



Designation: D3345 – 22

Standard Test Method for Laboratory Evaluation of Wood for Resistance to Subterranean Termites¹

This standard is issued under the fixed designation D3345; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the laboratory evaluation of treated or untreated wood for its resistance to subterranean termites. This test is considered as a screening test for treated material and further evaluation by field methods is required.

1.2 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use. (Warning—See 7.1.4.)*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*²

D1413 Test Method for Wood Preservatives by Laboratory Soil-Block Cultures (Withdrawn 2016)³

2.2 *Other Documents:*⁴

AWPA E10 Laboratory Method for Evaluating the Decay Resistance of Wood-Based Materials Against Pure Basidiomycete Cultures: Soil/Block Test

¹ This test method is under the jurisdiction of ASTM Committee D07 on Wood and is the direct responsibility of Subcommittee D07.06 on Treatments for Wood Products.

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² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

³ The last approved version of this historical standard is referenced on www.astm.org.

⁴ Available from the American Wood Protection Association (AWPA), PO Box 361784, Birmingham, AL 35236-1784, <http://www.awpa.com>.

AWPA E1 Laboratory Method for Evaluating the Termite Resistance of Wood-Based Materials: Choice and No Choice Tests

3. Choice or No-Choice Methodology

3.1 There are two types of basic termite bioassays:

3.1.1 *Choice test*—termites are presented with two feeding targets which may or may not contain insecticidal components. Difference in mass loss may be indicative of preference or avoidance of one material over another.

3.1.2 *No-choice test*—termites are presented with a singular choice and mass loss indicates ability of the insect to consume the material. In no-choice tests, mortality data is needed to determine dose mortality based on consumption of the test material, which is often treated with suspected insecticidal compounds.

NOTE 1—It is recommended that both choice and no-choice tests be performed when evaluating materials for resistance to subterranean termites as the different tests address different behavioral responses of the termites.

3.2 Details regarding test methodology shall be included in the final report, and use of choice or no-choice, or both, shall be indicated in the report title.

4. Apparatus

4.1 *Containers, Clear, Clean Glass or Plastic*, with loosely fitting tops with liners removed, 220 cm³ to 450 cm³ (225 mL (8 oz) to 480 mL (16 oz)).

4.2 *Tray*, enamel, stainless steel, or plastic, 0.25 m by 0.51 m (10 in. by 20 in.) and bucket.

4.3 *Paper Towels or Corrugated Cardboard*.

4.4 *Incubation Room (or Cabinet)*, maintained at a temperature between 25 °C to 28 °C (77 °F to 82 °F).

5. Reagents and Materials

5.1 *Benzalkonium Chloride Solution (1:750)*—Add 1 part benzalkonium chloride to 750 parts water. A comparable surface antiseptic is satisfactory.

5.2 *Distilled or Deionized Water*, heat-sterilized.

5.3 *Sand*, brown or white, screened, washed, and heat-sterilized.

5.4 *Southern Yellow Pine (SYP) (Pinus spp.)*, measuring 25.4 mm (1.00 in.) square by 6.4 mm (0.25 in.) in the longitudinal or tangential direction. Sapwood, no visible defects, smoothed surfaces equivalent to planed or sanded, 2 to 3 rings/cm (4 to 6 rings/in.). All test samples shall come from same parent board (1, 2).⁵

5.4.1 Other wood species may be used, but in each separate test using other species as the major test wood, untreated SYP sapwood blocks shall be used as additional controls to permit the correlation of test results among laboratories.

5.5 *Subterranean Termites*—Use a major common species of the region being studied.

5.5.1 Specific identification of any termites used shall be obtained and reported with the test data. Methods used for termite identification, as well as source of termites/collection location, shall also be reported.

6. Determining Amount of Water Based on Moisture Content

6.1 Determine the quantity of sterile distilled or deionized water to be added to the sand based on genus of termite used in testing. A moisture content between 8 % to 10 % and 15 % to 20 % shall be used for tests using *Coptotermes* spp. and *Reticulitermes* spp., respectively (2).

6.1.1 Water shall be added to oven dried sand in the following ratios:

6.1.1.1 *Coptotermes* spp., 100 g sand + 8 mL to 10 mL water.

6.1.1.2 *Reticulitermes* spp., 100 g sand + 15 mL to 20 mL water.

7. Collection of Termites

7.1 *Subterranean Termites*, (for example, *Reticulitermes* spp., *Coptotermes* spp.)—Collect from an active, large colony in a natural forest situation (for example, fallen logs, stumps), or from strong laboratory cultures. It is vital to the execution of this assay that healthy termites are collected for use in the laboratory tests. Non-aggressively feeding termite data can skew results and often lead to improper conclusions. Careful attention shall be paid to untreated control data over the duration of each test and if mass loss due to feeding is not in an acceptable range based on past studies (3, 4) the test shall be repeated with a fresh collection of termites (see also 15.1.2). (Note: Survival, wood consumption, and behavior can vary by termite colony. Therefore, depending on availability, tests may be run using termites from multiple colonies.)

