Designation: D1413 – 07ε1

Standard Test Method for Wood Preservatives by Laboratory Soil-Block Cultures[1]

This standard is issued under the fixed designation D1413; 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.

ε[1] NOTE—Editorial corrections were made throughout in April 2008.

1. Scope

1.1 This test method covers determination of the minimum amount of preservative that prevents decay of selected species of wood by selected fungi under optimum laboratory conditions.

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

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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D841 Specification for Nitration Grade Toluene

D1193 Specification for Reagent Water

D1758 Test Method for Evaluating Wood Preservatives by Field Tests with Stakes (Withdrawn 2015)[3]

D3345 Test Method for Laboratory Evaluation of Wood and Other Cellulosic Materials for Resistance to Termites

D3507 Test Methods for Penetration of Preservatives in Wood and for Differentiating Between Heartwood and Sapwood (Withdrawn 2008)[3]

E11 Specification for Woven Wire Test Sieve Cloth and Test Sieves

2.2 *Other Standards:*[4]

AWPA E10 Testing Wood Preservatives by Laboratory Soil-Block Cultures

3. Summary of Test Method

3.1 Conditioned blocks of wood are impregnated with different concentration solutions of a preservative in water or suitable organic solvent to produce a series of retentions of the preservatives in the blocks. After periods of conditioning or weathering, the impregnated blocks are exposed to one or more strains of wood-destroying fungi, one fungus for each test series. The minimum amount of preservative that in the prescribed testing protects the impregnated blocks against decay by a given test fungus is defined as the threshold retention for that organism. Failure to protect is evidenced by loss of wood from the treated wood blocks, as indicated by a loss in weight.

3.2 Provision must be made for coordinated preparation of the test cultures and for impregnation, conditioning, or weathering and conditioning, of the test blocks.

4. Significance and Use

4.1 This test method is useful in the development of new wood preservatives and preservative systems by evaluating the minimum preservative retention to prevent decay under laboratory conditions. The results are used to facilitate target retentions in subsequent tests for effectiveness against termites

[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 American Wood Protection Association (AWPA), P.O. Box 361784, Birmingham, AL 35236-1784, http://www.awpa.com.

(see Test Method D3345) and in field stakes (see Test Method D1758). The sections on Treatment and Preservative Permanence are referenced by other ASTM standards. The test method assumes that the test blocks exposed to certain conditions after treatment will achieve equilibrium, and will return to the same equilibrium after exposure to fungi. This assumption may lead to weight loss that is not due to decay. The test uses live cultures of fungal organisms that require careful colonization, storage, and feeding to remain viable strains.

5. Apparatus

5.1 *Conditioning Chamber or Room* , maintained at a selected temperature between 20 and 30°C (68 and 86°F) and a selected relative humidity between 25 and 75 %. The selected temperature shall not vary more than ±1°C (±2°F) and the selected humidity not more than ±2 %.[5]

5.2 *Incubation Room or Incubation Cabinet,* maintained at a selected temperature between 25 and 27°C (77 and 81°F) and a relative humidity between 65 and 75 %. The selected temperature shall not vary more than ±1°C (±2°F) and the selected humidity percentage not more than 2.

5.3 *Drying Oven*—A suitable, vented oven, maintained at a temperature of 105 ± 2°C (220 ± 4°F).

5.4 *Steam Sterilizer.*

5.5 *Balances,* fast-acting types preferred, sensitive and accurate to 0.01 g.

5.6 *Vacuum Pump or Water Suction Pump,* capable of reducing pressure to 100 mm (3.94 in.) mercury (Hg), or less.

5.7 *Impregnation Apparatus*—A suitable desiccator or bell jar shielded to protect personnel in event of breakage, provided with suitable separatory funnel or auxiliary flask for holding the treating solution and vacuum gage or manometer (Fig. 1).

5.8 *Trays or Racks, or Pin Bars*—Trays or racks made from suitable screening to permit free air movement around each block during initial drying and for convenient handling of the test blocks. Pin bars facilitate handling (see 7.2).

5.9 *Weathering Apparatus:*

5.9.1 *Forced Draft Oven.*

5.9.2 600 cm^3 breakers for weathering of oil-type preservatives.

5.9.3 225 cm^3 wide-mouth screw cap bottles for weathering water-borne preservatives.

5.10 *Culture Bottles,* cylindrical or square (Note 1), capacity nominal 225 or 450 cm^3 (8 or 16 oz), fitted with screw caps without liners (Fig. 2). An alternate lid fitted (Note 2) with a 25-mm autoclavable filter with a pore size of 0.2 microns is permitted to reduce or prevent mite infestation during the test.

