SLOVENSKI STANDARD
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Zaščitna sredstva za les - Določanje meje učinkovitosti proti glivam odprtotrosnicam

Wood preservatives - Determination of toxic values of wood preservatives against wood destroying Basidiomycetes cultured on an agar medium

Holzschutzmittel - Bestimmung der Grenze der Wirksamkeit gegenüber holzzerstörenden Basidiomyceten, die auf Agar gezüchtet werden

Produits de préservation du bois - Détermination du seuil d'efficacité contre les champignons Basidiomycetes lignivores cultivés sur milieu gélosé

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English version

DETERMINATION OF TOXIC VALUES OF WOOD PRESERVATIVES AGAINST WOOD DESTROYING BASIDIOMYCETES CULTERED ON AN AGAR MEDIUM

Détermination du seuil d'efficacité contre les champignons basidiomycètes lignivores cultivés sur milieu gélosé

Bestimmung der Grenze der Wirksamkeit gegenüber holzzerstörenden Basidiomyceten, die auf Agar gezüchtet werden

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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B R I E F H I S T O R Y

This European Standard was drawn up by the Technical Committee CEN/TC 38 " Methods of test for wood preservatives", the Secretariat of which is held by AFNOR.

This European Standard was adopted by CEN on the strength of its acceptance by the following Member countries :-

Austria - Belgium - Denmark - Finland - France - Germany - Netherlands - Norway - Portugal - Spain - Switzerland - United-Kingdom.

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INTRODUCTION

This European Standard describes a laboratory method of test which gives a basis for the assessment of the effectiveness of a wood preservative against wood destroying Basidiomycetes. By using this method it is possible to determine the loading at which impregnated wood of a susceptible species may be regarded as adequately protected under the conditions of test.

This laboratory method provides one criterion by which the value of a product can be assessed, and this criterion should be used to judge the value of the preservative taking into account the methods of application likely to be used. It is also recommended that this information be supplemented by data from other relevant tests and above all practical experience.

1. SCOPE

This European Standard specifies a method for determining the toxic values of wood preservatives previously introduced into the wood by full impregnation against wood destroying Basidiomycetes cultured on an agar medium.

2. FIELD OF APPLICATION

This method is applicable to

- water-insoluble chemicals which are being studied as active ingredients
- organic water-insoluble formulations, as supplied or prepared in the laboratory from concentrates
- water-soluble products, for example salts.

This method is applicable whether or not the test specimens have been subjected to appropriate ageing procedures.

3. PRINCIPLE

Impregnation of several series of test specimens of a susceptible wood species wit solutions in which the concentrations of preservative are ranged in a given progression.

Exposure of these test specimens to attack by Basidiomycetes in pure culture to establish the toxic values of the product under test.

4. TEST MATERIALS

4.1 Biological material. The test fungi to be used as follows:

4.1.1 Obligatory fungi in all cases. (see also Appendix E)

Coniophora puteana (Schumacher ex Fries) Karsten (BAM Ebw. 15) on softwood.

Coriolus versicolor (Linnaeus) Quélet (CTB 863 A) on hardwood.

4.1.2 Two species to be chosen compulsorily on the basis of the nature of the product (see also Annex E) :

For creosotes and similar products

Lentinus lepideus Fries ex Fries (BAM Ebw. 20) on softwood.

Lentinus cyathiformis (Schaeffer ex Fries) Bresadola (CTB 67-02 B) on hardwood.

For all other products

Poria placenta (Fries) Cooke sensu J. Eriksson (FPRL 280) on softwood.

Gloeophyllum trabeum (Persoon ex Fries) Murrill (BAM Ebw. 109) on softwood.

4.1.3 For specific regional uses or conditions, it is also possible to select other fungi on an optional basis 1).

4.1.4 Maintenance of strains. The strains shall be maintained and treated (frequency of subculturing, alternation of culture media, etc.) in accordance with the instructions from their laboratory of origin (see Annex E).

If a strain shows signs of degeneration, it shall no longer be used and the laboratory shall obtain a new standard culture of the strain.

4.2 Products and reagents

4.2.1 Culture medium. The culture medium is a malt agar medium with the following composition:

- malt extract containing 0,9 ± 0,3 % nitrogen: concentrated: 50 g
  : in powder form: 40 g
- agar containing approximately 0,3 % total nitrogen and causing no inhibition of growth of fungi: 20 g to 30 g
- distilled or deionized water; quantity to make up to 1000 ml.

Prepare this medium by warming the mixture in a boiling water bath or a steam bath, stirring until completely dissolved.

Place in each culture vessel a sufficient quantity of the medium to provide a depth of 3 mm to 4 mm when the vessel is laid flat. Plug the vessel as specified in 4.3.9 and sterilize in the autoclave at 121 °C for 20 minutes. Let the vessels cool lying flat.

