

DETERMINATION OF FUNGITOXIC VALUE OF PRESERVATIVES IN LABORATORY WOOD-BLOCK TESTS

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ABSTRACT

Laboratory-modified agar-block tests were made to determine the fungitoxic value of the wood preservative CCA against the test fungus *Serpula lacrymans*. The procedure applied to determine the results accounted for different standards: EN (toxic limit), ASTM (threshold retention), GOST (threshold retention and protection probability), PN (toxic dose), and JIS (value of efficiency). In order to improve the objectivity and repeatability of the toxic value results, statistical methods were used. Regression equations related sample mass loss and preservative retention.

Keywords: CCA, toxicity data, agar-block method, *Serpula lacrymans*, statistical estimation.

INTRODUCTION

Ever since the first methods were proposed to test the toxic value of wood preservatives (the agar-block method—Liese et al. 1935; the soil-block method—Flerov and Popov 1933; Leutritz 1939), a number of modifications have been introduced, differing only in details (Bravery 1974; Ważny 1975, 1976; Levi 1978).

Among the numerous aspects of the complex analytical procedure, particular importance is attached to the determination of the toxic value. The procedures used to determine this value, as well as the way it is presented, differ in the existing national standards and in the methods proposed by various authors. These differences in methodology make the comparison of data from different laboratories rather difficult.

The West German (DIN 52 176: 1972) and British standards (BS 838: 1961), both based on the agar-block method, use "toxic limit" to obtain the final result, defined as the interval between that concentration of preservative in kg/m³ that just permits decay and the next highest concentration in the series that inhibits all decay. The same approach is used to present data in the majority of European countries' standards (Ważny 1975, 1976).

In the Polish standard (PN 79/C 04903, 1978) and the proposed standard for the Council for Mutual Economic Aid countries (ST SEW), toxic value is shown on a chart as an intersection point between the empirical dependence curve of sample mass loss in relation to preservative retention, and the line denoting the

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level of a 3% sample mass loss. The same form was used in soil-block method by Rak and Unligil (1978) and Cookson and Greaves (1986).

The American standard (ASTM D 1413-76), based on the soil-block method, uses the notion of threshold retention, which is graphically determined as a point of intersection between two lines: one of which is the dependence line between the operational mass loss in treated fungus-free samples and preservative retention in kg/m^3 ; the other refers to the same relationship, but only for mass loss in treated fungus-infected samples.

In the Soviet standard GOST 16712-71 (the soil-block method) threshold retention is also graphically determined, expressed as the percentage of preservative concentration at which the mass loss constitutes 5% of the total mass loss in untreated control samples infected by the fungus.

In another Soviet standard GOST 24008-80 (the agar-block method), the concept of the timber protection probability is used. This value is first expressed in percentages and later transformed according to the probit scale. An acceptable fungicide will have a timber protection probability of 95% or 6.64 on the probit scale. Essentially, the method is used to evaluate the effectiveness of the protection of wood against stain and mould fungi, but it can also be successfully applied for wood-destroying fungi (Belenkov 1968; Seletskaya and Belenkov 1971; Sozonova and Belenkov 1972).

To be accepted by the Japanese standard (JIS A 9302) a given wood preservative is evaluated in terms of the "value of efficiency" denoting 80 or 90% protection of samples as compared with the untreated ones.

None of the existing national standards for laboratory testing of the fungitoxic value of preservatives against basidiomycetes uses mathematical methods of data processing. A few papers, however, have been produced over the years containing suggestions in this respect, mainly for the soil-block method. McKnight (1957, 1958) and Nance and Amburgey (1976) applied base to logarithmic functions, Link and DeGroot (1987) natural logarithms, and Belenkov (1968) probit function to this problem. For the agar-block, Igmandy (1974) proposed probit, Gyarmati (1977) and Gyarmati and Gyarmati (1982) probit, logarithmic and angular transformation in a statistical approach.

The purpose of the present research was to compare the toxic values of a chosen wood preservative as calculated according to different standard methods.

The comparison of results obtained in different countries and in different laboratories, with different analytical methods, is an important problem of international scientific and technological co-operation.

