INTERLABORATORY TESTING OF WOOD PRESERVATIVES USING ASTM D1413-61[1]

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ABSTRACT

Toxic thresholds for three wood preservatives determined at the Western Forest Products Laboratory, Vancouver, Canada, and at the U. S. Forest Products Laboratory, Madison, Wisconsin, showed considerable interlaboratory variation. At the Vancouver laboratory, toxic thresholds were always lower, and wood weight losses for the untreated control blocks were higher than the corresponding data for the Madison laboratory. Toxic thresholds calculated from both oven-dry weights and conditioned weights were similar within each laboratory. Ethylene oxide was unsuitable for the sterilization of creosote-treated wood because of the resulting increase in its toxicity to fungi.

Keywords: Soil block culture, creosote, pentachlorophenol, copper-chrome-arsenate, wood-decaying fungi, ethylene oxide, propylene oxide, sterilization, toxic threshold, decay.

INTRODUCTION

The American Society for Testing and Materials standard method for testing wood preservatives by laboratory soil-block cultures (ASTM D1413-61) was developed during the 1950's (Duncan and Richards 1950; Duncan 1953), adopted as a tentative standard in 1956, and then accepted as a full standard in 1961 (ASTM 1961). It is currently under revision by a joint ASTM and American Wood Preservers' Association task force, but as yet the revised standard has not been accepted as a replacement for the 1961 standard.

Because the concept of a standard test implies some level of reproducibility and because no mention of reproducibility is given in ASTM D1413-61, it would be assumed that a reference preservative would give a reproducible toxic threshold when repeatedly tested using this standard. Also, regardless of where this test is conducted, the measured threshold value should be approximately the same. However, there is evidence that standard tests for determining the toxicity of wood preservatives have some inherent variations that result in a certain lack of reproducibility (Anon. 1956; Da Costa et al. 1969; Liese et al. 1935; Savory and Bravery 1970; Schulze et al. 1950). Hilditch and Hambling (1971), in their review of wood-preservative tests, state that toxic values determined for preservatives by these methods are precise only within one or two concentration steps. This important fact had been observed and accepted more than two decades previously by Schulze et al. (1950), but it has seldom received comment since that time.

These observations prompted a collaborative study between the Western Forest Products Laboratory, Vancouver, Canada, and the U.S. Forest Products Laboratory, Madison, Wisconsin, during 1966–1971.

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The purposes of this study were:

1. to compare toxic thresholds obtained at the Vancouver and Madison laboratories, using the D1413-61 soil-block test, the same strains of fungi, the same three wood preservatives, and equivalent samples of wood blocks;
2. to compare toxic thresholds for the three preservatives, on the basis of:
   (a) conditioned wood blocks;
   (b) oven-dry wood blocks;
3. to compare ethylene oxide and moist heat methods of wood-block sterilization.

EXPERIMENTAL METHODS

Basically, both laboratories followed the methods laid down in ASTM D1413-61. Any significant deviations from this standard are described under each test method.

Test I

Preservatives tested: Copper-chrome-arsenate (CCA–II)—provided by the Vancouver laboratory for use by both laboratories. Dilutions were made on a weight/weight basis with distilled water. The following concentrations were used: 0.02%, 0.04%, 0.16%, 0.32%, and 0.80%.

Pentachlorophenol—provided by the Madison laboratory for use by both laboratories. It was supplied as a 5% concentrate in P9 oil and diluted as required on a weight/weight basis with toluene. The following concentrations were used: 0.075%, 0.15%, 0.30%, and 0.60%.

Creosote—conformed to Class I type of the U.S. Federal Specifications (1967) and was provided by the Madison laboratory for use by both laboratories. Dilutions were made as required on a weight/weight basis with toluene. The following concentrations were used: 1.0%, 2.0%, 4.0%, and 8.0%.

Wood used: Air-dried lodgepole pine (*Pinus contorta* Dougl.) sapwood was obtained from fresh-felled logs and provided by the Vancouver laboratory for use at both laboratories.

