

LABORATORY METHODS FOR EVALUATING THE TERMITE RESISTANCE OF WOOD-BASED MATERIALS: CHOICE AND NO-CHOICE TESTS

1.0 Scope:

1.1 This standard specifies a method for the laboratory evaluation of treated or untreated cellulosic material, cellulosic composite material, or non-cellulosic material for its resistance to subterranean termites. As described below, either a single-choice (also referred to as a "no-choice") test and/or a two-choice test procedure can be used for each material to be tested. Since specific information can be gained from each test, it is recommended that both tests be run. This test method is intended to be a screening test. It may also be used to provide information for standardization of protection treatments.

1.2 The method is applicable to both a single-choice (or no-choice) test and a choice test to determine toxicity or acceptance as a food source of cellulosic or cellulosic composite materials to termites.

1.3 The requirements for preparation of the material for testing and the test procedure appear in the following order:

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2.0 Outline of Method:

2.1 The single-choice (or no-choice) test procedure. As described below, each replicate container contains damp sand, termites, and a single test block. This procedure is useful for determining the toxicity of materials to termites, especially the threshold concentrations necessary for toxicity, and whether materials are sufficiently repellent to prevent termite feeding even when no other choices are available.

2.2 The two-choice test procedure. As described below, each replicate container contains damp sand, termites, and two test blocks - normally a treatment block and an untreated or preferably solvent-treated control block. This procedure is useful for determining whether materials are repellent to

termites when other choices are available, as is the case in most real world environments; or whether the toxicity is sufficient to deter termite feeding when that feeding is not limited to the single toxic material.

3.0 Apparatus and Material:

3.1 Recommended containers are glass screw-top jars, 80 mm diameter by 100 mm in height. Containers should not exceed this size. If smaller containers are used, the amount of sand and water may have to be adjusted according to Section 4.

3.2 Test samples can be treated with various chemicals and retentions of interest or may be from other cellulosic material of interest.

3.3 Screened, washed, heat-sterilized, silica sand, 150 g per container.

3.4 Distilled or deionized water.

3.5 Subterranean termites in the genera *Reticulitermes*, *Coptotermes*, or *Heterotermes*. Test results obtained with a particular termite species may be applicable to other species and genera as well, but this cannot be assumed to be the case. Tests should emphasize the use of termite species commonly encountered in the geographic region(s) of interest. *Reticulitermes flavipes* has a very broad geographic distribution in eastern North America; *Reticulitermes hesperus* is found on the west coast; *Heterotermes aureus* is a common desert termite; and *Coptotermes formosanus* is found in Hawaii, the tropics and subtropics, and the southern states.

3.6 Enamel, stainless steel, or plastic tray, e.g. 250 mm x 500 mm (10 x 20 in.) and bucket.

3.7 Paper towels.

3.8 Benzalkonium chloride solution (one part Benzalkonium chloride to 750 parts water), or comparable surface antiseptic.

4.0 Collection of Termites:

4.1 Subterranean termites, (e.g. *Reticulitermes*, *Coptotermes*, or *Heterotermes*) are collected from logs, stumps, buried containers of cellulosic materials near termite nests, or from wood or paper placed in the field as baits to aggregate foraging termites. If termites are taken from a laboratory culture, then culture maintenance conditions and the original date and place of collection must be reported with the test data. If possible, the original date, place of termite collection, length of time in storage prior to the test and conditions of that storage, and species determination should be reported with the test data. A sample of termite soldiers and (if possible) the winged alate (swarmer) caste from each collection should be preserved in 70% ethanol, labeled with the date and place of collection, and either maintained by the testing laboratory or deposited in a recognized entomological collection at a public or government institution.

4.2 Termites should be separated from collection debris as described below, or by other methods commonly used in the test laboratory (must be described with data).

4.2.1 Wood or paper collection materials are removed to the laboratory with termites and carefully broken open. The insects are shaken out onto a tray or trays. After distributing the debris and insects evenly on the tray(s), damp paper towels, sheets of Kraft paper, etc. are laid over the debris. The termites will cling to the damp paper after a few minutes.