MATERIALS AND METHODS

For the evaluation of toxicity data, a modified agar-block test was used. Blocks of $20 \times 20 \times 15$ mm were cut from the sapwood of two trees of *Pinus radiata* D. Don. The blocks were conditioned to constant mass at 12% moisture content before being treated by vacuum impregnation (30 min vacuum of 88 kPa followed by 30 min soak at atmospheric pressure). The water-soluble wood preservative copper-chrome-arsenate (CCA composition: $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 35%, $\text{K}_2\text{Cr}_2\text{O}_7$ 45%, $\text{As}_2\text{O}_5 \cdot 2\text{H}_2\text{O}$ 20%) was used. Twelve concentrations of preservative (from 0.15 to $5.0 \text{ kg}/\text{m}^3$) in distilled water were selected in steps of logarithmic progression. Six replicate blocks at each concentration were used. The blocks were then held for six weeks at room temperature for fixation of preservative, reconditioned to

constant mass (at 12% MC), weighed, and sterilized by γ -irradiation before their addition to the test vessels. Screw-capped jars of 150 ml volume were used, containing 100 ml of malt agar (1% malt extract, 2.5% agar), and were steam-sterilized, with plastic mesh separators being sterilized by γ -irradiation. The agar was inoculated with mycelium of the dry rot fungus *Serpula lacrymans* (Schum. ex Fr.) S. F. Gray strain FPRL 12E (obtained via PRL from BAM as Eberswalde 315). Jars of blocks with each preservative concentration, and those with sterile control blocks, were allocated to separate trays, each containing 500 ml tapwater. Trays were covered with foil and incubated at 20 ± 1 C for 12 weeks, after which blocks were removed, cleaned, reconditioned to 12% MC and weighted. Final mass loss was calculated for each concentration.

Mass losses at different preservative retentions for all samples (according to ASTM), or their average values (according to the remaining methods) were based on two retention scales: base 10 and logarithmic. Regression lines were estimated by the method of visual-graphic interpolation.

Toxic values were established by six different methods, as required by appropriate standards, i.e., that of toxic limit—according to EN, threshold retention—ASTM, toxic value (doses)—PN, threshold retention—GOST 16 712, protection probability—GOST 24008 and value of efficiency—JIS, respectively.

Statistical elaboration of results was conducted in a number of ways. Regression equations, correlation coefficients and variances were determined for mass loss and preservative retention. The above values were calculated using the least square method (Steczowicz 1986). In order to choose the best method of data processing, regressions were analyzed in terms of mass loss percentages, and then transformed into probits, base 10 and natural logarithms. For the sake of comparison, a general unified form of a typical polynomial equation was used.

The significance of regression was found with the F test according to Snedecor (1957), at the confidence level of 0.95. The values of mass loss in the range of 0–1% were transformed into their decimal and natural logarithmic equivalents, with their value changed to 1.1%, avoiding negative values of logarithms without changing the total number of observations as was in the case of Nance and Amburgey (1976). The above transformation does not affect the dependence character, as fungitoxic value was determined for the mass loss level of 3%.

As for retention values, all mathematical ways of data processing were presented on the decimal scale (kg/m^3) and the decimal logarithmic scale. The decimal multiplication of retention made it possible to avoid negative values that arise in logarithmic transformations in the 0–1 kg/m^3 range, without changing the essence of the relationship itself. For comparison, manual-graphic interpolation was also used.

In all cases, the fungitoxicity was either calculated by means of regression equations or determined graphically according to the Polish standard (PN-79/C-04303), as an intersection point found by interpolation, or as a calculated regression line between sample mass loss and preservative retention, at the mass loss level of 3%.

RESULTS AND DISCUSSION

The results of toxic evaluation of the CCA obtained by different standard methods are shown in Table 1. These results, except for those referring to EN 113, were obtained by means of visual-graphic interpolation of the regression line

TABLE 1. Toxic data of CCA preservative (against *Serpula lacrymans* in sapwood of *Pinus radiata*) estimated by various standard procedures.

Standard	Value	Toxic data kg/m ³ from	
		Decimal scale	Logarithmic scale
EN 113	Toxic limit	1.00–1.50	
ASTM D 1430-76	Threshold retention	1.60	1.42
PN-79/C-04903	Toxic value (dose)	1.50	1.40
GOST 16712-71	Threshold retention	1.30	1.30
GOST 24008-80	Probability of protection	1.60	1.55
JIS A 9302	Value of efficiency	1.45	1.40

between sample mass loss and preservative retention (Fig. 1), as required by the applied standards.

EN and PN posed the least difficulty in obtaining results. In the former case, there was no problem in establishing the retention limit between the lowest retention value at which mass loss was higher than 3% and the next one at which it was lower than 3%. Likewise, when the PN procedure was followed, there was no problem in finding the intersection point between the empirical line of the 3% loss and the curve of dependence between sample mass loss and preservative retention.