Fungi used with the indicated preservatives:

Fungi		Preservatives
Lentinus lepideus Fr.	FPLV No. 44A	Creosote
L. lepideus	MAD No. 534	Creosote
Lenzites trabea Pers. ex Fr.	FPLV No. 47B	Pentachlorophenol
L. trabea	MAD No. 617	Copper-chrome-arsenate, pentachlorophenol and creosote
Poria carbonica Overholts	FPLV No. 111A	Copper-chrome-arsenate, pentachlorophenol and creosote
P. monticola Murr.	FPLV No. 120D	Copper-chrome-arsenate
P. monticola	MAD No. 698	Copper-chrome-arsenate and pentachlorophenol

Fresh isolations of the above fungi were exchanged by the two laboratories just prior to this study. Therefore, there was no reason to suspect abnormalities in any of the culture used by either of the two laboratories.

Deviations from ASTM D1413-61 methods: Besides using conditioned weights for determining the weight of wood lost during the incubation period with each fungus, both laboratories used a replicate set of test blocks, measuring oven-dry weights before and after incubation to provide an index of wood substance lost. Corrections for the weight of preservative in these test blocks were applied, where applicable, following BS 838 (British Standards Institution 1961).

At the Vancouver laboratory, 16-oz (450-ml) cylindrical pickle jars, containing approximately 200 g (dry weight) of soil, were used as culture bottles. Feeder strips of lodgepole pine $\frac{1}{16} \times 1 \times 2$ inches ($0.16 \times 2.54 \times 5.08$ cm) were used, one per bottle

with two test blocks on each feeder strip. These modifications have been suggested for acceptance in the proposed revision of the ASTM D1413-61 test, since they have been used for many years by the Eastern Forest Products Laboratory, Ottawa, Canada, in testing wood preservatives without any indication of effect on the determined toxic thresholds.

Test II

Preservative tested: Creosote, as for Test I but using higher treating concentrations, was provided by the Madison laboratory for use at both laboratories.

Wood used: Air-dried ponderosa pine (*Pinus ponderosa* Laws.) sapwood was obtained separately by each laboratory from fresh-felled logs.

Fungi used:

L. lepideus	MAD No. 534
L. trabea	MAD No. 617

Deviations from ASTM D1413-61 methods: Changes were the same as those described for Test I, but also included the following:

At the Vancouver laboratory, wood-block sterilization was achieved by using ethylene oxide under vacuum (Smith 1965), followed by ventilation in a horizontal laminar air-flow clean bench.

Test III

Preservative used: Creosote, as in Test I but using higher treating concentrations, was provided by the Madison laboratory for use at both laboratories.

Wood used: Air-dried ponderosa pine sapwood was obtained from fresh-felled logs and supplied by the Vancouver laboratory for use at both laboratories.

Fungi used:

L. lepideus	MAD No. 534
L. trabea	MAD No. 617

Deviations from ASTM D1413-61 methods: Changes were the same as those described for Test I, but also included the following:

At the Vancouver laboratory, both the ASTM D1413-61 specified steam-sterilization technique and the ethylene-oxide sterilization method were tested. At the Madison laboratory, only the ethylene-oxide method was used.

RESULTS AND DISCUSSION

The threshold retentions for copper-chrome-arsenate, pentachlorophenol and creosote were found to be higher when determined at the Madison laboratory than at the Vancouver laboratory (Tables 1 and 3). This interlaboratory variation was found for both the conditioned and oven-dry methods of determining threshold retentions. The Princes Risborough Forest Products Research Laboratory carried out cooperative trials between three laboratories in England (Anon. 1956) in which they tested several preservatives using a soil-block method and the same or different soils. Their conclusions suggested that greater variations in determined threshold retentions occurred when tests were carried out at different laboratories than when the tests were conducted at the same laboratory, but with different soils. The unimportance of the soil used, within reasonable limits, has been suggested by the work of Duncan (1958). She concluded that soil texture and organic content do not cause serious variations in the determined threshold values, provided that the soils have a water-holding capacity within the range of about 20 to 40%. However, the moisture content of the soil, if considerably greater than its water-holding capacity, could cause serious increases in the moisture content of the wood blocks, thereby resulting in decreased toxic thresholds. Since soils from both laboratories in our study had been adjusted according to the procedure recommended in ASTM D1413-61, soil moisture content would not appear to have affected the measured threshold values.