NOTE 1—*Culture Bottles:*
(1) 225-cm^3 (8-oz) French square, for use with one block only.
(2) 225-cm^3 (8-oz) cylindrical, for use with one or two blocks.
(3) 450-cm^3 (16-oz) cylindrical, for use with two blocks only.

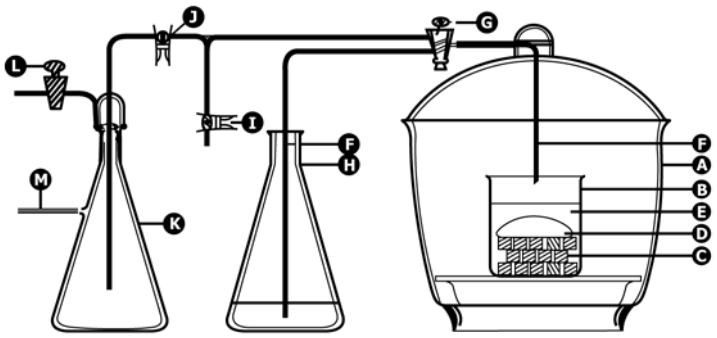
NOTE 2—Prepare the lids by drilling a 6.4-mm hole in the center and lightly sanding the underside with medium grit paper. Adhere the filter with a small amount of high temperature silicon or slow-curing epoxy and cure overnight. Ensure that the adhesive does not covered the drilled hole.

5.11 *Soil Sieves*—U.S. No. 6 sieve in accordance with Specification E11.

6. Reagents

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that

[5] Scheffer, T. C., "Humidity Controls for Conditioning Rooms," Forest Products Laboratory Report No. 2048, U.S. Forest Service, 4 pp., 5 Figs., January 1956.



A—Vacuum desiccator, internal diameter 250 mm.
B—Plastic or glass treatment beaker.
C—Test wood blocks.
D—Glass or other suitable weight.
E—Treating solution.
F—Polyethylene tubing.
G—Three-way stopcock with TFE-fluorocarbon plug.
H—Flask containing treating solution.
I—Glass joint with O-ring leading to either vacuum gage or manometer.
J—Glass joint with O-ring.
K—Flask for vacuum trap.
L—Stopcock to atmosphere.
M—Line to source of vacuum.

FIG. 1 Apparatus for Vacuum Impregnation

A—Wood cubes, 19-mm or 0.75-in.
B—Test fungus growing over feeder block.
C—Wood feeder strip, one feeder strip for each culture bottle..
D—Soil.

FIG. 2 French Square and Cylindrical 225 cm³ (8 oz) and cylindrical 450-mm (16-oz) Culture Bottles with Metal Screw Lids

all reagents shall conform to the Specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type IV of Specification D1193.

6.3 *Toluene,* conforming to Specification D841.

7. Wood and Test Blocks

7.1 *General Properties*—The selection of either a hardwood or softwood is dependant on the products to be treated for the selected preservatives. Selected wood shall be free of knots and visible concentration of resins, and showing no visible evidence of colonization by mold, stain, or wood-destroying fungi, with 2½ to 4 rings/cm (6 to 10 rings/in.). Whenever practicable, begin selection of the wood for the test blocks at the sawmill. Quarter-sawed boards are preferable. Newly cut boards, nominally 25 mm (1 in.) thick, that are immediately kiln dried without antistain treatment provide chemical-free wood that has had minimum opportunity for fungus infection prior to use in the soil-block culture test.

7.1.1 For softwoods, pine sapwood shall be used for tests intended to show comparative wood preserving values under the test. If southern pine is used, it should be 40 to 50 % summerwood, Report the species of wood and growth characteristics of selected specimens.

7.1.2 For hardwoods, use sapwood from sweet-gum (*Liquidambar styraciflua* L.) or yellow poplar (*Liriodendron tulipeifera* L.).

7.1.3 *Sapwood Identification*—When the boundary between heartwood and sapwood is difficult to recognize, use a color test (see Test Methods D3507 to distinguish between the two. Sample blocks shall be all sapwood.

7.2 *Test Blocks* (Note 3)—Mill-test blocks as accurately as possible to 19 mm on each face. The volume of the blocks (without holes) shall be 6.9 ± 0.2 cm³ as determined by calipers.