There were some problems with the GOST and JIS standards in tracing the dependence line when visual-graphic interpolation was made. The arrangement of data points allowed for an “N” number of lines to be traced. The choice of the most appropriate line depended largely on the objectivity of interpolation. There were fewer problems with the GOST 24008 method, where the transposition of results from percentages into those of probits made the curves, to some extent, more regular (Finney 1971).

As for the tracing of the regression line, most of the questions arose on the ASTM procedure, especially with reference to the correlating of analytical samples. A widespread scattering of measuring points on the chart made it possible to draw an “N” number of lines, and consequently an “N” number of points of their intersection with the line of operational mass loss.

In this particular case, as in the other procedures, the application of logarithmic scale for preservative retention (X axis) made our task easier. The above form of graphic representation of data, as has already been shown (Ważny 1977; Rak and Unligil 1978; Ważny and Thornton 1989), regularizes to some extent the dependence curve, especially for lower retention values.

Despite some major differences between individual procedures of toxic value assessment required by various standards, the results obtained are similar to a large extent (Table 1). With the fungus *Serpula lacrymans*, the toxic values of the CCA preservative ranged from 1.30 to 1.60 kg/m³ on the decimal scale, and from 1.30 to 1.55 on the logarithmic scale. The toxic limit obtained for the EN standard was 1.00–1.50 kg/m³, thus all the other values fall close to the main limit value or slightly above. The above results, especially those obtained by interpreting the dependence lines between sample mass loss and preservative retention, are not free, however, from subjectivity, due to visual-graphic regularization.

