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Wood preservatives — Determination of the preventive efficacy against wood destroying basidiomycetes fungi

ICS 71.100.50

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National foreword

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— present to the responsible international/European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;

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TECHNICAL REPORT

RAPPORT TECHNIQUE

TECHNISCHER BERICHT

CEN/TR 14839

February 2004

ICS 71.100.50

English version

Wood preservatives - Determination of the preventive efficacy against wood destroying basidiomycetes fungi

Produits de préservation du bois - Détermination de l'efficacité préventive vis-à-vis des champignons lignivores basidiomycètes

Holzschutzmittel - Bestimmung der vorbeugenden Wirkung gegen holzzerstörende Basidiomyceten

This Technical Report was approved by CEN on 12 November 2003. It has been drawn up by the Technical Committee CEN/TC 38.

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Foreword

This document (CEN/TR 14839:2004) has been prepared by Technical Committee CEN/TC 38 “Durability of wood and wood-based products”, the secretariat of which is held by AFNOR.

The status of this document as Technical Report has been chosen because it has been judged useful to maintain this old method former ENV 839:1993 superseded by ENV 839:2002 for the use of laboratories.

Introduction

This Technical Report specifies a laboratory method of test which gives a basis for assessing the effectiveness of a wood preservative, when applied mainly as a surface treatment, against basidiomycetes fungi. In contrast the method for determining the toxic values against wood rotting fungi (EN 113) provides a mean of determining the loading at which impregnated wood of a susceptible species can be regarded as adequately protected under the test conditions.

This laboratory method provides one criterion by which the effectiveness of a product can be assessed. In making this assessment the methods by which the preservative may be applied should be taken into account. It is also recommended that results from this test should be supplemented by those from other relevant tests and above all by practical experience.

The procedures described in this standard method are intended to be carried out by suitably trained and/or supervised specialists. Appropriate safety precautions should be observed throughout the use of this Technical Report.

1 Scope

This Technical Report specifies a method of test for the determination of the preventive action of a wood preservative against basidiomycetes fungi when the preservative is applied as a surface treatment to wood.

This method is applicable to formulations of preservatives in a ready to use form as :

- water-insoluble chemicals which are being studied as active fungicides ; or
- organic formulations, as supplied or as prepared in the laboratory by dilution of concentrates ; or
- organic water-dispersible formulations as supplied or as prepared in the laboratory by dilution of concentrates ; or
- water-soluble materials, for example salts.

NOTE This method can be used in conjunction with an appropriate ageing procedure, for example EN 73.

2 Reference

EN ISO 3696, *Water for analytical laboratory use — Specification and test methods* (ISO 3696:1987).

3 Terms and definitions

For the purposes of this Technical Report, the following terms and definitions apply.

3.1
representative sample
sample having its physical or chemical characteristics identical to the volumetric average characteristics of the total volume being sampled

3.2
supplier
sponsor of the test

3.3
superficial application process
process which does not include particular features or procedures intended to overcome the natural resistance of wood to penetration by a wood preservative product in its ready to use form

4 Principle

The test preservative is applied by brushing to the longitudinal faces of a series of test specimens of a susceptible wood species. The treated test specimens are exposed to feeder blocks colonized by pure cultures of basidiomycetes fungi. The lateral penetration of the different fungi through the exposed surface of the test specimens is assessed from sawn cross-sections of the test samples at the end of the exposure period.

5 Test materials and apparatus

5.1 Biological material

The test fungi to be used are as follows :

5.1.1 Obligatory test fungi (brown rots) on Scots pine sapwood.

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

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

*Poria placent*a (Fries) Cooke sensu J. Eriksson (FPRL 280).

The strains shall be obtained and maintained in accordance with annex B.

5.1.2 Obligatory fungus (white rot) on beech, if tests including a white rot fungus are also to be undertaken.

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

The strain shall be obtained and maintained in accordance with annex B.

5.1.3 Additional fungal species

If additional fungi are used, a description of the strain(s) equivalent to that of the obligatory fungi given in annex B shall be recorded in the test report.

The strain(s) shall be maintained in accordance with the instructions from their laboratory of origin.

5.2 Feeder blocks

5.2.1 Wood species

Scots pine sapwood (*Pinus sylvestris* Linnaeus) shall be used for feeder blocks for brown rot fungi and beech (*Fagus sylvatica* Linnaeus) for white rot fungi.

5.2.2 Wood quality

The wood shall be sound and without knots. The wood shall not have been water-stored, floated, chemically treated or steamed. The Scots pine shall be exclusively sapwood containing little resin.

NOTE Wood that has been kiln dried at temperatures below 60 °C may be used.

5.2.3 Dimensions

The dimensions of feeder blocks measured at 12 % (*m/m*) moisture content shall be :

(50 0,5) mm (25 0,5) mm (15 0,5) mm

5.2.4 Number of feeder blocks

Four feeder blocks shall be prepared for each test specimen that is to be used (see 7.5).

5.3 Products and reagents

5.3.1 Water complying with grade 3 of EN ISO 3696.

5.3.2 Culture medium for feeder blocks :

¾ malt extract containing (0,9 0,3) % (*m/m*) nitrogen :

- ¾ concentrated : 50 g ;
- ¾ in powder form : 40 g ;

¾ agar containing approximately 0,3 % (*m/m*) nitrogen and causing no inhibition of growth of fungi : 20 g ;

¾ water : 1 000 ml.

Combine the ingredients and heat to dissolve. Dispense into each culture vessel (5.4.5) a sufficient quantity to give a depth of between 3 mm and 4 mm. Close the vessels and sterilize in the autoclave (5.4.7) at 121 °C for 20 min. Cool to room temperature whilst lying flat.