7.1.1 Transport short sections of infested logs or cellulosic collection materials (for example, corrugated cardboard traps) to the laboratory and carefully break open. Shake the insects out onto a tray or trays and distribute the debris and insects evenly on the tray(s). Lay damp paper towels, sheets of kraft paper, and so forth, over the debris. The termites will cling to the damp paper after a few minutes.

7.1.2 Prepare an 8 L to 11 L (2-gal to 3-gal) pail by placing about ten unfolded, slightly crumpled, damp paper towels in

the bottom of the pail. Rinse these towels in distilled water and squeeze a number of times until damp. Cover these towels with about ten unfolded, dry paper towels.

7.1.3 Shake the damp towels covering the tray debris into the above described pail. After 2 to 4 h, remove the dry towels and any insects and debris on them from the pail and discard. Gently shake insects clinging to the lower, damp towels into a clean tray. Using a small piece of paper (approximately 7.5 cm by 7.5 cm (3 in. by 3 in.)), gently slide the paper under the termites and lift up, tilting the paper slightly so any remaining debris falls off. Healthy termites will cling to the paper. Collect termites for testing into a clean tray by lightly tapping the hand holding the paper, which will cause the termites to fall off.

7.1.4 Add termites to test containers as soon as possible once separated from debris. Do not hold termites in the pail or tray longer than 24 h before using. (**Warning**—Exercise reasonable care to ensure that any termites discarded (for example, 7.1.3) are dead. Oven-drying debris and towels used at 100 °C for 6 h is sufficient. When a test is finished, exercise reasonable care to ensure that living insects are not discarded.)

8. Weathering of Test Blocks

8.1 If the test material is weathered prior to termite exposure, the weathering procedure shall follow that of Test Method D1413 or AWPA E10.

8.1.1 Complete details on the weathering procedure used shall be included in the final report and noted in the report title.

9. Treatment and Conditioning of the Test Blocks

9.1 Treatment of test blocks shall follow standard treatment methods detailed in AWPA E10, initial, wet, and final weights shall be reported to determine uptake and retentions of treatments used in bioassays.

9.2 All specimens shall be placed on a screen or mesh rack and maintained under open laboratory room conditions or under a laminar flow hood for at least 48 to 72 h prior to conditioning.

9.2.1 If volatile formulations are tested, appropriate solvent controls shall be included as treatments to identify possible solvent/carrier effects.

9.2.2 Blocks treated with volatile formulations should be placed on a screen or mesh in a flow hood for at least 1 week to allow for outgassing.

9.3 Condition all test blocks, following weathering if used, to a constant weight within the equilibrium moisture content range from 6 % to 14 % to ensure that all solvent is removed prior to exposure to insects.

9.4 Conditioned weights shall be recorded for all specimens prior to termite exposure.

9.5 The conditioning procedure found in Test Method D1413 shall be used prior to termite exposure.

10. Block Quantity and Identifications

10.1 No-choice testing:

10.1.1 Prepare a minimum of five replicate blocks for each variable being tested, for example, for each retention of each preservative, chemical, or solvent.

⁵ The boldface numbers in parentheses refer to a list of references at the end of this standard.

10.1.2 Use a minimum of five untreated blocks as described in 5.4 as controls for each separate study. If SYP is not used as the species in 10.1.1, then five additional blocks of untreated SYP shall be included as a second set of controls.

10.2 Choice-testing:

10.2.1 Prepare blocks so that there are a minimum of five containers evaluating each set of treatment pairings (Table 1).

TABLE 1 Example Treatment Pairings for Choice Testing

Hypothetical Test Groups	Treatment Comparison Pairings	
Untreated Control (C)	C—C	T1—T2
Treatment #1 (T1)	C—T1	T1—T3
Treatment #2 (T2)	C—T2	T2—T3
Treatment #3 (T3)	C—T3	

10.2.2 Use a minimum of five paired untreated blocks as described in 5.4 as controls for each separate study. If SYP is not used as the species in 10.2.1, then five pairs of untreated SYP shall be added as a second set of controls.

10.3 Identify all blocks with a number in a suitable manner.

10.3.1 Block numbers shall be noted on the outside of choice-test containers to identify placement of individual specimens within the container.

11. Assembling Containers

11.1 Prior to use, wash all containers with an appropriate surface antiseptic solution, and dry.

11.2 Add sand to each container in accordance with 6.1.1.

11.3 Add sufficient water to each container as determined in Section 6. After addition of the water, allow the containers to stand for approximately 2 h prior to adding termites.

11.4 Add test blocks to the containers as follows (Fig. 1):

11.4.1 For no-choice tests, test blocks shall be placed on the surface of the sand with one edge of the block up against the side of the container.

11.4.2 For choice tests, blocks should be placed on the surface of the sand at opposite ends with edges of the blocks up against the side of the container.

11.5 Use a suitable numbering system on each container.

11.6 If volatile chemicals are to be tested, a 4.8 mm (No. 12 or approximately 3/16 in.) hole shall be drilled in the center of the container lid.