4.2.2 A 7 to 20 liter (2 to 5 gallon) pail is prepared by placing about 10 unfolded, slightly crumpled, damp paper towels in the bottom of the pail or similar container. These towels should be rinsed in distilled water and squeezed damp. Cover these towels with about 10 unfolded, dry paper towels.

4.2.3 The damp towels covering the tray debris are shaken into the above described pail. After two to four hours, the dry towels and any insects and debris on them are removed from the pail and discarded. Insects clinging to the lower, damp towels are used in the test.

4.2.4 Termites broken out of field colonies should not be held in the pail or container longer than 24 hours before being used.

4.2.5 Caution: Exercise reasonable care to ensure that all termites discarded (e.g. Step 4.2.3) are dead. Debris and towels may be microwaved, autoclaved, or oven dried. When a test is finished, reasonable care should also be exercised that living insects are not discarded.

5.0 Test Specimens: The standard species for testing is southern yellow pine (*Pinus spp.*) and samples should be cut as accurately as possible from kiln dried sapwood, providing blocks with smooth surfaces. Initial sample sizes to be tested for solid wood should be 25 mm (1 inch) by 25 mm (1 inch) by 6 mm (1/4 inch) in the radial direction. The wood shall be free of sapstain chemicals, mold, stain, decay and insect attack with no visible defects, and have four to six rings per inch. For cellulosic composites, thickness may vary. Samples should measure 25 mm (1 inch) by 25 mm (1 inch) by the thickness of the material. Treatment of samples for testing should follow the procedures of AWWA E10 Sections 9 through 12, although using the samples sizes and numbers as described below. For testing of cellulosic composites, the material for testing should be selected from manufactured sheets or profiles that are representative of the tested material as used in service, with test samples having no large surface area glue-line exposure to termites.

5.1 Block Quantity and Identifications

5.1.1 Five replicate blocks should be prepared for each variable under test, e.g. for each retention of each preservative or chemical to be tested.

5.1.2 Five untreated blocks as described in 5.1.1 must be used as controls for each separate study.

5.1.3 If using the indirect method of determining initial oven-dry weight of the test blocks (Section 5.4), then **five** extra blocks for each variable tested will need to be included, that can be sacrificed by oven-drying to determine initial moisture content of blocks before exposure to termites

5.1.4 If southern yellow pine is not used as the species in 5.1.1 and 5.1.2, then five blocks of untreated southern yellow pine must be added to each study to permit a comparison to studies using southern yellow pine as the major species.

5.1.5 All blocks should be identified with a number in a suitable manner.

5.2 Weathering of Test Blocks. If the test material needs to be weathered prior to exposure to the insects, the complete details on the weathering shall be reported. The AWP E10 weathering procedure for the soil-block test is recommended.

5.2.1 If volatile chemicals are to be tested, test samples should be aged prior to termite testing (e.g., see AWP E10 12.1.2 Volatility Test) to aid in removal of volatile components. Aging methods must be reported with test data, as well as any preservative depletion, as indicated by appropriate chemical analyses. Solvent-only control samples shall be included in the test, and termite mortality in controls must be reported.

5.2.2 If leachable chemicals are to be tested, test samples should be protected from contact with the sand by placement on a plastic or aluminum foil square exceeding the dimensions of the sample by approximately 3 mm (1/8 in.) on each side.

5.3 Conditioning of the Test Blocks. All test blocks, following weathering if used, need to be conditioned to constant weight (see AWP E10 Section 11 soil-block conditioning procedure). The conditions used will be dependent on the nature of chemicals used to treat the test blocks. The initial conditioned weight can be used indirectly (Section 5.3.1) to calculate the oven-dry weight of the test specimens, which is then used with the post-test oven-dry weight to obtain percent weight loss due to termite attack. Alternatively, the initial conditioned weight can be used with the post-test conditioned weight to directly calculate percent weight loss by the equilibrium conditioning method (Section 5.3.2).