It could be interpreted from the work of Duncan (1958) that soils with a low organic content gave unusually low decay rates, accompanied by higher toxic thresholds, than would be expected from a nor-

TABLE 1. ASTM designation D1413-61 soil-block test of copper-chrome-arsenate, pentachlorophenol and creosote preservatives, conducted at the Vancouver and Madison forest products laboratories, using both conditioned and oven-dry weights in calculations of the toxic thresholds.

Fungus	Threshold retention[1] (pcf)			
	Vancouver laboratory		Madison laboratory	
	Conditioned	Oven-dry	Conditioned	Oven-dry
		Copper-Chrome-Arsenate		
Lenzites trabea : 617	0.08 (4.2)[2]	0.08 (0.6)	0.38 (0.1)	<0.38 (0.0)
Poria carbonica : 111A	0.02 (5.0)	0.02 (0.6)	0.08 (0.5)	0.08 (0.3)
Poria monticola : 120D	>0.36 (9.6)	>0.36 (3.6)	0.39 (1.6)	0.39 (0.0)
Poria monticola : 698	<0.15 (4.8)	0.08 (0.0)	0.15 (3.4)	0.15 (1.5)
		Pentachlorophenol		
Lenzites trabea : 47B	0.10 (4.4)	0.10 (0.3)	0.19 (3.3)	0.19 (0.0)
Lenzites trabea : 617	0.10 (3.5)	0.05 (3.0)	0.19 (1.7)	0.19 (2.6)
Poria carbonica : 111A	<0.02 (3.2)	<0.02 (1.5)	0.05 (0.1)	<0.05 (0.0)
Poria monticola : 698	0.10 (3.7)	0.10 (0.0)	0.19 (2.7)	0.19 (0.0)
		Creosote		
Lentinus lepideus : 44A				
Lentinus lepideus : 534	Threshold retentions not reached, therefore more than 2.6 in all cases.			
Lenzites trabea : 617				
Poria carbonica : 111A				

[1] Where a threshold retention is not reached, an indication of this value is given based on the closest preservative concentration tested.

[2] Figures in parentheses are the percentage weight losses corresponding to the estimated threshold retentions.

mal loamy soil where the converse was indicated. Since the Vancouver soil was taken from a horticultural mixture prepared by the University of British Columbia and known to have a high organic content, it is possible that the organic content of the Vancouver soil was higher than that of the Madison soil.

TABLE 2. Percentage dry weight losses of control, untreated wood blocks in Test I, calculated from both conditioned and oven-dry weight measurements. Values are normally averages of six replicates; larger numbers of replicates are indicated by figures in parentheses.

Fungus	PERCENT WEIGHT LOSS			
	Vancouver laboratory		Madison laboratory	
	Conditioned	Oven-dry	Conditioned	Oven-dry
Lentinus lepideus : 44A	44.2	44.5	40.0	40.7
Lentinus lepideus : 534	42.2	39.0	35.9	38.0
Lenzites trabea : 47B	58.6	61.1	34.3	40.0
Lenzites trabea : 617	52.4(18)	51.2(18)	32.3	37.7
Poria carbonica : 111A	52.2(18)	46.2(18)	39.1	30.7
Poria monticola : 120D	16.5	15.6	11.3	11.7
Poria monticola : 698	58.0(12)	56.5(12)	26.0	40.0

There was a considerable variation between laboratories in wood-weight loss following decay by the same strains of fungi (Tables 2 and 4). The wood-weight loss values from Vancouver were generally 30 to 70% greater than those measured at Madison. This could be attributed to the possible higher organic content of the Vancouver soil. Since wood-destroying fungi, both Basidiomycetes and soft-rot fungi, are cellulolytic in their degradation of wood and since some of the involved enzymes are inducible, it would be reasonable to expect a higher activity of wood degradation following culture of these organisms on a substrate somewhat enriched in cellulose. Considering the soft-rot fungi, Savory and Bravery (1970) observed indications with sterile soil as a growth medium and using the fungus *Chaetomium globosum* that decay was directly related to the organic content of the soil. Similarly, Wälchli (1970) tested several soil types and also concluded that the degradation of wood by soft-rot fungi was directly related to the organic content of the soil. Therefore, it is conceivable that both the soft-rot fungi and the wood-destroying Basidiomycetes show a similar response to the presence of cellulosic organic matter in the soil.