4.2.2 Solvents and diluents. Distilled or deionized water or, as appropriate, volatile liquids 2) leaving in the wood no residue which would have a toxic effect on the fungi at the end of the post-treatment conditioning period.

4.2.3 Fumigant (if necessary), Xylene technical grade

4.2.4 Sterilant (if necessary) An ethylene-oxide-based sterilant or propylene oxi

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1) See Annex F for a recommended non-comprehensive list of optional fungi.
2) Do not use benzene as solvent because it poses a health risk to those conduct the test.

4.3 Apparatus

4.3.1 Conditioning chamber, well ventilated and controlled at 20 ± 2 °C and 65 ± 5 % r.h.

4.3.2 Culture chamber (incubator or room), dark and capable of being controlled at 22 ± 1 °C and 70 ± 5 % r.h.

4.3.3 Drying oven capable of being controlled at 103 ± 2 °C.

4.3.4 Treatment vessels, of a material that does not react with their contents, for example of glass for organic products and of plastics materials for salts containing fluorine.

4.3.5 Weights, chemically inert for ballasting the test specimens.

4.3.6 Protective gloves

4.3.7 Vacuum vessels fitted with stopcocks.

4.3.8 Vacuum pump fitted with a pressure gauge and capable of maintaining a pressure of 7 mbar 3)

4.3.9 Kolle flasks or equivalent vessels (see figures 1 and 2 in Annex D) with a capacity of between 400 ml and 650 ml, providing a flat surface area of between 90 cm^2 and 120 cm^2 for the medium.

Kolle flasks are usually plugged with a wad of cotton wool. The other containers are usually fitted with leakproof lids, the centres of which are to be pierced with a round hole of approximately 20 mm diameter and plugged with a wad of cotton wool.

4.3.10 Test specimen supports made of glass, stainless steel or any other inert material, that is to say, with no risk of having any effect on the culture medium, the fungus, the wood or the product impregnated, or of being itself modified. The supports are used to prevent direct contact of the specimens with the culture medium, but shall not separate them from it by more than 3 mm.

4.3.11 Ordinary laboratory equipment, including:

a desiccator with an efficient desiccant (silica gel for example) and an analytical balance

5. SAMPLE OF THE PRESERVATIVE

The sample shall be representative of the product to be tested.

6. TEST SPECIMENS

6.1 Species of wood. The species of wood to be used shall be susceptible to attac by fungi and shall be readily impregnated by liquids.

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3) 1 mbar = 10^{-1} kPa.

The reference species are Scots pine (Pinus sylvestris Linnaeus) representing softwoods and beech (Fagus sylvatica Linnaeus) representing hardwoods.

Additional tests may be undertaken using other species corresponding to the above characteristics, and of particular importance for certain countries, but if so this shall be stated in the test report.

6.2 Quality of the wood. Use sound, straight-grained wood without knots, which in the case of Scots pine shall be exclusively sapwood containing little resin and in the case of beech shall be even-grained wood free from tyloses and displaying no red heart.

Use wood showing an average rate of growth of 2,5 to 8 annual rings per centimetre in the case of Scots pine and of 2 to 6 annual rings per centimetre in the case of beech.

The proportion of summer wood in the annual rings shall not exceed 30 % of the whole for pine.

The wood shall not have been floated, stored in water or heated above 60 °C or treated with chemical agents.

6.3 Provision of test specimens. Cut the specimens from planed strips having a cross section of 25 mm x 15 mm, on which the growth rings may run in any direction, with the exception of a completely tangential orientation on the broad faces which is unacceptable.

The longitudinal faces shall be parallel to the direction of the grain. Transverse faces shall be cut neatly and have sharp edges.

Take the specimens required for a test at random from a stock originating from at least 2 trees.

6.4 Dimensions and density of specimens. The nominal dimensions of each specimen, measured at a moisture content of 12 % are as follows:

50 mm x 25 mm x 15 mm

The volume of each specimen is theoretically 18, 75 cm^3, but the dimensions of each test specimen shall be checked so that the actual volume is known.

In a batch of treated specimens, the density may differ from the mean value by ± 10 %. This tolerance is increased to ± 20 % for the control specimens. The mean density of the specimens used for the test shall be recorded in the test report.

6.5 Number and distribution of test specimens. The specimens are divided into:

e_1 – treated test specimens: these are the impregnated specimens subjected to attack by the wood destroying fungi. Use at least 4 treated test specimens for each preservative concentration and for each fungus.

e_2 – untreated test specimens

$e_{2.1}$ - control specimens: these are non-impregnated test specimens, equal in number to the treated test specimens e_1, and are placed one in each culture vessel with the treated test specimens.