In a situation like this, one can say that the results of toxic value assessment

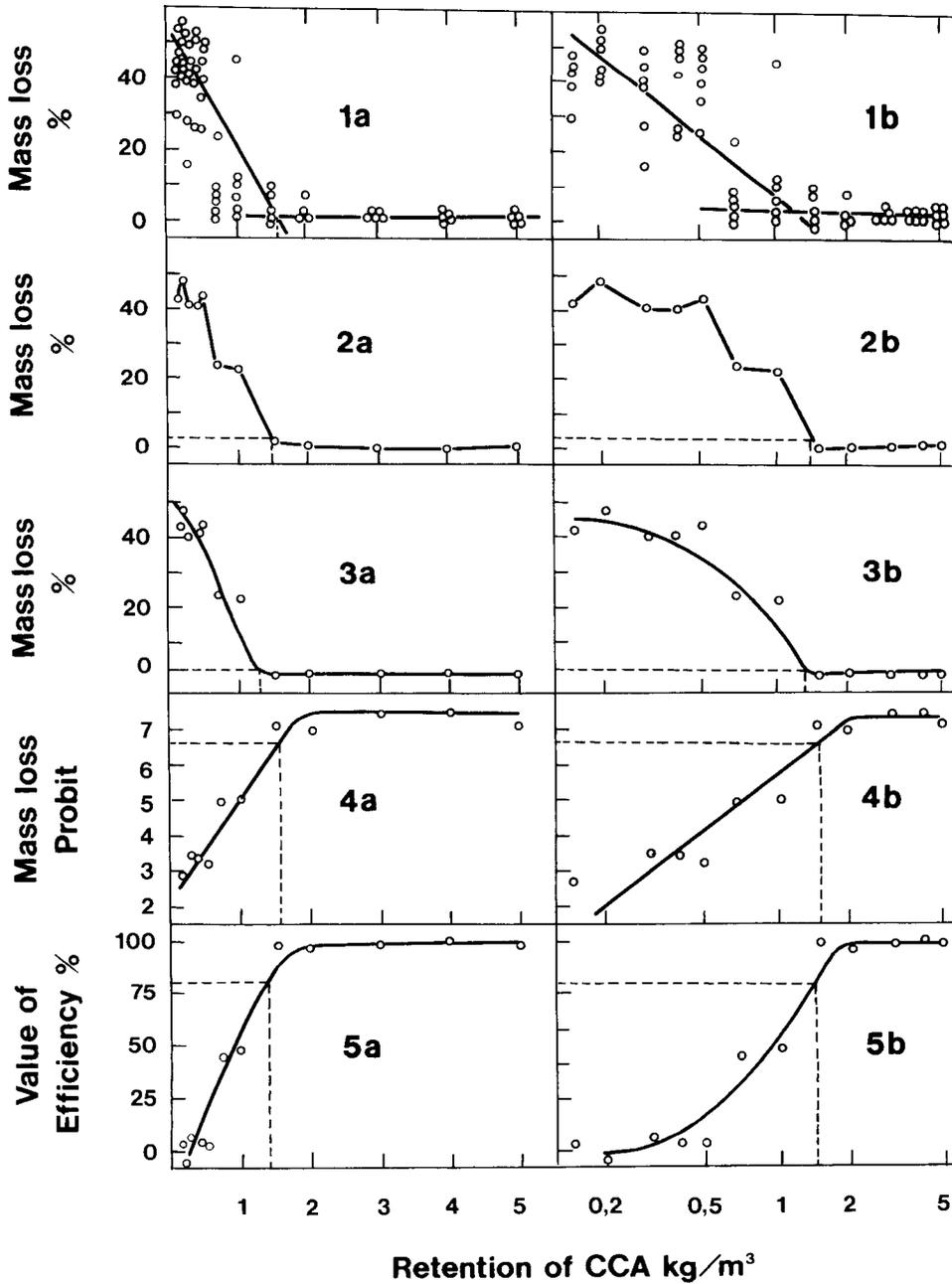


FIG. 1. The correlation lines of mass loss of blocks (against *Serpula lacrymans* in sapwood of *Pinus radiata*) and CCA retention estimated by various procedures: 1. ASTM, 2. PN, 3. GOST 16712-71, 4. GOST 24008-80. 5. JIS; retention in decimal scale (a) and log scale (b).

TABLE 2. Results of the estimation of the toxic value of CCA preservatives (against *Serpula lacrymans* in *Pinus radiata* sapwood) by various methods.

Mass loss (y) given as	Retention (x)	Equation of regression	Coef. of correlation	Variation S_y^2	Toxic value	
					Log 10 (10 R)	kg/m ³
Percent ^a	1 ^b	—	—	—	—	1.50
	2 ^c	—	—	—	—	1.60
Percent	1	$y = -1.572x^3 + 16.080x^2 - 51.458x + 51.328$	0.834	135.380	—	1.66
	2	$y = 33.700x^3 - 82.517x^2 + 17.967x + 44.181$	0.836	134.540	1.265	1.84
Probit	1	$y = 0.250x^2 - 17.751x + 5.175$	0.853	0.416	—	1.49
	2	$y = -2.048x + 5.576$	0.834	0.460	1.190	1.55
Log 10	1	$y = 0.139x^2 - 1.012x + 1.758$	0.873	0.128	—	1.64
	2	$y = -1.263x + 2.037$	0.865	0.135	1.235	1.72
Log n	1	$y = 0.319x^2 - 2.311x + 4.047$	0.873	0.681	—	1.64
	2	$y = -2.908x + 4.690$	0.865	0.713	1.235	1.72

^a — Visual graphic interpolation.

^b — Expressed in kg/m³.

^c — Expressed in log 10 (10 R).

of wood preservatives, obtained by different standard procedures, are comparable, provided there are no other differentiating factors involved. In order to be able to obtain full objectivity, however, it seems absolutely essential to apply the methods of mathematical statistics in the future.

As determined by different statistical methods of calculating the relationship between mass loss and preservative retention, the fungitoxic values for the CCA preservative are shown in Table 2. It contains regression equations of mass loss and preservative retention as well as correlation coefficients and variances, and the toxic value in kg/m³. All statistical methods used confirmed the regression to be significant at the 0.95 confidence level, as expressed by linear, quadratic or cubic equations and by a high correlation coefficient.

Calculated by means of regression equations or determined graphically as an intersection point between the regression line and the mass loss level of 3%, the fungitoxic values were found to be approximate to one another and within the 1.49–1.84 kg/m³ range (Fig. 2). The lowest values were found in the probit transformation of mass loss.

The toxic value found by visual-graphic interpolation equalled ca. 1.5 kg/m³ (Fig. 2 - 1a), so it remained within the limits of minimum values obtained by mathematical methods. It is possible, however, for the dependence curve to be drawn somewhat differently, thereby resulting in a slightly different fungitoxic value.

Seeing that approximate fungitoxic values were obtained by different transformation methods of sample mass loss and preservative retention, one can say that all forms applied can be, in principle, useful in our search for greater objectivity of assessment. As it requires less labor (being a linear equation) and allows simpler forms of calculations to be used, one should use common logarithms to transform the preservative retention values, as well as probit, common, and natural logarithmic transformations in case of mass loss.