NOTE 3—For convenience in handling, blocks may be drilled through the center of a tangential face with a 3-mm drill (approximately 0.125 in. or a No. 30 drill). Pin bars may then be used for handling. Store working stocks of test blocks and feeder strips in the conditioning room. It is desirable to weigh the blocks after they come to approximate equilibrium moisture content in storage or in the conditioning room, and to sort them into fairly narrow-range weight groups. Since the blocks are cut accurately to size this division into weight groups is, in effect, a segregation into density groups (see 11.4).

7.3 *Feeder Strips:*

7.3.1 *General Considerations*—One feeder strip is required for each culture bottle (Fig. 2). If test blocks other than pine are used for special investigations, the sapwood selected for feeder strips shall be capable of furnishing a satisfactory growth of the test fungus; for example, sweetgum sapwood often is used with hardwood test blocks.

7.3.2 *Size*—The feeder strips are cut 3 by 28 by 35 mm (⅛ by 1⅛ by 1⅜ in.) with the grain of the wood parallel to either of the longer dimensions. The exact dimensions are not critical, but in bottles with two test blocks, the blocks shall not contact each other.

8. Test Fungi

8.1 *General Considerations*—Always include a comparatively tolerant fungus (see 8.2 and 8.3) in testing a preservative.

NOTE 4—Other economically important fungi may be used in addition to the tolerant fungus in special investigations, or in some cases, substituted for it (see AWPA E10). The following numbers refer to standard strains of test fungi maintained in the American Type Culture Collection (ATCC).[7]

8.2 *Fungus Species for Softwood Sapwoods:*

8.2.1 *Neolentinus lepideus (Fr:Fr.) Redhead and Ginns. (Madison 534, ATCC No. 12653)*—A fungus particularly tolerant to creosote or to mixtures containing creosotes.

8.2.2 *Gloeophyllum trabeum* (Pers. ex. Fr.) Murr. = [*Lenzites trabea* Pers. ex. Fr.] (Madison 617, ATCC No. 11539)—A fungus particularly tolerant to phenolic and arsenic compounds.

8.2.3 *Postia placenta* (Fr.) M. Larsen et Lombard = [*Poria monticolor* Murr.] (Madison 698, ATCC No. 11538)—A fungus particularly tolerant to copper and zinc compounds.

8.3 *Fungus Species for Hardwood Sapwoods:*

8.3.1 The three fungi listed in 8.2.

8.3.2 *Trametes versicolor* (L. ex Fr.) Pilát = [*Polyporus versicolor* L. ex. Fr.] (ATCC No. 42462), a white-rot fungus prevalent on hardwood products.

9. Culture Media

9.1 *Malt Agar Substrate*—For both stock test-tube and petri dish cultures of the test fungi, use a nutrient medium consisting of about 2 weight % malt extract and 1.5 weight % agar.

[6] *Reagent Chemicals, American Chemical Society Specifications* , American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[7] Available from American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, VA 20108, http://www.atcc.org.

Sterilize the medium at 103 kPa (15 psi) for 20 min and allow to cool before inoculations.

9.2 *Soil Substrate*—Use a soil substrate with a water-holding capacity between 20 and 40 % and pH between 5.0 and 8.0 and weighing not less than 90 g/120 cm^3. After breaking up all clumps, mix and screen the soil through the U.S. No. 6 sieve and store in large covered containers. The soil should not be so wet when it is sifted that the particles again stick together.

9.2.1 *Determination of Water-Holding Capacity of Soil*—The water-holding capacity of a soil is that percentage of water, based on the ovendry weight of the soil, that is retained after subjecting the soil to the following procedure based on a method of G. J. A. Bouyoucos.[8] Pass a sample of air-dry soil through a U.S. No. 6 sieve. Determine the water-holding capacity as follows. Use the sieved soil to fill a small Buchner funnel approximately 50 mm in diameter and 25 mm in depth, and fitted with rapid-filtering paper, to somewhat more than capacity. Compact the soil by dropping the funnel three times through a height of 10 mm (0.4 in.) on a wooden tabletop. Level the soil surface by cutting off excess soil with a spatula at the top of the funnel without further compaction. Then place the filled funnel in a 400-cm^3 beaker and retain in an upright position by wedges at the sides of the funnel. Add water to the beaker to a depth slightly beyond the level of the filter paper. Allow the soil to wet by capillarity so as to reduce the danger of entrapping air within the column. When the upper soil surface shows signs of wetting, add more water until the water level approximates the upper surface of the funnel. Place a cover over the beaker, and allow the soil to soak for 12 h or overnight. Then place the funnel in a suction flask which is connected to a water aspirator or vacuum pump, and apply full suction for 15 min. During suctioning, cover the funnel with a moist cloth on which an inverted cup is placed to prevent evaporation of water from the exposed soil surface. After 15 min remove the funnel from the suction flask, scrape the soil into a weighed receptacle, and weigh to obtain the wet weight, W_1. Ovendry for 24 h at 105 ± 2°C (220 ± 4°F) and reweigh soil (W_2). Determine water-holding capacity based on the ovendry weight of soil.