$e_{2.2}$ - specimens for virulence control of the fungi used: 6 of these non-impregnated specimens are subjected to attack by each wood destroying fungus.

e_3 - treated check test specimens for calculation of correction co-efficient: these are test specimens treated in exactly the same way as the e_1 test specimens, at least four per concentration, which are placed, after drying, conditioning and any appropriate ageing in uninoculated culture vessels two in each vessel. Variations in mass of these specimens will make it possible to determine the correction factor (C) of the variations in mass of the treated test specimens e_1, resulting from factors other than attack by the test fungi. At a given treating concentration, factor C is the mean change in mass of the e_3 test specimens.

7. PROCEDURE

7.1 Preparation of test specimens

7.1.1 Conditioning of specimens before treatment. Place the numbered test specimens in the oven (4.3.3) and leave them there for 18 h (4). Cool in a desiccator and weigh to the nearest 0,01 g to determine the initial dry mass, m_0. Replace the test specimens in the desiccator and store them there in order to keep them dry until impregnation.

7.1.2 Treatment of test specimens

7.1.2.1 Preparation of treatment solutions. Prepare a series of concentrations (by mass) of the preservative in the appropriate solvent or diluent (4.2.2).

Prepare a series of at least 5 concentrations distributed about the expected toxic values. A solvent or diluent control, i.e. treatment at concentration 0 shall also be used. If the approximate toxic values are unknown, the concentrations shall form a widely spaced geometric series for a first test, and a more closely spaced geometric or arithmetic progression for subsequent tests.

All treatment solutions shall be freshly prepared.

7.1.2.2 Impregnation. Carry out impregnation in ascending order of concentration, starting with the solvent control (concentration = 0).

The following procedure ensures the required complete impregnation of test specimens by the test solutions:

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4) In the case of supplementary tests (6.1) using species of wood other than beech and pine sapwood, this drying time may need to be longer than 18 h; the drying time shall be such that the test specimens reach constant mass.

- for each concentration place the test specimens, kept dry as described in 7.1.1 and of known dry mass m_0, in one of the treatment vessels (4.3.4) so that as much of their surface as possible is exposed (e.g. by piling them crosswise). Ballast the stack of specimens with the weights (4.3.5) to prevent them from floating later when the liquid is admitted.

- place each treatment vessel in one of the vacuum vessels (4.3.7) and after reducing the pressure to 7 mbar hold it at this pressure for 15 min [5] After this period, close the stopcock to the vacuum pump (4.3.8) and open the other stopcock to allow the solution of preservative to be drawn into the treatment vessel within the vacuum vessel. Keep the specimens covered completely by the solution throughout the remainder of impregnation process.

- next, admit air to bring the vacuum desiccator back to atmospheric pressure, remove the treatment vessel with its submerged specimens from the vacuum desiccator, cover it and leave it for 2 h, adding further solution if necessary to keep the specimens fully covered by the liquid.

After this impregnation treatment, remove the test specimens one by one, remove the excess liquid by light blotting with absorbent paper. Immediately weigh each to the nearest 0,05 g to ascertain the mass after impregnation (i.e. m_1).

In the case of water-soluble preservatives, for example salts, and water-insoluble chemicals which are being studied as active products calculate the mass of preservative retained for each test specimen, from the mass of solution absorbed ($m_1 - m_0$) and its concentration 6).

In the case of water-insoluble formulations the retention is expressed for each test specimen in terms of the corresponding mass of the formulation ready for use but if a concentrate is supplied the retention is expressed in terms of the solution prepared ready for use as specified by the manufacturer.

Calculate the mass of preservative retained per unit volume of wood.

If, in a series of simultaneously impregnated test pieces, the quantity of product absorbed by certain test specimens varies by more than 15 % from the mean absorption of the series, the results obtained from these test specimens shall not be included in the mean. These test specimens shall be replaced by supplementary specimens.

7.1.3 Drying and conditioning of specimens after treatment. The drying and conditioning procedures used depend on the nature of the product to be tested and the nature of the diluent or solvent. The procedures recommended below are usually applicable, but should it prove necessary to change them, mention of the alterations made shall be included in the test report.

After impregnation, dry the specimens for 4 weeks in the conditioning chamber (4.3.1). Arrange the test specimens resting on the narrow faces on two glass rods so that they do not touch each other in covered vessels 100 mm to 200 mm high. Invert the test specimens twice a week.

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5) Observe the proper safety measures for vacuum vessels.

6) When dealing with preservative formulations whose constituents are absorbed selectively by wood, it is necessary to carry out chemical analysis of the solution before and after impregnation. Simarly, analysis is recommended when very dilute solutions are used.