NOTE Alternatively, the medium can be sterilized then dispensed into sterile vessels under aseptic conditions.

5.3.3 Test substrate for test specimens

An hydrated, laminar, aluminium-iron-magnesium silicate[1)] exfoliated to yield particles up to 3 mm diameter. Particles less than 1 mm shall be removed by sieving. Before use, thoroughly mix the sample of test substrate.

The test substrate shall be used only once.

5.3.4 Acidifying solution for the test substrate

An acidifying solution shall be prepared with the following composition :

¾ potassium chloride (KCl) solution, 0,1 mol/l : 950 ml ;

¾ hydrochloric acid (HCl), solution 0,1 mol/l : 50 ml.

5.3.5 End-sealing product

A material resistant to the penetration of the test preservative and the test fungi and without any fungistatic or fungicidal activity within the test specimen.

NOTE Three coats of a 2-component epoxy lacquer have been found to be suitable.

5.3.6 Fungicidal solution for assessment

Prepare a stock solution of the following composition :

Phenol	0,5 g
(methyl-1-(butylcarbamoyl) benzimidazol-2-ylcarbamate):active ingredient (benomyl[2)])	0,16 g
2, 6-dichloro-4-nitroaniline	0,16 g
Ethanol solution 50 % (V/V)	50 ml

Prepare the ready-for-use solution by adding 5 ml of the stock solution to 1 l water (see 5.3.1).

5.3.7 Staining solution[3)]

A solution of 0,04 % (*m/m*) bromophenol blue in ethanol solution, 50 % (V/V).

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1) Vermiculite is suitable.

2) Propriety products normally contain 50 % (m/m) benomyl.

3) Optionally as an aid to assess penetration of mycelium into the wood after exposure.

5.4 Apparatus

5.4.1 Conditioning chamber, well ventilated and controlled at (20 2) °C and (65 5) % relative humidity.

5.4.2 Culture chamber (incubator or room), dark and controlled at (22 2) °C and (70 5) % relative humidity.

5.4.3 Laboratory work area, well ventilated, where treatment of the test specimens is carried out

CAUTION — It is essential to follow safety procedures for handling flammable and toxic materials.

5.4.4 Test containers

Made of a material which does not have a toxic effect on the test fungi, and provided with a ventilated lid. They shall have a volume of between 2 l and 3 l, and minimum dimensions of (140 120) mm and 85 mm in depth.

NOTE 1 These dimensions are necessary to allow a minimum of 20 mm between the two test specimens and 10 mm between each test specimen and the container.

NOTE 2 A suitable container is shown in Figure 5.

NOTE 3 It is convenient if the containers can be sterilized by autoclaving.

5.4.5 Culture vessels

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 and provided with a ventilated closure.

NOTE Culture vessels used in EN 113 are suitable.

5.4.6 Ordinary laboratory equipment including a balance capable of weighing to the nearest of 0,01 g.

5.4.7 Autoclave

Capable of being controlled and maintaining a temperature of 121 °C.

5.4.8 Sawing equipment

Fine toothed sawing machine for cutting of test specimens for evaluation at the end of the test.

5.4.9 Test specimen supports

Made of a material which has no effect on the test fungi and does not react with the preservative. They shall be of an open texture, have a thickness of (3 0,5) mm and of a sufficient area to support securely the test specimen on the feeder blocks.

NOTE Polyethylene mesh has been found to be suitable.

5.4.10 Feeder block supports

Made of a material which does not react with the culture medium and has no effect on the test fungi. They shall be of an open texture, have a thickness of (3 0,5) mm and of sufficient area to support securely the feeder blocks.

NOTE Polyethylene mesh, glass rods and stainless steel rods have been found to be suitable.

5.4.11 Drying supports, that will give a minimum contact with the treated test specimens to be placed on them. The supports shall be made of a material which does not react with the test solvent or test preservative, for example glass for organic products or plastics material for salts containing fluorine.

5.4.12 Equipment for chemical gas sterilization or access to a radiation service (see annex C).

5.4.13 Facilities for vacuum filtration

Comprising vacuum source, filter flask, Büchner funnel and coarse grade fitting filter papers of 125 mm diameter.

6 Sampling of the preservative

The sample of preservative shall be representative of the product to be tested. Samples shall be stored and handled in accordance with any written instructions from the supplier.

NOTE For the sampling of preservatives from bulk supplies, the procedure given in EN 212 should be used.

7 Test specimens

7.1 Species of wood

The test shall be carried out on Scots pine sapwood (*Pinus sylvestris* Linnaeus) for softwoods and beech (*Fagus sylvatica* Linnaeus) for hardwoods.

7.2 Wood quality

The wood shall be free from cracks, stain, decay, insect damage or other defects. The wood shall not have been water-stored, floated, chemically treated or steamed.

NOTE 1 Wood that has been kiln dried at temperatures below 60 °C can be used.

The Scots pine shall be exclusively sapwood containing little resin. The growth rate shall be between 2,5 and 8 annual growth rings per 10 mm. The proportion of latewood in the annual rings shall not exceed 30 % of the whole.

The beech shall be even-grained, free from tyloses, discolouration and red heart. It shall have between 2 and 6 annual growth rings per 10 mm.

NOTE 2 It is recommended that specimens of similar growth rate are used within a single test.

7.3 Provision of the test specimens

Cut the test specimens from planed strips having a cross section (30 50) mm.

The orientation of the test specimen shall be with the annual rings having a minimum contact angle of 10° to the same broad face.