$$\text{Water} - \text{holding capacity}\,(WHC), \% = \left[(W_1 - W_2)/W_2\right] \times 100 \quad (1)$$

9.2.2 *Preparation of Soil Culture Bottles*—Half fill a culture bottle with sifted soil substrate and lightly compact by tapping. This amount of soil, about 120 cm^3 for an 8-oz culture bottle, should weigh not less than 90 g when ovendried. Use a proportionate volume when the larger 450 cm^3 bottles are used. The water in the completed soil culture bottle should be 130 % of the water-holding capacity of the soil. To determine the amount of additional water needed, weigh the volume of soil that will be used to half-fill a culture bottle, W_3. Dry this soil at 105 ± 2°C (220 ± 4°F) for 12 h and reweigh, W_4. Calculate the amount of water to be added to each culture bottle with that particular soil as follows:

$$\text{Water required, g} = \left(WHC \times 0.013 \times W_4\right) + W_3 - W_4 \quad (2)$$

where WHC is entered as a number, not a decimal.

9.2.3 Add the required amount of water to each culture bottle. Use a funnel bottle with a stem of large diameter that reaches nearly to the bottom of the culture bottles to add the corresponding volume of soil to minimize dust settlement on the glass. Level the soil surface and place directly on the soil one sapwood feeder strip for each test block to be used. Steam sterilize the prepared bottles, with caps loosened, at 103 kPa (15 psi) for 30 min. This sequence of steps generally leaves the inside surfaces of the culture bottles clean above the soil level and the water diffuses through the soil during sterilization without puddling.

10. Preparation of Test Cultures

10.1 After the sterilized soil culture bottles are thoroughly cooled, cut approximately 10-mm square fungus inoculum sections from a petri dish culture that is not more than 3-weeks-old (Note 5). Immediately place the square of inoculum in contact with an edge of the feeder strip on the soil. Close the culture bottles with lids released one-fourth turn from a tightened position (see 5.10, Note 2), and incubate at the desired temperature for approximately 3 weeks or until the feeder strips are covered by mycelium. The culture bottles are now ready to receive the test blocks.

NOTE 5—When not in active use, store the test cultures in test tube agar slants in a refrigerator maintained between 2 and 5°C (35 and 40°F). When the slants are used to inoculate petri dishes, inoculate and incubate replacement slants until the surface of the slant is covered by mycelium prior to refrigeration. Test tubes that are 150 by 16 mm in diameter, equipped with a plastic screw cap work well. It is recommended that the liner in the cap be removed before using. Depending on the type of refrigerator used, check the agar slants every 1 to 2 months for loss of moisture. When the culture appears excessively dry, prepare new slants and inoculate (see 9.1). It is suggested that three test tube slants of each test fungus be maintained as outlined above. It is sometimes helpful to place small sapwood sticks into the plants to maintain fungal viability.

11. Preparation and Impregnation of Test Blocks

11.1 *Initial Conditioning and Initial Weights*—Before impregnation, condition the test blocks by either of the following methods:

11.1.1 *Conditioning at Specified Temperature and Relative Humidity*—Mark each block (for example, with waterproof ink) and bring the test blocks to a constant moisture equilibrium in the conditioning room. Weigh the blocks to the nearest 0.01 g just before treatment. This weight (T_1) is referred to as the initial or untreated weight of the test block (Note 6). After weighing keep the test blocks in the conditioning room until they are to be impregnated with the preservative.

NOTE 6—Coding the different weights as T_1, T_2, and so forth, avoids confusion and simplifies recording data. The suggested system of T designations is as follows, record all weights in grams:

T_1 = initial weight of the conditioned or oven-dried test block before impregnation,

T_2 = weight of the test block immediately after impregnation and wiping (equals T_1 plus grams of treating solution absorbed),

T_3 = weight of test block plus remaining preservative after conditioning and before exposure to the test fungus,

[8] Bouyoucos, G. J. A., "A Comparison Between the Suction Method and the Centrifuge Method of Determining the Moisture Equivalent of Soils." *Soils Science,* Vol 40, 1935, pp. 165–170.