In our study it is possible that the observed differences in decay rate were caused by variations within the soils of other nutrients, such at nitrogenous compounds, essential for the growth of fungi. However, in the absence of any evidence to support this latter theory, preference would be given to the theory implicating the level of organic matter within the soil. Future studies should consider comparative soil analyses, which were not possible in our tests.

However, the present results exemplify an apparent anomaly, where cultural conditions favoring large wood weight losses by a fungus result in a lower and not a higher resistance of this fungus to a fungicide.

Significant differences between replicate determinations of the toxic thresholds for

TABLE 3. ASTM D1413-61 soil-block tests of creosote conducted at the Vancouver and Madison forest products laboratories, using both conditioned and oven-dry weights in calculations of the toxic thresholds and either ethylene oxide or heat as a means of sterilizing the wood blocks.

Fungus	Threshold retention (pcf)			
	Vancouver laboratory		Madison laboratory	
	Conditioned	Oven-dry	Conditioned	Oven-dry
Test II				
Lentinus lepideus : 534	2.0(E)[1]	1.7(E)	6.9(H)[2]	5.8(H)
Lenzites trabea : 617	2.0(E)	1.7(E)	5.2(H)	2.1(H)
Test III				
Lentinus lepideus : 534	-	0.7(E)	3.7(E)	3.7(E)
	-	2.2(H)	-	-
Lenzites trabea : 617	-	1.0(E)	4.0(E)	3.5(E)
	-	1.0(H)		

[1]Wood blocks sterilized using ethylene oxide.

[2]Wood blocks sterilized using heat.

creosote, using a modified ASTM soil-block technique, were described by Da Costa et al. (1969). They attributed these discrepancies to intertree variability of the *Pinus radiata* wood blocks used in their experiments, despite the blocks being closely matched for density and solution uptake. However, in our studies with both Test I (lodgepole pine) and Test III (ponderosa pine), one homogeneous mixture of wood blocks of each tree species was taken and random samples were used by each laboratory. Therefore, the intertree variability observed by Da Costa et al. is unlikely to be causing the interlaboratory variations in thresholds observed in our studies.

Considering the two methods for calculating the preservative toxic thresholds, there were no consistent important differences between results obtained using either conditioned or oven-dry weights (Tables 1 and 3). Therefore, there is no evidence to suggest that the measurement of threshold retentions, based on oven-dry weights and using corrections for the preservative uptake where necessary, should not be an alternative in the existing ASTM D1413-61 test. The oven-dry method is already used in some European standards (British Standards Institution 1961; Deutsche Normenausschuss 1939; Nordic Wood Preservation Council 1970; Urad pro Normalizaci 1961),

TABLE 4. Percentage dry weight losses of control, untreated wood blocks in tests II and III, calculated from both conditioned and oven-dry weight measurements. Each value is an average of six replicates.

Fungus	PERCENT WEIGHT LOSS			
	Vancouver laboratory		Madison laboratory	
	Conditioned	Oven-dry	Conditioned	Oven-dry
Test II				
Lentinus lepideus : 534	42.6(E)[1][2.7][2]	41.0(E)[3.4]	33.2(H)[3]	32.1(H)
Lenzites trabea : 617	57.4(E) [2.6]	52.4(E)[3.1]	33.8(H)	45.6(H)
Test III				
Lentinus lepideus : 534	-	45.4(E)[1.9]	34.5(E)	33.8(E)
	-	48.6(H)[3.6]	-	-
Lenzites trabea : 617	-	50.0(E)[1.7]	42.1(E)	39.1(E)
	-	50.3(H)[2.3]	-	-

[1]Wood blocks sterilized using ethylene oxide.