NOTE The preferred orientation is with the annual rings at (45 10)° to the broad face which is to be exposed to the test fungus.

The cross sections shall be cut cleanly and have sharp edges. Avoid using test specimens from the butt or crown of the tree.

7.4 Dimensions of test specimens

The dimensions of each specimen after conditioning to a moisture content of (12 2) % (m/m) shall be:

(100 0,5) mm (50 0,5) mm (30 0,5) mm

NOTE A moisture meter of the two-pronged electrical conductivity type is suitable for assessing moisture content.

Mark each specimen so that it can be identified throughout the test.

7.5 Number of tests specimens

The specimens shall be divided into :

- e_1: treated test specimens : use at least four treated test specimens for each combination of preservative, test fungus ;
- e_2 : untreated test specimens : use at least four test specimens for each fungus.

NOTE These untreated test specimens are used to confirm the virulence of the test fungi. They are exposed in separate test containers to avoid possible effects due to the test preservative.

8 Procedure

8.1 Preparation of test specimens

8.1.1 Conditioning of test specimens before treatment

Place the test specimens (7.4) in the conditioning chamber (5.4.1) until consecutive weightings at 24 h intervals are within ± 0,1 g.

8.1.2 End-sealing

Apply the end-sealing product (5.3.5) to both end-grain surfaces of each test specimen. End-sealing shall be applied three times, allow to dry between each application.

8.1.3 Treatment with the test product

Treat the e_1 test specimens (7.5) on all longitudinal faces with the test preservative under test by brushing, weighing each test specimen before and after treatment to the nearest 0,01 g. The application rate shall follow the supplier's instructions.

NOTE The test specimens can be used to examine other treatment processes, for example dipping, and double vacuum; the method used should be recorded in the test report.

Calculate the uptake of preservative and express it in grams per square metre of treated surface.

8.1.4 Drying

Following treatment (8.1.3), place the treated test specimens on drying supports (5.4.11) with the broad face which subsequently will be remote from the feeder blocks in contact with the supports. Dry the test specimens until weightings at 24 h intervals are within 0,1 g.

NOTE 1 The length of drying period will vary with the nature of preservative.

NOTE 2 If test specimens are to be subjected to an ageing procedure or the assessment procedure described in annex E is to be used, the appropriate procedures should be carried out at this stage.

8.2 Preparation of test containers

8.2.1 Inoculation of feeder blocks

Sterilize the feeder blocks (5.2) by one of the methods described in annex C.

Inoculate the culture medium (5.3.2) with the test fungi (5.1) no more than 7 days after preparation. Obtain the inocula from cultures which are less than 4 weeks old. Place the inoculated culture vessels in the culture chamber (5.4.2) and allow the fungi to grow until they have covered the agar surface; in no case shall this period exceed 4 weeks.

Introduce aseptically into each culture vessel a single layer of sterile feeder blocks on sterile feeder blocks supports. Return to the incubation chamber for between 3 weeks and 6 weeks.

NOTE According to material, the feeder supports (5.4.10) can be sterilized by one of the methods described in annex C or by heating in an oven at 160 °C during 4 h.

8.2.2 Preparation of the test substrate

Into each test container (5.4.4) place a quantity of the test substrate (5.3.3) equivalent to 40 % of the volume of the container. Close and sterilize by autoclaving at 121 °C for 20 min, or by using one of the methods described in annex C.

Determine the water holding capacity (WHC) of the test substrate (5.3.3) using the method described in annex D. The WHC of the vermiculite shall not to be less than 300 %.

Calculate the quantity of acidifying solution (5.3.4) required to adjust the moisture content of the test substrate in each test container as follows :

Gloeophyllum trabeum	-	WHC of test substrate only ;
Coniophora puteana	-	WHC of test substrate plus half the conditioned dry mass of the test specimens ;
Poria placenta	-	WHC of test substrate plus half the conditioned dry mass of the test specimens ;
Coriolus versicolor	-	WHC of test substrate plus the total conditioned dry mass of the test specimens.

NOTE The test fungi have different moisture requirements. The moisture requirements of any optional fungi should be determined prior to testing.

Sterilize the calculated quantities of acidifying solution (5.3.4) by autoclaving at 121 °C for 20 min. Cool to room temperature. Under aseptic conditions, distribute evenly over the surface of the test substrate.

8.2.3 Installation of feeder blocks

Under aseptic conditions transfer eight feeder blocks previously exposed to the same test fungus into each charged test container (8.2.2). Place the feeder blocks in two sets of four, separating the sets from one another by at least 20 mm and from the sides of the test container by at least 10 mm . Gently press down the feeder blocks until they penetrate half way into the test substrate (see Figure 1). Place a test specimen support, previously sterilized by one of the methods described in annex C or by autoclaving at 121 °C for 20 min, onto each set of feeder blocks.

8.3 Exposure of test specimens in containers

In each test container, place on the feeder blocks (see Figure 2), two test specimens (e_1) treated with the same test preservative, or two untreated test specimens (e_2), previously sterilized by one of the methods described in annex C.

8.4 Culture conditions and duration of test

After introducing the test specimens place the test containers in the culture chamber (5.4.2) for during 12 weeks.

8.5 Assessment of fungal development in the test specimens

After exposure, remove the test specimens from the test container and clean gently of adhering mycelium. Record evidence of water logging, the presence of contaminating fungi or inhibition of growth. Saw each test specimen transversely at 20 mm intervals (see Figure 3). Immediately, dip each section in fungicidal solution (5.3.6) assemble in their original positions and secure with an elastic band.