T_{3w} = weight of the test block plus remaining preservative after weathering or leaching and conditioning and before exposure to the test fungus,
T_m = weight of the test blocks immediately after removal from the test bottle and after adherent mycelium has been brushed off, and
T_4 = weight of the test block after test and after final conditioning.

11.1.2 *Ovendrying*—Dry the marked blocks in the drying oven (see 5.3) for 24 h. Remove the blocks to a desiccator and when cool weigh each block to the nearest 0.01 g. This weight is the initial or untreated weight of the block (T_1). Keep the ovendried blocks over desiccant in a sealed chamber until impregnated.

11.2 *Preparation of Treating Solutions of Preservatives Under Test*—Make up the treating solutions of the preservatives in appropriate concentrations so as to leave in the blocks, after removal of the solvent, a range of retentions running from below to above the anticipated threshold. The lowest retention (exclusive of blocks treated with solvent only, see 11.6) shall be low enough to permit fungus attack with consequent decay and definite weight loss. When the preservative is soluble in water make the required concentrations with distilled water. Dilute Preservatives that are insoluble in water, such as creosote, creosote-coal tar solutions, and solutions of pentachlorophenol or copper naphthenate in an oil carrier, with toluene. The dilutions are necessary to provide a uniform distribution of preservative at retentions low enough to permit fungus attack and to determine threshold values for the various test fungi employed. All preservatives should be in such a state of solution before use that the active ingredients will be well distributed throughout the treated wood. The number of concentrations to be made up for any given preservative depends on whether it is possible to anticipate a threshold and how close it is necessary to determine it. The preferred procedure is to run a preliminary test to locate an approximate threshold, and then to run a critical series of tests, narrowing the interval between concentrations around the level of the approximate threshold.

11.3 *Number of Blocks in a Treatment Group*—It is desirable to treat the least number of blocks per concentration required to prepare no less than four test bottles. The number of blocks to be treated with a given concentration of preservative, for testing by a single fungus, may vary. The smaller the interval between concentrations of treating solution, the smaller the number required. The primary concern should be to see that the number of blocks is sufficient to define clearly the relation between preservative retention and weight change in the blocks during test.

11.4 *Treatment Procedure*—Choose blocks for treatment that have the narrowest practicable spread in density. Weight differences not exceeding 0.5 g among blocks in a given test are desirable. Place the blocks to be treated with a given concentration of preservative in a suitable beaker and weight them down to prevent eventual floating on the treating solution. Place the beaker in the desiccator or bell-jar of the impregnation apparatus (Fig. 1) directly below the outlet from the separatory funnel or treating solution flask. Attach the apparatus to the vacuum or suction pump and reduce the pressure in the treating chamber to 100 mm (3.94 in.) Hg or less and hold this pressure for 20 to 30 min. Pour the prepared solution of the preservative into the separatory funnel or solution flask, using sufficient solution so that the blocks will remain covered after the treatment is completed. At the end of the holding period close the stopcock to the vacuum or suction pump and open the access to the separatory funnel or solution flask so that the treating solution flows into the beaker with the test blocks and covers them. The partial vacuum is broken and the beaker is removed from the treating chamber and covered with a watch glass or plastic film to minimize loss of treating solution by evaporation. Leave the blocks submerged in the treating solution for at least 30 min. A longer time is necessary for some treating solutions in order to obtain maximum and uniform absorptions in the blocks (Note 7). Remove the blocks from the solution individually, wipe lightly to remove surface preservative solution, and immediately weigh to the nearest 0.01 g (T_2). Record the gain in weight ($T_2 - T_1$) as the grams of treating solution absorbed (Note 6).

NOTE 7—Calculated retentions are based on equal distribution of the preservative in the wood. Such distribution is obtained only if the absorptions represent the total amount of liquid a block will hold. Most of the air has been evacuated from the wood before the preservative solution is introduced, leaving the cell cavities free to be filled with the solution. The amount of air space available to hold liquids has been determined for woods of different density and moisture content.[9] The approximate maximum absorption to be expected can therefore be computed from the percentage of air space and the specific gravity of the treating solution. The greater the volume of air space (the lower the density), the greater the absorption that should be obtained if all air cavities are filled. With water-soluble preservatives, absorptions are higher than for oil-type preservatives because water not only fills the air spaces, but is also absorbed in the cell walls.