[2]Figures in brackets are standard deviations.

[3]Wood blocks sterilized using heat.

where saving of experimental time and storage area for conditioning the wood blocks is achieved.

The use of ethylene oxide as a sterilizing agent for wood blocks has many advantages (Smith 1965, 1968) and, apart from its unreliable reaction with older cultures of some strains of *L. lepideus* (Smith 1965), its use does not seem to alter the subsequent rate of decay of wood by some brown- or white-rot fungi (Smith and Sharman 1971). However, following the observation of Da Costa and Osborne (1969) that propylene oxide can increase the apparent toxicity of creosote in wood, thereby giving an erroneously low threshold value, it was suspected that ethylene oxide might react in a similar way. Both ethylene and propylene oxides are alkylating agents and effective in sterilizing wood (Smith 1965). From Tests II and III (Table 3), the ethylene oxide-sterilization treatment on creosote-impregnated wood clearly increased its subsequent toxicity to *L. lepideus*. Threshold values based on oven-dry weights, at the Vancouver and Madison laboratories, decreased from 2.2 to 0.7 pcf and about 5.8 to 3.7 pcf, respectively, when heat and ethylene oxide-sterilization methods were compared. Within the Vancouver laboratory, there was no evidence of any similar effect with the fungus *L. trabea* (Table 3), nor was there any evidence of residual ethylene oxide toxicity with control, unimpregnated wood blocks (Table 4). Therefore, the ethylene oxide (and propylene oxide)-creosote reac-

TABLE 5. The effect of ethylene oxide and heat sterilization of creosote impregnated wood blocks on their final moisture content, after three months' incubation in soil jars in the absence of fungi. Each value is an average of four blocks from two jars. Results from the Vancouver laboratory only.

Treating concentration of creosote (%)	Final average moisture contents (percentage oven-dry weights) and standard deviations			
	Sterilizing method			
	Ethylene oxide		Heat	
0.0	52.6	2.4	38.1	1.9
1.5	44.2	2.4	37.3	3.4
2.0	53.9	2.0	38.7	3.2
3.0	47.5	4.5	39.3	3.2
5.0	51.0	1.0	36.6	1.7
7.0	51.4	4.8	39.2	4.3

tion seems to be specific to the fungus *L. lepideus*. The use of propylene oxide (1:2-epoxypropane) as a sterilizing agent for preservative-treated blocks was included in the British Standard 838 (1961), but our results and those of Da Costa and Osborne (1969) and Smith (1965) would suggest that neither propylene nor ethylene oxide should be used as sterilizing agents when creosote is being tested, especially against the fungus *L. lepideus*.

The ethylene oxide-sterilization method also appeared to cause the creosote-treated and untreated blocks to achieve a higher final moisture content (Table 5). This might be connected to small residues of ethylene oxide or ethylene glycol in the blocks after sterilization. No previous observations concerning this effect of ethylene oxide on the moisture content of wood have been found. Conversely, however, McMillin (1963) found that ethylene oxide treatment of hard maple wood, using trimethylamine as a catalyst at elevated temperatures and pressures, resulted in a decreased hygroscopicity of the wood. Whether real or illusory, the presently observed differences in moisture content seemed to have no effect on the weight loss of wood blocks during decay (Table 4).

CONCLUSIONS

Using the ASTM D1413-61 soil-block test:

1. Interlaboratory variation of determined preservative threshold retentions could occur to the magnitude of two to four times.
2. Considerable variation can occur in the rate of decay of unimpregnated control wood blocks measured at different laboratories, this possibly being directly related to the organic content of the soil. Further research is required to clarify this possible influence of soil type on wood-decay toxicity tests.
3. Ethylene oxide is unsuitable for the sterilization of creosote-treated blocks, since it appears considerably to increase their toxicity to the fungus *L. lepideus*.
4. Either conditioned weights or oven-dry

weights (with a correction factor for volatile preservatives) can be used to calculate threshold retentions for preservatives.

5. Standard tests should be modified ultimately to include some indication of possible interlaboratory variation in derived preservative toxic thresholds.

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