Place the test specimens in an empty test container (5.4.4) in the culture chamber (5.4.2) for one week. Separate the sections and assess the fungal penetration on one face revealed by each saw cut (4 per test specimen). Additionally, note if the test fungus has colonized via the sealed end-grain faces.

NOTE 1 Mycelium, which has developed on the sawn faces during incubation and which is indicative of colonization by the test fungus may be viewed using a X10 magnification hand lens.

NOTE 2 Additionally, it is possible also to assess fungal development using bromophenol blue solution (5.3.7). It is recommended to allow the sawn test specimens to dry in the laboratory work area (5.4.3) for 48 h. It is recommended to coat the section to be examined with the bromophenol blue solution. The area colonized by the test fungus is indicated by a yellow colour and the uncolonized area is indicated by a blue colour. This method of detection is sensible at only early stages of decay and should be considered as an aid for visual assessment. However this method does not work in all cases.

NOTE 3 Assessment can be done according to annex E.

Rate the level of attack of treated test specimens (e_1) in accordance with the rating scale given in Table 1 (see also Figure 4).

Estimate the percentage of the cross-section of the untreated test specimens (e_2) that has been colonized to the nearest 5 %.

NOTE 4 This operation can be assisted by using tracing graph paper.

Reject any test specimen which has a rating of less than 2 and which was noted as being waterlogged, affected by contaminating fungi or by volatiles from the preservative.

Table 1 — Rating system for evaluation of fungal attack

Rating	Condition and appearance
0	No evidence of colonization or decay.
1	Limited points or continuous line of mycelium along the broad face nearest the feeder blocks.
2	Penetration of mycelium more than 1/5 of the cross section.
NOTE If assessment according to annex E is used, rating 2 corresponds to a penetration of mycelium more than 5 mm.	

9 Validity of test

The results for a given test fungus shall be accepted as valid if more than 75 % of the cross-section of at least three untreated test specimens (e_2) have been colonized by the test fungus and if the results from at least three treated test specimens (e_1) have been accepted.

10 Calculation of results

Calculate the notional mean rating for each set of replicate test specimens excluding any rejected replicates.

NOTE The notional mean will normally be based on four ratings on each of four test specimens.

11 Test report

The test report shall include at least the following (see also annex A for an example) :

a) the number of this Technical Report and date of its publication ;

b) the name of the supplier of the wood preservative product under test ;

c) the type (clause 1) of the wood preservative product tested together with its unique name or code and with an indication of whether or not the composition has been declared ;

d) the name and concentration of the active ingredient ;

e) the solvent or diluent used ;

f) the species of wood used and its density ;

g) the species and strain numbers of the fungi used for the test ;

h) the concentrations tested ;

i) the date of the application of the wood preservative product ;

j) for each treated test specimen :

- ¾ the corresponding quantity, in grams per square metre, of the wood preservative product under test ;

k) the conditioning period after treatment ;

l) any ageing procedures carried out, specifying the type, conditions and duration, with possible reference to a standard ;

m) the date when the test specimens were exposed to the test fungi ;

n) the date of examination of the test specimens ;

o) the method used for assessment ;

p) the assessment rating on each cross-section of each replicate examined ;

q) the notional mean value obtained for the total 16 cross-sections for each test fungus ;

r) the percentage of the cross-sections colonized on control test specimens and a statement as to whether or not these data validate the test ;

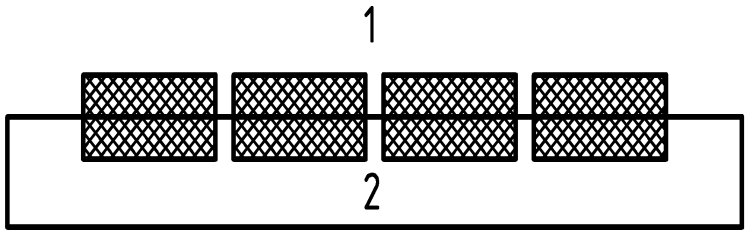
s) the name of the organization responsible for the test report and the date of issue ;

t) the name and signature of officer(s) in charge of testing ;

u) the following note :

"The interpretation and practical conclusions that can be drawn from a test report demand a specialized knowledge of the subject of wood preservation and, for this reason, the test report cannot of itself constitute an approval certificate".

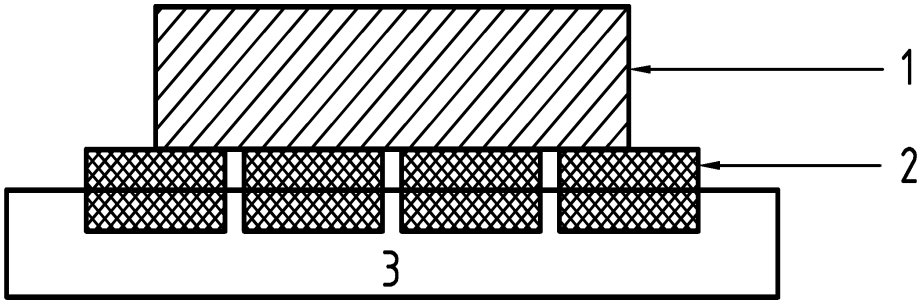
The test report shall list any variation from the described test method and any factors that may have influenced the results.