11.5 *Calculation of Retentions*—Calculate the amount of preservative absorbed by the block, that is, the retention, as kilograms per cubic metre (kg/m^3) of wood as follows:

$$\text{Retention, kg/m}^3 = (GC/V) \times 10 \quad (3)$$

and as pounds of preservative per cubic foot (lb/ft ^{3}) of wood as follows:

$$\text{Retention, lb/ft}^3 = (GC(62.4)/100\ V) \quad (4)$$

where:

G = $(T_2 - T_1)$ = grams of treating solution absorbed by the block (initial weight of block before treatment subtracted from the initial weight plus the treating solution absorbed),
C = grams of preservative in 100 g of treating solution,
V = volume of block, cm^3, and
62.4 = factor for converting grams per cubic centimetre to pounds per cubic foot.

To convert kg/m^3 to lb/ft^3, divide by 16.0.

11.6 *Control Blocks*—For each fungus used in a preservative test, condition and treat with solvent only at least five blocks taken from the density lot being used in that particular test. Put these control blocks through all steps of the decay test. The uniformity of weight loss caused in them by the test fungus

[9] MacLean, J. D., "Effect of Moisture Changes on the Shrinkage, Swelling, Specific Gravity, Air or Void Space, Weight, and Similar Properties of Wood," Forest Products Laboratory Report No. 1448, U.S. Forest Service, 1958.

serves as an indication of the normalcy of the individual tests and an indication of the stability of test conditions from one test to another. The control blocks also provide weight change data for use when it is desired to correct the weights of blocks for changes in moisture content in solvent retention. Similarly, untreated control blocks in the same density range are put through all stages of the decay test when evaluating, for example, undiluted creosote or some chemical modification of wood.

12. Conditioning Treated Blocks

12.1 After the blocks have been impregnated and weighed to obtain absorption, space them on trays or racks and expose them under open laboratory room conditions for 48 to 72 h. Then place all such treated blocks, whether initially conditioned or ovendried, in the conditioning room and leave them there for 21 days, unless the blocks are to be weathered (see 13.2).

12.2 Weigh the individual blocks to the nearest 0.01 g (T_3) just before they are sterilized and subsequently placed in contact with the test fungus on the feeder strip (see Section 14). This weight (Note 6) will be used in determining the loss during the decay test (see Section 17).

13. Preservative Permanence

13.1 Weathering of test blocks is viewed as an additional variable for the preservative systems. If oil-type preservatives are evaluated, the weathering procedure involves a leaching test, followed by a volatility test, followed by conditioning to constant weight. If water-borne preservatives are evaluated, the procedure involves a leaching test followed by conditioning to constant weight.

13.2 *Weathering Procedure for Oil-Type Preservatives*—Start the weathering procedure 3 days after treatment of the blocks. Expose the blocks first to a leaching test and then to a volatility test. The schedule for both totals is approximately 14 days.

13.2.1 *Leaching Test*—Space equally all blocks of a given retention group, but no more than eight per beaker, on hardware cloth supports in 600-cm^3 beakers. Weight down the blocks in each beaker and add water to fill the beaker. Keep the water at room temperature for 2 h. Then pour off the water, remove the weights and proceed with the volatility test.

13.2.2 *Volatility Test*—Prior to placing the beakers containing the blocks in the forced draft oven, check to make sure that the blocks are still spaced equally on the hardware cloth without touching the side of the beaker or one another. Weather the blocks for 334 h (13.9 days) at a suitable temperature to maintain the blocks at 48.9 ± 1.1°C (120 ± 2°F).

13.3 *Leaching Procedure for Water-Borne Preservatives*—Expose the blocks to leaching by reagent water in a constant-temperature room maintained at 27 ± 1°C (80 ± 2°F). For each retention group, place four treated blocks in a 225-cm^3 (8-oz), widemouth, screw-capped bottle and weight them down with inert material and cover the blocks with 50-cm^3 of water for each block. Place the bottles containing the blocks covered with water in a vacuum desiccator and evacuate to a pressure of 100 mm (3.94 in.) Hg or less for ½ h or until air bubbles cease to escape from the submerged blocks. Then break the vacuum to allow the impregnation of blocks by the water, and remove the weights from the blocks. After 6, 24, and 48 h, and thereafter at every 24-h interval for a period of 2 weeks remove the leach water from the bottle, measure in a graduate and save for analysis if desired. Replace the amount of leach water removed by a fresh change of water.[4]

13.3.1 *Loss of Preservative*—Remove any film, especially in the case of copper bearing preservatives, adhering to the glass walls of the bottles with hydrochloric acid and add to the leach water for analysis. Check 10-cm^3 aliquots of the 6-h leach water qualitatively for each of the components in the original salt formulation. When the presence of leached components has been established qualitatively, determine their amount by appropriate chemical analysis. Calculate the loss from the original retention, as determined by the weight increase of the blocks ($T_2 - T_1$).