11.7 For choice and no-choice tests, assemble five containers with sand, water, and termites but without test blocks.

12. Adding Termites

12.1 Weigh out 1 g ± 0.05 g of subterranean termites (Section 7) and add to the center of each of the previously

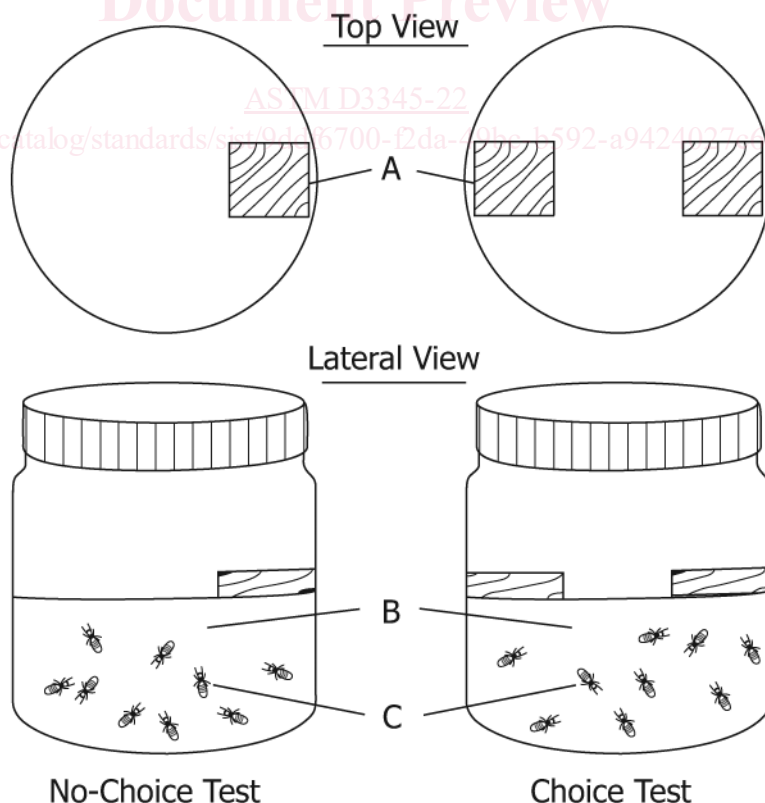


FIG. 1 Termite Test Set-up for No-choice (Left) and Choice (Right) Tests. General Testing Components Include: Wood Block Specimen (A), Wetted Sand Substrate (B), and Subterranean Termites (C)

prepared containers. Select termites so that the proportion of soldiers is approximately 1 % to 3 % and shall not exceed 10 % (5).

12.2 Replace the container tops loosely.

13. Container Storage and Inspections

13.1 Maintain all containers in the dark at a temperature between 25 °C to 28 °C (77 °F to 82 °F) for the duration of the four week study.

13.2 At the end of the first and fourth weeks, examine the containers and record the presence of tunneling, termite mortality, and position of the termites in the container as follows:

13.2.1 After one week, containers assembled in accordance with 11.7 shall show virtually complete survival, thereby indicating that test procedures have been followed and vigorous termites used.

13.2.2 *Tunneling Present*—Yes or no. (Tunneling indicates vigor of insects.)

13.2.3 *Majority Termite Position*—(On or beneath surface. Position in test container can suggest response of termites to an antagonizing effect such as repellency of preservative.)

13.2.4 *Approximate Termite Mortality*— Slight (0 % to 33 %), moderate (34 % to 66 %), heavy (67 % to 99 %), complete (100 %).

14. Container Disassembly

14.1 After four weeks disassemble the containers and remove and clean the blocks. Prior to and during the disassembly, note the items in 13.2. Discard the used sand and termites (**Warning**— See 7.1.4.) and do not reuse for subsequent tests.

15. Evaluation of Test Blocks

15.1 Examine and visually rate each block using the rating system below. Representative blocks for each level of attack are shown in Fig. 2.

15.1.1 For more details on block ratings see additional assessment results in AWPA E1.

Rating
10
9
7
4
0

Block Description
Sound, surface nibbles permitted
Light attack
Moderate attack, penetration
Heavy
Failure

15.1.2 To support test validity and to verify that adequate testing methods were followed, the following criteria shall be met at the end of the test period (6):

15.1.2.1 For no-choice tests, untreated SYP control specimens shall achieve a visual rating of 0 to 4 and visually estimated termite mortality shall be none to slight.

15.1.2.2 For choice tests, in containers comparing two SYP controls, at least one SYP block per container shall achieve a visual rating of 0 to 4. Visually estimated termite mortality shall be none to slight.

15.2 Weight loss of wood specimens shall be determined following appropriate re-conditioning methods (see Test Method D1413).

16. Report

16.1 The following information should be included in the final report:

16.1.1 Termite species used and method(s) of identification.

16.1.2 Wood species and treatments.

16.1.3 Testing method used (choice or no-choice).

16.1.4 Weathering procedure (if applicable).

16.1.5 Estimated visual termite mortality (and appropriate observations made during inspections).

16.1.6 Visual ratings, rating system used (Test Method D3345 or AWPA E1), and mass losses for each specimen.

16.1.6.1 Actual change in dry mass (mg) of block at end of test.

16.1.6.2 Percentage change in dry mass at end of test.

17. Keywords

17.1 wood; subterranean termites