Key

1 Feeder block supports
2 Test substrate

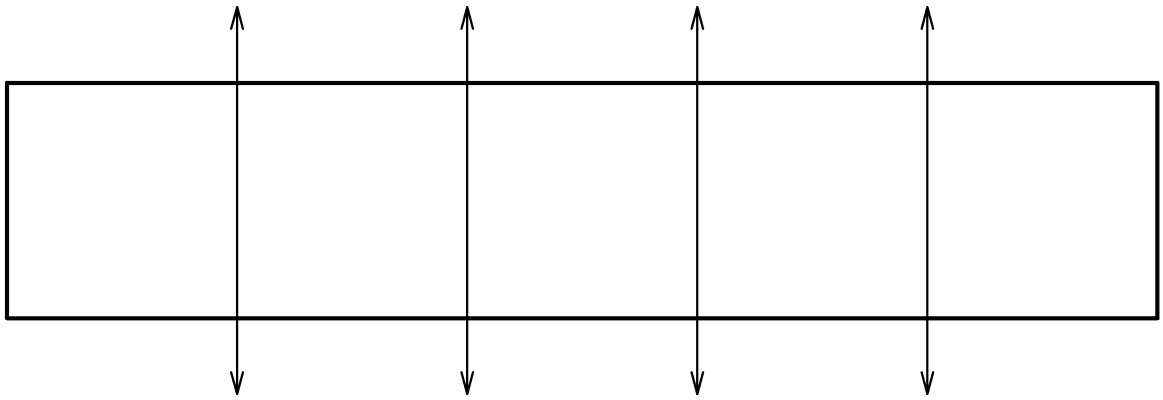
Figure 1 — The positioning of feeder blocks supports on test substrate



Key

1 Test specimen
2 Feeder block supports
3 Test substrate

Figure 2 — The positioning of test specimen on four feeder blocks supports



Key

Cut lines every 20 mm

Figure 3 — The position of cutting for evaluation

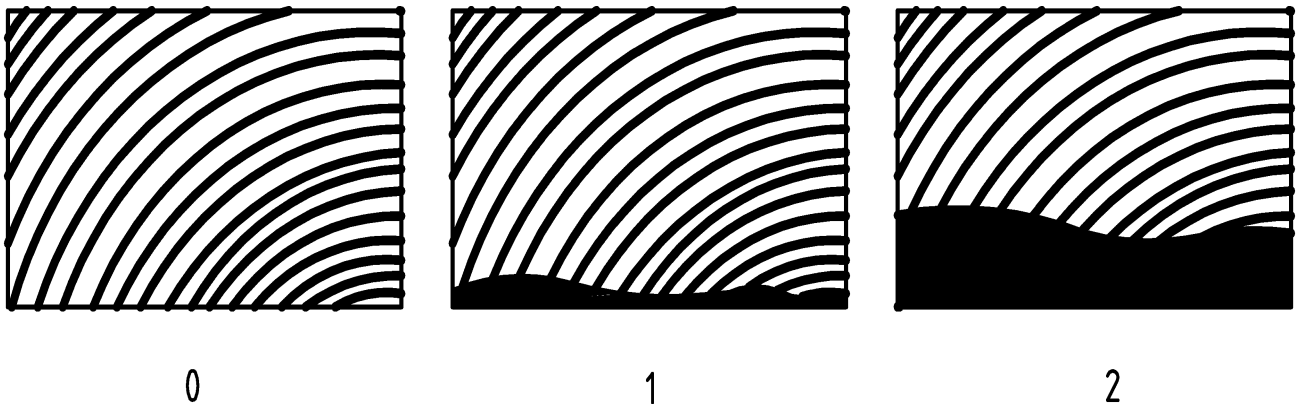


Figure 4 — The rating of fungal colonization in exposed test specimens

Figure 5 — Example of convenient containers for the test, containing two test specimens on the test substrate (the lid had been removed)

Annex A

Example of a test report

Number and date of this Technical Report :	CEN TR 14839: 2003.
¾ name of the supplier :	Company A
¾ name and type of the wood preservative product : X -	organic solution, the full formulation of which was declared ;
¾ name and concentration of the active ingredient :	Y a mass fraction of 1,5 % ;
¾ solvent or diluent used :	none, supplied ready to use ;
¾ species of wood used :	Scots pine sapwood (*Pinus sylvestris* L.), and the density 485 kg/m^3 ;
¾ species of fungi used :	
	- *Coniophora puteana* (BAM Ebw.15) ;
	- *Gloeophyllum trabeum* (BAM Ebw.109) ;
	- *Poria polacenta* (FPRL 280) ;
¾ concentration (s) tested :	ready to use ;
¾ date of application :	2002.09.16 ;
¾ quantity of wood preservative product retained :	see Table A.1 ;
¾ duration of conditioning period after treatment :	10 days ;
¾ ageing test carried out :	Evaporative ageing according to EN 73 ;
¾ date of exposure to fungi :	2002.09.26 ;
¾ date of examination :	2002.12.19 ;
¾ method of assessment :	Dipping in fungicidal solution followed by incubation for one week in a culture chamber ;
¾ assessment rating of fungal development on each cross section :	see Table A.1 ;
¾ notional mean value for each fungus :	see Table A.2.
¾ mean percentage of the cross-section colonized by the test fungi for control test specimens :	
	- e_2 : 80 % of the surface of the (4 4) cross-sections ;
¾ this report has been prepared by :	laboratory B ;

¾ location and date : C 2002.12.28 ;

¾ name and signature of the officer(s) in charge : Mr. D.

NOTE The interpretation and the practical conclusions that can be drawn from this test report demand a specialized knowledge of the subject of wood preservation and, for this reason, this test report cannot of itself constitute an approval certificate.