5.3.1 Oven-dry calculation method. This is an indirect method using a separate sets of sample blocks (section 5.1.3) that are conditioned with the test blocks to constant weights. These extra sample blocks are then oven-dried at 103°C, and their average percent MC determined. The average percent MC for each set is used to calculate the oven-dry mass of the individual test specimens in that set, using the equation in 5.3.1.1. This protects the test specimens from potentially destructive effects of high temperature oven-drying but depends upon high uniformity between conditioning of the sample blocks and test specimens. Initial conditioning of the samples can be in a forced draft oven at 40°C, or at lower temperature with controlled humidity, until a constant mass is obtained. Prior to conditioning, all blocks must be dried to at least 2% below the EMC of the conditioning chamber for all samples (see AWP E10 Section 16). This is because wood exhibits a hysteresis effect, which can result in different final moisture contents at equilibrium, depending on whether the wood is absorbing or desorbing water. With this method, there is no requirement for post termite exposure conditioning and test blocks can be oven-dried at 103°C to determine their final oven dry weights.

5.3.1.1 Calculation of oven-dry (OD) weights of individual test specimens in exposure sets before termite exposure:

$$\text{Calculated OD weight} = \frac{\text{Conditioned weight of test sample}}{\left(1 + \left(\frac{\text{Average percent MC}}{100}\right)\right)}$$

5.3.2 Equilibrium conditioning method. Conditioning of all test specimens to constant weight under uniform temperature ($\pm 1^{\circ}\text{C}$) and humidity ($\pm 2\%$ RH) conditions can also be used to determine pre-exposure weights as long as the exact conditions are repeated after termite exposure. As in 5.3.1 all test specimens must be dried to at least 2% below the target EMC of the conditioning chamber before conditioning.

5.3.3 If wood-plastic composite (WPC) specimens can sustain high temperatures, the test specimens can be dried to constant weight at 103°C for determination of their pre- and post-test oven-dry weights without needing to calculate average percent MC, otherwise, a separate set of sample blocks need to be cut and conditioned with the test specimens at 40°C in a force-draft oven to obtain the calculated oven-dry weights as specified in Section 5.3.1. See AWPA E10 Section 11.2 for details on increasing the WPC moisture levels if necessary.

6.0 Procedure:

6.1 Assembling Containers

6.1.1 Prior to use, all containers shall be rinsed in a surface antiseptic solution or autoclaved, washed and dried.

6.1.2 One hundred and fifty (150) grams of sand are added to each container.

6.1.3 Suitable numbers are used to identify each container. Five containers shall be assembled with sand, water, and test blocks, but without termites.

6.1.4 For *Coptotermes*, 30 ml of sterile water is added to the 150 grams of sand in each container to bring the sand to 20% MC. For *Reticulitermes*, 27 ml of sterile water is added to the 150 grams of sand in each container to bring the sand to 18% MC. Water and test samples should be added to the sand in each container at least 2 hours prior to placing termites in the container.

6.1.5 In the single-choice procedure, the test block is placed on the surface of the sand with two corners of the block against the side of the container.

6.1.6 In the two-choice procedure, the two test blocks are placed on the surface of the sand on the opposite sides of the test container, with two corners of each block against the side of the container.

6.1.7 If leachable chemicals are to be tested, test samples including controls should be protected from contact with the sand by placement on a plastic or aluminum foil square exceeding the dimensions of the sample by approximately 3 mm (1/8 in.) on each side.

6.2 Termite Exposure

6.2.1 Termite Source: Termites from a single colony or single field location should be used consistently in each individual test. If termites are collected from multiple locations or colonies, then each treatment should consist of the same number of replicates from each individual collection.