13.4 *Weight After Weathering or Leaching*—At the end of the weathering or leaching procedure, place the blocks directly in the conditioning room. As soon as the blocks have reached a constant moisture equilibrium, weigh each block to the nearest 0.01 g (T_{3w}).

14. Sterilization of Treated Test Blocks and Placement in Culture Bottles

14.1 *Sterilization of Test Blocks*—Sterilization by ionizing radiation is the preferred method and avoids driving off volatiles that may be removed using other methods. the specimen shall be arranged parallel with each other and flat within a polyethylene envelope sealed with hot iron welding. The polyethylene sheeting shall be at least 90 microns in thickness (Note 8). the envelopes are subjected to a radiation level of 2.0 or 2.5 Mrad when using radioisotopes or 2.0 to 5.0 Mrad if electron accelerators are used. After irradiation, the envelopes may be stored for several weeks. When ready to insert the blocks into the bottles, open the envelope under aseptic conditions.

NOTE 8—While the sheet can be welded on three sides, it is more practical to use sheeting available as a roll. It is advisable to reduce the oxygen content of the envelope (see 14.1). Before putting the test blocks in the culture bottles, place them by retention groups into closed containers and steam at 100 ± 2°C (212 ± 4°F) for 20 min. After cooling, aspetically place the test blocks, with a cross-section face centered in contact with the mycelium-covered feeder strip, in the previously prepared culture bottles (see 5.10 and 9.1).

14.1.1 Other methods of sterilization are acceptable (steam, microwave, gases) but volatiles in the blocks may be driven off. If steam sterilization is used, put the conditioned and weighed test blocks into tightly closed containers and steam them at 100°C (212°F) for 20 min.

14.1.2 After cooling, aseptically place the test blocks, with a cross-section face centered in contact with the mycelium-covered feeder strip, in the previously prepared culture bottles (see 5.10 and 9.1). Label the culture bottles with the block numbers they contain.

15. Incubation and Duration of Test

15.1 Place screw cap snugly on each culture bottle, then loosen one-quarter turn to allow for aeration (see 5.11, Note 2,

and Section 10). Place the culture bottles containing the test blocks in the incubation room (see 5.2) and keep them there for 12 weeks.

16. Handling Test Blocks After Exposure to Test Fungi

16.1 At the end of the incubation period (see Section 15), remove the blocks from the culture bottles. Carefully brush off the mycelium. If data on moisture content in the blocks are desired, weigh the individual blocks to the nearest 0.01 g (T_m). Then place the blocks on trays or racks in the conditioning chamber or room until they reach equilibrium weight. (Heat in excess of 32°C (90°F) should not be used.) Then weigh the blocks individually to the nearest 0.01 g (T_4).

17. Calculation of Weight Losses

17.1 Calculate the weight loss from the conditioned weights of the block immediately before and after testing, as follows (see Note 5):

$$\text{Weight loss, } \% = (100\,(T_3 - T_4)/T_3) \quad (5)$$

Use $T_{3\ w}$ instead of T_3 in the case of weathered blocks.

18. Evaluation of Test Results

18.1 *Threshold Retention*—Determine the minimum amount of preservative that is effective in preventing significant decay, under the conditions of the test, by a particular fungus. This amount of preservative in terms of kilograms per cubic metre (kg/m^3) or pounds per cubic foot (lb/ft^3) of wood, is referred to as the " threshold retention." The threshold is determined by visual inspection and by estimating the point at which weight loss caused by decay does not occur.

18.2 *Visual Evidence of Decay*—Examine the blocks after they have been conditioned and weighed at the completion of the test. Distortion, shrinkage, and softening of the blocks should be considered as evidences of decay. The abnormalities are usually pronounced in the blocks with the lower retentions of preservative, but they become progressively less evident with higher retentions, until they are no longer apparent. Visual inspection should not be relied on since decay is sometimes not readily seen, especially near the threshold retention level.

18.3 *Use of Weight-Loss Percentages*— The calculated weight-loss percentages may contain certain operational weight losses as a result of loss of preservative during the test period or failure of the blocks to come to exactly the same moisture equilibrium as before the test period. Such losses, which are not due to decay, may show a progressive increase from lower to higher retentions (Fig. 3), particularly in the case of a volatile preservative. When the weight losses in blocks show an increase, and the increase is progressive as the retention decreases, decay loss, in addition to any operational loss, is indicated. The threshold value is then considered to be the average retention at which this transition in weight loss is indicated. Slight surface decay that is not progressive may be shown by blocks having retentions that are somewhat above the threshold. In such instances, decay losses leading to determination of thresholds are not considered to occur until there is a definite increase in weight losses over and above those relatively low ones that result from surface decay.