Table A.1 — Results for wood preservative X on Scots pine sapwood against *Coniophora puteana* (BAM Ebw.15)

Number of test specimens	Absorption of solution (g/m^2)	Rating on each cross-section
$e_1 - 1$	154	0 0 1 0
$e_1 - 2$	147	0 0 1 1
$e_1 - 3$	149	0 0 0 0
$e_1 - 4$	148	1 0 0 1
NOTE Notional mean rating 0,31.		

Table A.2 — Results for wood preservative X for the three brown rot fungi

Test fungus	Strain N°	Timber species	Mean absorption of the series (g/m^2)	Notional mean rating
Coniophora puteana	BAM 15	Scots pine	150	0,31
Gloeophyllum trabeum	BAM 109	Scots pine	140	0,06
Poria placenta	FPRL 280	Scots pine	142	0,12

Annex B

Test fungi

B.1 General information on maintenance and acquisition of test strains

Laboratories holding the parent strain should re-isolate the strain if it shows any sign of weakness.

Laboratories which run tests regularly may maintain their strains themselves but if the strain shows signs of weakness, a fresh culture should be obtained from the laboratory of origin. At least every two year, test strains shall be re-isolated from untreated wood which is actively being attacked. Sterilize two feeder blocks and expose them to attack as described in 8.3, for a period of 6 weeks to 8 weeks. Without drying, split open the blocks and remove small sticks of wood from the centre of the blocks. Partly embed the small sticks in the culture medium (5.3.2) prepared in test tubes, and allow the fungi to grow. Use these cultures for future tests. The culture medium used to maintain test strains may contain 1 % (*m/m*) of sterilized saw-dust.

All laboratories maintaining test fungi should test the virulence once a year.

The laboratory sending test cultures should provide a description of all growth features characteristic of the respective fungus.

NOTE When sending cultures special care should be taken to avoid any harmful effects during transport, e.g. by freezing during air-transport. To avoid the effects of X-rays the cultures should be packed in aluminium containers or wrapped in aluminium foil.

International Regulations exist concerning the transport of cultures. Information on these can be obtained from any recognized culture collection for example a member of the European Culture Collection Organization.

B.2 *Coniophora puteana* (Schumacher ex Fries) Karsten (Synonym : *Coniophora cerebella* (Persoon) Duby)

Strain : BAM Ebw. 15 (Bundesanstalt für Materialforschung und-prüfung - D 12200 - BERLIN).

Activity :

Fungus causing a cuboidal brown, rot of hardwood and softwood.

Simple laboratory culture, rapid growth on malt agar medium, or malt agar-peptone.

Loss in mass in 16 weeks of Scots pine sapwood specimens the size of feeder blocks : minimum 20 % (*m/m*).

Maintenance and treatment :

Maintain stock cultures on test tube slopes of a medium containing 3 % (*m/m*) agar, 5 % (*m/m*) malt extract and 1 % (*m/m*) Scots pine sapwood or spruce (*Picea* spp) sawings at a temperature of 5 °C to 8 °C.

Subculture every six months. Every two years cultivate on wood chips with a few millilitres of a solution of malt extract.

B.3 *Gloeophyllum trabeum* (Persoon ex Fries) Murrill (Synonyms : *Lenzites trabea* (Persoon ex Fries) Fries - *Trametes trabea* (Persoon ex Fries) Bresadola)

Strain : BAM Ebw. 1O9 (Bundesanstalt fur Materialforschung and-prüfung - D - 12200 - BERLIN).

Activity :

Fungus causing a cuboidal brown, rot of hardwood and softwood.

Cultivation in well-ventilated conditions, rapid growth on malt agar medium.

Loss in mass in 16 weeks of Scots pine sapwood specimens the size of feeder blocks : minimum 20 % (*m/m*).

Maintenance and treatment :

Maintain stock cultures on test tube slopes of a medium containing 3 % (*m/m*) agar, 5 % (*m/m*) malt extract and 1 % (*m/m*) of softwood sawings at a temperature of 5 °C to 8 °C.

Subculture every six months on malt agar medium.

B.4 *Poria placenta* (M. Larsen et Lombard) Cooke sensu J. Eriksson (Synonym : *Poria monticola* Murrill)

Strain : FPRL 280: (Building Research Establishment Ltd - Garston - WATFORD Herts WD259 XX - U.K)

Activity :

Fungus causing a cuboidal brown rot of softwood.

Loss in mass in 16 weeks of Scots pine sapwood specimens the size of feeder blocks : minimum 20 % (*m/m*).

Maintenance and treatment :

Maintain at 5 °C to 20 °C, keep reserve cultures on 5 % (*m/m*) malt agar medium under mineral oil and subculture every four years.

Keep stock cultures on 5 % (*m/m*) malt agar medium and subculture every three months. Every two years, or more frequently if necessary, inoculate the subcultures on wood or sawdust and subculture on malt agar after 6 weeks to 12 weeks of development on this substrate.

B.5 *Coriolus versicolor* (Linnaeus) Quélet

(Synonym *Polyporus versicolor* Linnaeus ex Fries) – *Polystictus versicolor* (Linnaeus) Saccardo – *Trametes versicolor* (Linnaeus ex Fries) Pilat)

Strain : CTB 863 A (Centre Technique du Bois et de l'Ameublement – Allée de Boutaut BP 227 F 33028 Bordeaux Cedex).

Activity :

Fungus causing a fibrous white rot of hardwood.

Simple laboratory culture, rapid growth on malt agar medium.

Loss in mass in 16 weeks of beech specimens the size of feeder blocks: minimum 20 % (*m/m*).

Maintenance and treatment :

Sub-culture every six weeks on malt agar medium.