6.2.2 Number of Termites: Termites may be counted or weighed. Counting is preferred and should be performed to ensure accuracy of mortality. The ratio of soldier to worker termites should reflect the ratio typically found in a wild colony of the species used. For *Coptotermes*, the number of soldiers is approximately 10% (360 workers, 40 soldiers). For *Reticulitermes*, the soldier number is approximately 1-3% (396 workers, 4 soldiers).

6.2.2.1 Counting: When counting, four hundred termites are individually counted using an aspirator of the type commonly used to pick up insects and added to each of the previously prepared containers.

6.2.2.2 Weighing: For large tests weighing sets of termites may be used. Prior to the test, the average mass of individual termites should be determined and recorded with the test results. This is calculated by weighing five groups of at least one hundred termites each, and averaging the average weight of an individual termite in each group of one hundred or more. Then termites are added to each container until the additions reaches the mass calculated for 400 termites.

6.2.3 Addition of Termites:

6.2.3.1 In the single-choice procedure, termites should be placed on the opposite side of the container from the test block.

6.2.3.2 In the two-choice procedure, termites should be placed between the two test blocks.

6.2.3.3 The container tops are replaced loosely. If volatile chemicals are to be tested, the container lids may have to be removed and the containers inspected daily to prevent termites from escaping.

6.3 Container Storage and Inspection

6.3.1 All test containers are maintained at 24° to 28°C (75° to 82°F) for four weeks (28 days).

6.3.2 After one week, inspect control containers with termites. Termites from a healthy collection on untreated southern yellow pine should show close to 100% survival.

6.4 Container Disassembly

6.4.1 After four weeks the containers are disassembled and the blocks removed. Blocks can be cleaned using a small brush and rinsed with distilled or deionized water if necessary to remove sand.

6.4.2 Count and record the numbers of live termite workers and soldiers in each container.

6.4.3 The used sand and termites are discarded as described in Section 4.2.5 and should not be reused for subsequent tests.

7.0 Assessment of Results:

7.1 Block Evaluation

7.1.1 Each block is examined and visually rated using the following rating system (photographic references of various ratings are provided for comparison at the end of this standard):

10 Sound

9.5 Trace, surface nibbles permitted

9 Slight attack, up to 3% of cross-sectional area affected

8 Moderate attack, 3-10% of cross-sectional area affected

7 Moderate/severe attack, penetration, 10-30% of cross-sectional area affected

6 Severe attack, 30-50% of cross-sectional area affected

4 Very severe attack, 50-75% of cross-sectional area affected

0 Failure

7.2 In addition to visual rating as in Section 7.1.1, each block is dried to a consistent weight according to which method was used to determine the block mass before exposure (Section 5.3), and that weight recorded.

8.0 Interpretation of Results:

8.1 Reported data for each sample should include:

8.1.1 Visual rating of block at end of test.

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

8.1.3 Percentage (%) change in dry mass of block at end of test.

8.1.4 Number of live termite workers at end of test.

8.1.5 Number of live termite soldiers at end of test.

8.1.6 Total (workers plus soldiers) percentage (%) termite mortality at end of test.

8.2 Mean changes in block masses for each variable under test may be statistically compared by analysis of variance (ANOVA) of actual (mg) mass changes, and an appropriate means separation test. A paired comparisons *t*-test may be used to compare the mass loss of treatment and control blocks within each treatment in the two-choice test.

8.3 Mean total termite mortalities for each variable under test may be statistically compared by analysis of variance (ANOVA) of proportional mortality values transformed by the arcsine of the square root, and an appropriate means separation test.

8.4 Statistical methods other than those described in Sections 8.2 and 8.3 may also be used, and a description of the statistical methods should accompany the test results.