19. Refining the Threshold

19.1 If the threshold is indeterminate because of wide intervals in the retention gradient chosen, or for any other reason, repeat the test using closer gradient intervals near the approximate threshold level, with a view to locating the threshold as accurately as possible.

20. Report

20.1 For each evaluation, report the following general conditions:

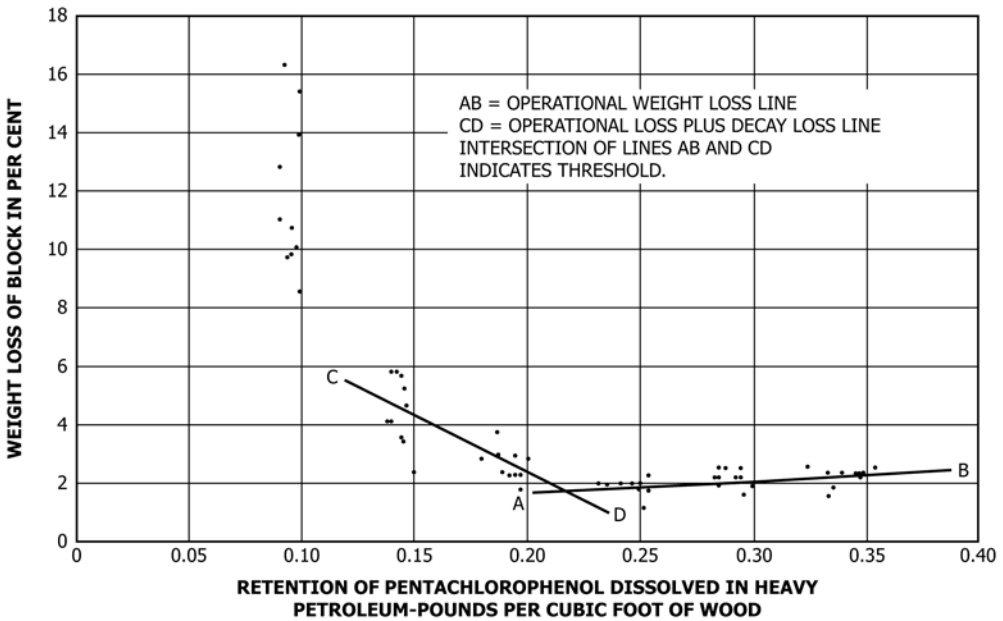


FIG. 3 Weight Loss for Pentachlorophenol Treated Blocks Put Through Soil-Block Test—Test Fungus Madison 617

20.1.1 Types of culture bottle used, and number of specimens per bottle;

20.1.2 Species of wood used for test blocks and feeder strips and available information or observations on source, growth rate, and so forth to indicate compliance with 7;

20.1.3 Chemical identification and treatment retentions for standard or control preservatives utilized;

20.1.4 Chemical identification and treatment retentions targeted for preservatives under investigation;

20.1.5 Moisture holding capacity of the soil used and amount of soil per bottle;

20.1.6 Test fungi and strains used for soft woods;

20.1.7 Test fungi and strains used for hardwoods;

20.1.8 Observations on viability of test fungi;

20.1.9 Weathering procedures, if used, and preservative loss due to weathering if determined; and

20.1.10 Method of block sterilization.

20.2 For each preservative evaluated, tabulate the following by retention group:

20.2.1 Solution concentration of the preservatives,

20.2.2 Mean retention (kg/m^3) of each group,

20.2.3 Post-treatment conditioning or storage, and

20.2.4 Any chemical analysis of blocks to verify treatment levels.

20.3 Tabulate the following for each preservative retention group and fungi used:

20.3.1 Weight loss % of each block and mean weight loss % for the group,

20.3.2 Weight loss % of untreated controls and standard preservative controls that were used, and

20.3.3 Observations of visible decay.

20.4 Tabulate the following utilized for each preservative by fungus:

20.4.1 Determination of threshold retention, and

20.4.2 Plots of weight loss versus retention for fungus exposure.

21. Precision and Bias

21.1 This test is dependent on the physiological action of living organisms, thus care is necessary to avoid inferring that the test results are quantitatively repeatable and reproducible. Comparing results with one formation with those of other formations in different soil block evaluations is not valid.

22. Keywords

22.1 cultures; laboratory; preservatives; soil block