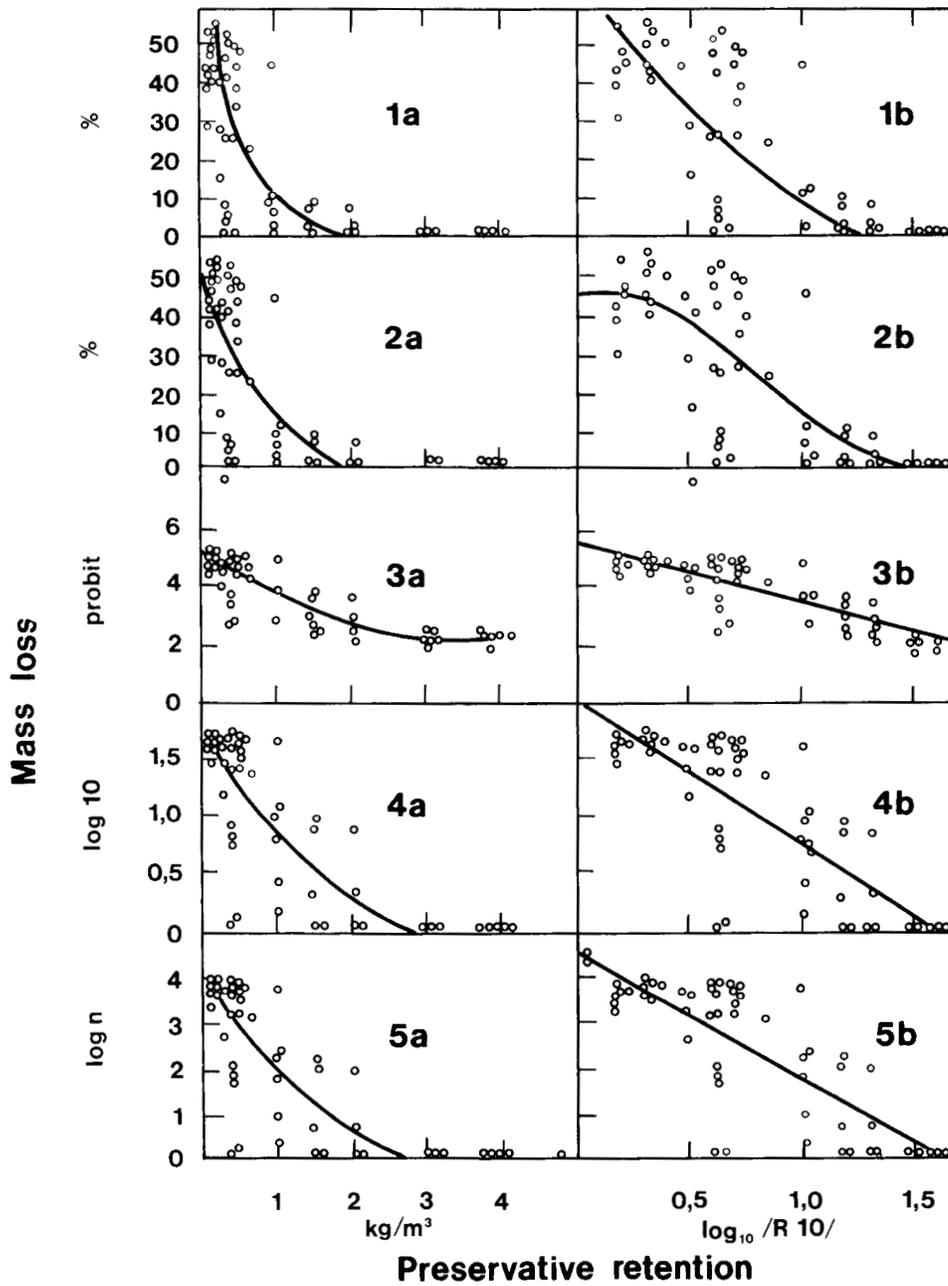


FIG. 2. The correlation lines of mass loss of blocks, caused by *Serpula lacrymans* in sapwood *Pinus radiata*, and CCA retention estimated by various procedures: (1) visual-graphic interpolations. (2) calculated in percent of mass loss. (3) calculated in probit of mass loss. (4) calculated in log 10 of mass loss. (5) calculated in log n of mass loss. (a) retention expressed in kg/m^3 . (b) in $\log_{10} /R 10/$.

The results presented here, in agreement with the experiences of others (Nance and Amburgey 1976; Link and DeGroot 1987) show the statistical methods to be useful in increasing objectivity and repeatability, while defining the fungitoxic values of wood preservatives in laboratory practice. Choosing the most appropriate mathematical procedure requires, however, further research, incorporating additional preservatives and other test fungi.

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