Annex C

Methods of sterilization

C.1 Epoxyethane - based sterilant

This method is not recommended for organic preservatives and is unsuitable for products containing boron compounds or chlorinated or phenolic substances.

NOTE The toxic and explosive nature of this product require special safety measures. Reference should be made to any national regulations governing its use.

Place the test specimens individually in low density polyethylene envelopes (thickness between 30 µm to 90 µm) which are sealed by hot iron welding.

Place the test specimens for 60 min in an appropriate apparatus where the epoxyethane is at a concentration of 1,2 g/l at a pressure of 550 kPa, the temperature being 55 °C and the relative humidity being 70 % to 80 %.

Ventilate the test specimens for 5 days by exposing them to a current of sterile air.

Do not open the envelopes until the precise moment when the contents are to be used.

C.2 Ionizing irradiation

This method is suitable for all preservatives and is especially preferred for organic preservatives and those preservatives for which the reactivity with epoxyethane is unknown.

Place the test specimens individually in polyethylene envelopes (at least 90 µm thick) and seal them by hot iron welding.

NOTE 1 Polyethylene sheeting can be used, folding the sheet over the test specimen bed and welding along three sides. It is more practical to use polyethylene tubing sold in rolls. The test specimens are introduce into this tubing which is then welded both sides of the test specimens.

Send the envelopes thus prepared to an irradiation centre. Advice with regard to the packing of the envelopes should be obtained from the irradiation centre.

Subject the envelopes to a dose between 25 kGy[4)] and 50 kGy when using radioisotopes (e.g. ^{60}Co sources) or between 50 kGy and 100 kGy when using electron-accelerators.

NOTE 2 There does not appear to be any difference between sterilization obtained with a high intensity for a short time or a low intensity applied over a prolonged period. After irradiation, the envelopes can be safely stored for several weeks without detrimental effect.

Do not open the envelopes until the precise moment when the contents are to be used.

4) 1 kGy = 1 kJ/kg = 0,1 Mrad.

Annex D

Determination of water holding capacity (WHC)

NOTE The calculation of water holding capacity in this CEN Technical Report is different from that given ENV 807.

D.1 Principle

The ability of a substrate to retain water against the pull of a vacuum pump has been accepted as a measure of its water holding capacity (WHC).

D.2 Procedure

Measure three 250 ml samples of the test substrate (5.3.3) and weigh each one to the nearest 0,1 g to determine the initial mass (m_o). Place each sample in a separate container, flood with water, and allow to soak for 18 h to 24 h.

Place a coarse filter paper in the bottom of a Büchner funnel (5.4.13) and moisten to seal the filter paper to the funnel. Transfer a prepared test sample into the funnel and spread evenly. Apply suction until no more water is being withdrawn from the sample, increasing the degree of suction slowly to avoid perforation of the filter paper. Determine the wet mass ($\underline{m}_1$) of the wet sample plus the wet filter paper. Determine the mass (m_2) of a wet filter paper after subjecting it to suction in the Büchner funnel. Repeat with the other samples.

D.3 Calculations

Calculate the amount of water (W_i) required to raise the moisture content of each sample to its water holding capacity using the following formula :

$$W_i = m_1 - (m_o + m_2)$$

Calculate the mean amount of water (W) from the individual (W_i) amounts for the three samples.

Annex E

Optional method of assessment of colonization of the test specimens by the test fungi

E.1 Principle

Colonization of the test specimens by the test fungi is monitored during the exposure period by recovering the test fungus on baits inserted into holes drilled in the test specimen to within 5 mm of the test face.

E.2 Materials

E.2.1 Sealing plugs

Small plugs between 4 mm and 10 mm in diameter, capable of being sterilized.

NOTE Silicone rubber seals have been found to be suitable; the diameter should be at least 1 mm larger than that of the drills.

E.2.2 Baits

Small inserts capable of being colonized quickly by the test fungi and of being sterilized.

NOTE Proprietary cotton wool buds or unwaxed wooden toothpicks have been found to be suitable.

E.2.3 Benomyl/malt agar medium

¾ malt extract containing (0,9 0,3) % (*m/m*) nitrogen ;

¾ as concentrate 50 g ; or

¾ in powder form 40 g ;

¾ agar containing approximately 0,3 % (*m/m*) total nitrogen and causing no inhibition of growth of fungi: 20 g to 30 g ;

¾ suspension of methyl-1-(butylcarbamoyl)benzimidazol-2-ylcarbamate, 0,1 g in 500 ml of water: 50 ml ;

¾ water to make up to 1 000 ml.

Combine the ingredients of the medium and sterilize in an autoclave at 121 °C for 20 min. Dispense under aseptic conditions into suitable test vessels, for example, in 90 mm diameter Petri dishes; use 20 ml.

E.3 Apparatus

E.3.1 Drilling equipment

Equipment capable of drilling holes into the test specimens between 4 mm and 10 mm in diameter and to within (5 0,1) mm of the test face.

E.4 Procedure

E.4.1 Drilling assessment holes

Following treatment (8.1.3) and drying (8.1.4), drill a minimum of three holes between 4 mm and 10 mm in diameter (see note) into each test specimen, from the broad face of the specimen opposite to the face that is to be exposed to the test fungus, leaving (5 0,1) mm of wood beyond the end of each hole (see Figure E.1). Space the holes evenly along the length of the test specimen.

NOTE The exact diameter of the assessment hole is dictated by the diameter of the sealing plug (E.2.1).

E.4.2 Inserting baits

Make the bait assemblies by inserting a bait through a sealing plug. Place one bait assembly in each hole in each test specimen, ensuring that the tip of the bait is in contact with the base of the hole. Sterilize the test specimens using one of the method I annex C containing the baits and place them on the feeder blocks (see 8.3).

