established for each of the internal controls, invalidate the test.

11.3 Any deviation from this procedure as written must be reported.

11.4 The criterion for passing the test must be determined by the interested parties.

12. Precision and Bias

12.1 *Precision.* Precision for this test method has not been established. Until a precision statement is generated for this test method, use standard statistical techniques in making any comparisons of test results for either *within-laboratory* or *between-laboratory* averages.

12.2 *Bias.* Anti-house dust mite properties of textiles under long-term test conditions can be defined only in terms of a test method. There is no independent method for determining the true values. As a means of estimating these properties, the method has no known bias.

13. Notes and References

13.1 Available from Publication Office, ACGIH, Kemper Woods Center, 1330 Kemper Meadow Dr., Cincinnati OH 45240; tel: +1.513.742.2020; web site: www.acgih.org.

13.2 Desiccated ox liver powder can be obtained from Oxoid Inc., 800 Proctor Ave., Ogdensburg NY 13669; tel: +1.800.567.8378; fax: +1.613.226.3728; e-mail: webinfo.us@oxoid.com; web site: www.oxoid.com.

13.3 Tangle Trap can be obtained from The Tanglefoot Company, 314 Straight Ave. S.W., Grand Rapids MI 49504-6485; tel: +1.616.459.4139; fax: +1.616.459.4139; e-mail: info@tanglefoot.com; web site: www.tanglefoot.com.

13.4 A mesh consisting of 447 denier nylon filaments, with 40×40 threads/inch, thickness of 0.36 mm and with a hole size of 0.63 mm has been found effective. This mesh can be obtained from Industrial Textiles Ltd., 62 Patiki Rd., Avondale, Auckland 1007 NZ; tel: +64.98283188; free fax: +64.98281022; e-mail: info@vakeattack.co.nz; web site: www.indtex.co.nz.

13.5 Other means of heating the samples, such as the use of an incandescent light source, can also be used. However, the process must be optimized to obtain maximum mite recovery for each type of sample before initiation of the test.