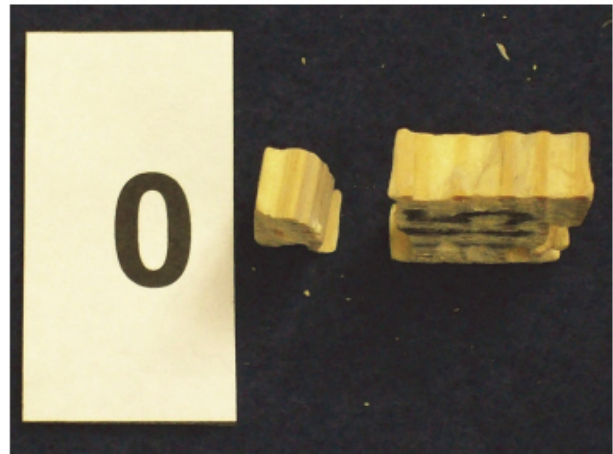
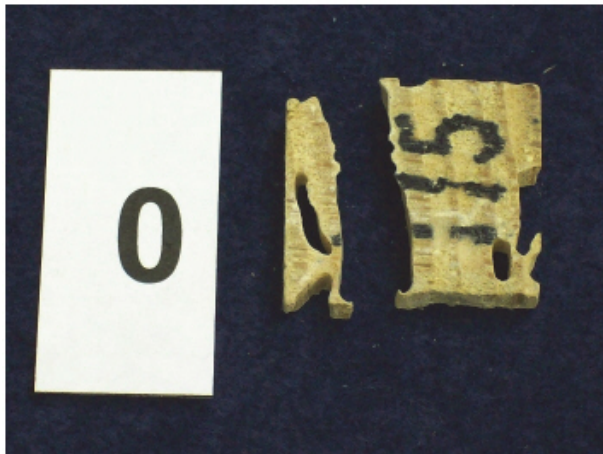
8.5 Note: Because of internal attack, visual ratings can be misleading and it is strongly recommended that both visual ratings and mass loss be included in the final report.

9.0 Additional Commentary:

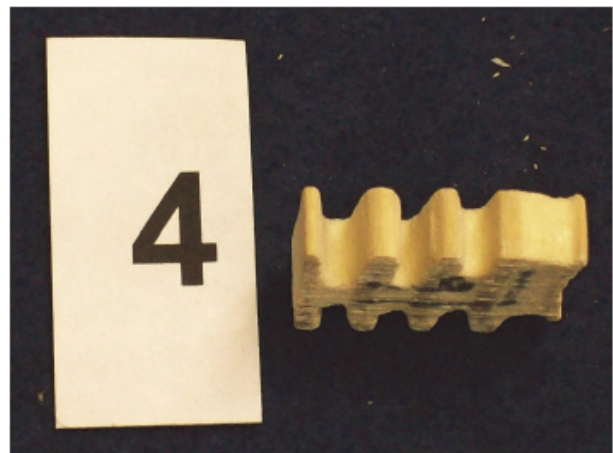
9.1 References

9.1.1 In general, details of AWP Standard E10 should be used for treating procedure and any other items in question not specifically mentioned in the above detailed termite test method.

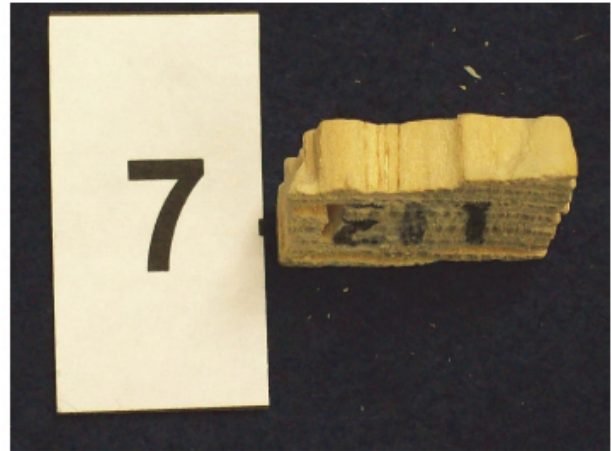
E1 Rating "0": Failure



E1 Rating "4": Very Severe



E1 Rating "7": Moderate / Severe



E1 Rating "9": Slight



E1 Rating "10": Sound