E.4.3 Assessment procedure

E.4.3.1 At minimum intervals of one week, under aseptic conditions, remove the bait assemblies from the test specimens. Remove the sealing plugs and place the baits on benomyl/malt agar medium (E.2.3) contained in Petri dishes; label the Petri dishes to retain the identity of the test specimen, the location of the assessment hole and the total period of incubation of the test specimens.

Place fresh sterile bait assemblies (see note 1) in each hole, ensuring that the baits are in contact with the base of each hole, and continue incubation.

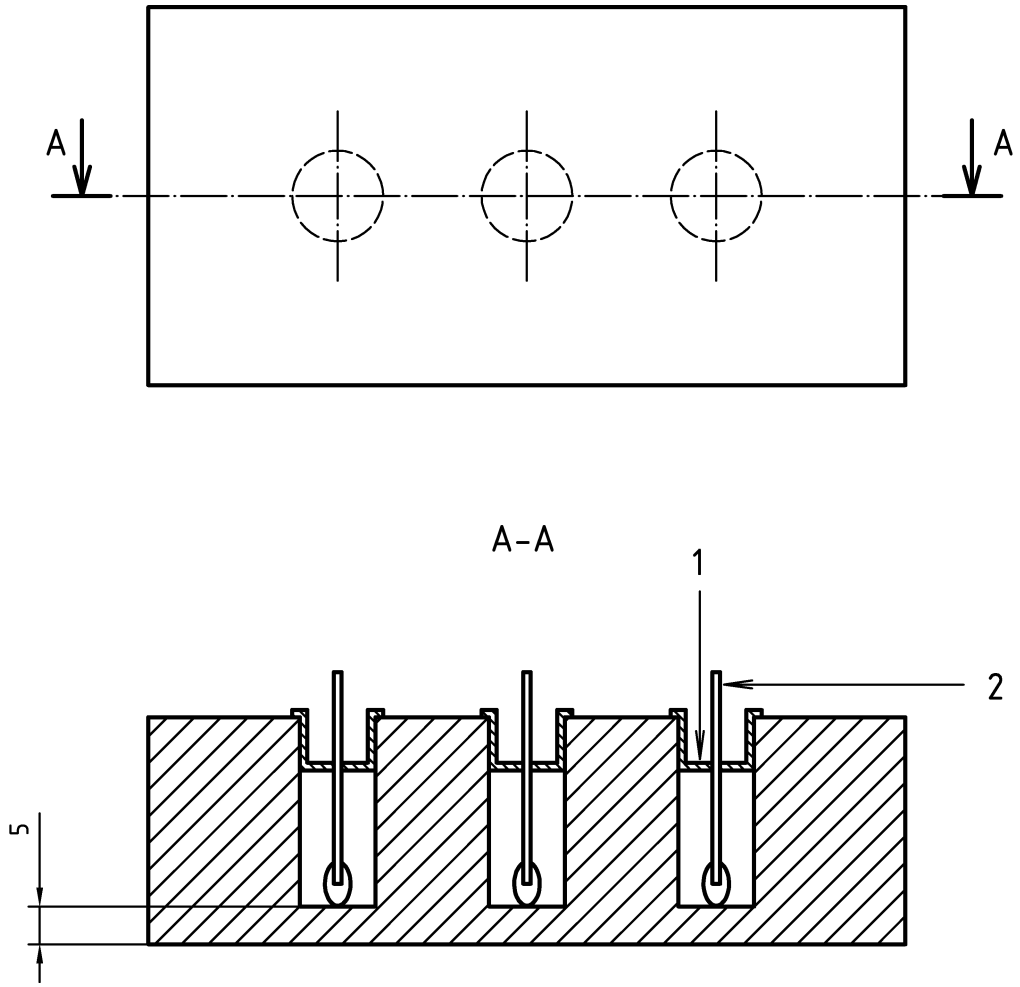
NOTE 1 Fresh bait assemblies can be prepared and sterilized by autoclaving.

Terminate the test after the incubation period specified by the supplier.

NOTE 2 On termination , the test specimens can be assessed using the method described in 8.5.

E.4.3.2 Place the Petri dishes containing the baits in the culture chamber (5.4.2) ; observe the baits every three to four days over a two week period and note whether growth of the test fungus has occurred. Once the test fungus has grown from a bait from a particular assessment hole, discontinue sampling of that assessment hole and close the hole with a sealing plug without bait.

Dimensions in millimetres



Key

1 Sealing plug

2 Bait

Figure E.1 — Insertion of baits in test specimens - Top location of drilled holes - Bottom positioning of baits

E.5 Expression of results

For each individual assessment hole, express the results as the recovery time, that is the minimum period of incubation of the test specimens (E.4.3.1) before growth of the test fungus was observed on baits from that assessment hole.

NOTE Growth of the test fungus from a bait is equivalent to that assessment position being given a rating of 2 (see Table 1).

Calculate the mean recovery time for all the assessment holes in each set of replicate test specimens. If the test is terminated before growth has occurred at all assessment holes, use the incubation period up to that time for the missing values in the calculation but express the mean recovery time as greater than the calculated value.

E.6 Test report

The test report should include, in addition to those data required for the obligatory test (see clause 11), the following :

a) the length of the assessment period ;

b) for each test specimen, the length of incubation period before the test fungus was first recovered from each assessment hole ;

c) for each test fungus, the mean time for the recovery of the test fungus from the untreated test specimens (e_2) and the treated test specimens (e_1).

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