DS-Information

DS/ENV 839

1. udgave

Godkendt: 1993-12-16

Træbeskyttelsesmidler
Bestemmelse af effektivitet mod forebyggelse af trænedbrydende svampe (Basidiomycetes)

Wood preservatives – Determination of the preventive efficacy against wood destroying basidiomycete fungi

DS
DANSK STANDARD

DS/ENV 839

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Nationalt forord

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Den er identisk med Europæisk Præstandard ENV 839:1993.

For en europæisk præstandard gælder, at der to år efter udgivelsen skal tages stilling til:

- om den skal have status som Europæisk Standard, eller
- om den skal gælde yderligere to år, eller
- om den skal erstattes af en revideret ENV, eller
- om den skal ophæves.

Kommentarer til indholdet i den europæiske præstandard kan sendes til Dansk Standard.

National foreword

This DS-Information has not been subject to public enquiry and does not have status as a Danish Standard.

It is identical with European Prestandard ENV 839:1993.

Two years after the publication of a European Prestandard, it shall be decided:

- whether it shall be transformed into a European Standard, or
- whether it shall be reconfirmed for another two-year period, or
- whether it shall be replaced by a revised ENV, or
- whether it shall be withdrawn.

Comments on the European Prestandard may be sent to the Danish Standards Association.

EUROPEAN PRESTANDARD
PRENORME EUROPEENNE
EUROPAISCHE VORNORM

ENV 839

June 1993

UDC

Descriptors :

English version

Wood preservatives : Determination of the preventive efficacy against wood destroying basidiomycete fungi

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

Holzschutzmittel : Bestimmung der vorbeugenden Wirkung gegenüber holzzerstörenden - Basidiomyceten

This European Prestandard (ENV) was approved by CEN on as a prospective standard for provisional application. The period of validity of this ENV is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the ENV can be converted into a European Standard (EN).

CEN members are required to announce the existence of this ENV in the same way as for an EN and to make the ENV available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the ENV) until the final decision about the possible conversion of the ENV into an EN is reached.

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

Central Secretariat : rue de Stassart 36, B-1050 Brussels

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Foreword

This European Prestandard has been drawn up by the Technical Committee CEN/TC 38 "Durability of Wood and derived materials", the Secretariat of which is held by AFNOR.

This European Prestandard was approved by CEN and in accordance with the CEN/CENELEC Internal Regulations the following countries are bound to implement this European Prestandard : Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

0 Introduction

This European Prestandard specifies a laboratory method of test which gives a basis for the assessment of the preventive action of a wood preservative, when applied mainly as a surface treatment, against basidiomycete 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 may 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 further recommended that results from this test should be supplemented by those from other appropriate tests, and above all by comparison with 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 standard.

1 Scope

This European Prestandard specifies a method for the determination of the preventive action of a wood preservative against basidiomycete 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 may be used in conjunction with an appropriate ageing procedure, for example EN 73.

2 Normative reference

This European Prestandard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to of revisions of any of these publications apply to this European Prestandard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

ISO 3696 : 1987 Water for analytical laboratory use - Specification and test methods

3 Definitions

For the purposes of this standard, the following definitions apply :

3.1 representative sample

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

3.2 supplier

The sponsor of the test.

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 basidiomycete 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

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 placenta (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 as 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 x (25 ± 0,5) mm x (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 ISO 3696

5.3.2 Culture medium for feeder blocks

- Malt extract containing (0,9 ± 0,3) % (*m/m*) nitrogen :

  . concentrated : 50 g ;

  . in powder form : 40 g.

- Agar containing approximately 0,3 % (*m/m*) nitrogen and causing no inhibition of growth of fungi : 20 g.

- Water : 1000 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 : Alternativement, the medium may 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. Particle 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 :

- potassium chloride (KCl) solution, 0,1 mol/l : 950 ml ;
- hydrochloric acid (HCl), 0,1 mol/l : 50 ml

5.3.5 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
- 50 % (*V/V*) ethanol solution	50 ml

1) Vermiculite is suitable.

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

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*) bromophenolblue in ethanol solution, 50 % (*V/V*).

5.4 Apparatus

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

5.4.2 Culture chamber (incubator or room), dark and controlled at (22 ± 1) ° C and at relative humidity (70 ± 5) %.

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 mm x 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.

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

5.4.6 Ordinary laboratory equipment including a balance capable of weighing to an accuracy 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 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

Giving a minimum of contact with the treated test specimens to be placed on them. They 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 annexe 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

The sample of preservative shall be representative of the product to be tested. Samples shall be stored and handled in accordance with any written recommendations 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 sound, straight-grained and without knots. 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 may be used.

The Scots pine shall be exclusively sapwood containing little resin. The growth rate shall be between 2,5 annual growth rings per 10 mm 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 annual growth rings per 10 mm 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 mm x 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 conditionning to a moisture content of (12 ± 2) % (*m/m*) shall be :

(100 ± 0,5) mm x (50 ± 0,5) mm x (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 4 treated test specimens for each combination of preservative, test fungus ;

e_2 : untreated test specimens : use at least 4 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 weighings at 24 h intervals are within ± 0,1 g.

8.1.2 End sealing

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

8.1.3 Treatment

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 recommendations.

NOTE : The test may 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 as grams per square metre of treated surface area.

8.1.4 Drying

Following treatment (8.1.3), place the treated 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 weighings at 24 h intervals are within ± 0,01 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 ageing procedures 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 :

G. trabeum - WHC of test substrate only ;

C. puteana - WHC of test substrate + half the conditioned dry mass of the test specimens ;

P. placenta - WHC of test substrate + half the conditioned dry mass of the test specimens ;

C. versicolor - WHC of test substrate + 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 8 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 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 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 (5.3.4) 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 x 10 magnification hand lens.

NOTE 2 :Additionally, it is possible also to assess fungal development using bromophenol blue solution (5.3.7). Allow the sawn test specimens to dry in the laboratory work area (5.4.3) for 48 h. 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 has to 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 5 : 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 3 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 4 ratings on each of 4 test specimens.

11 Test report

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

a) the number and date of this European Prestandard ;

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

c) the specific and unique name or code of the preservative tested, with an indication of whether or not the composition has been declared ;

d) the name and concentration of active fungicide ;

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 ;

h) the concentrations tested ;

i) the date of the application of the preservative

j) for each test specimen treated :

- the corresponding quantity, in grams per square metre, of the preservative 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 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 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 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".

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

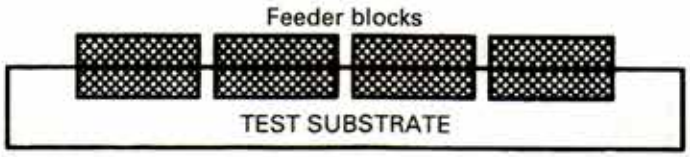


Figure 1 : The positioning of feeder blocks on substrate

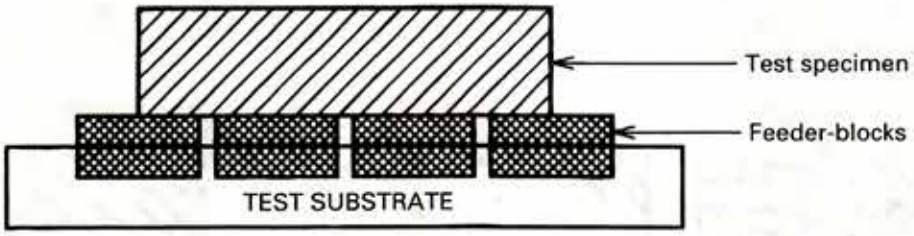
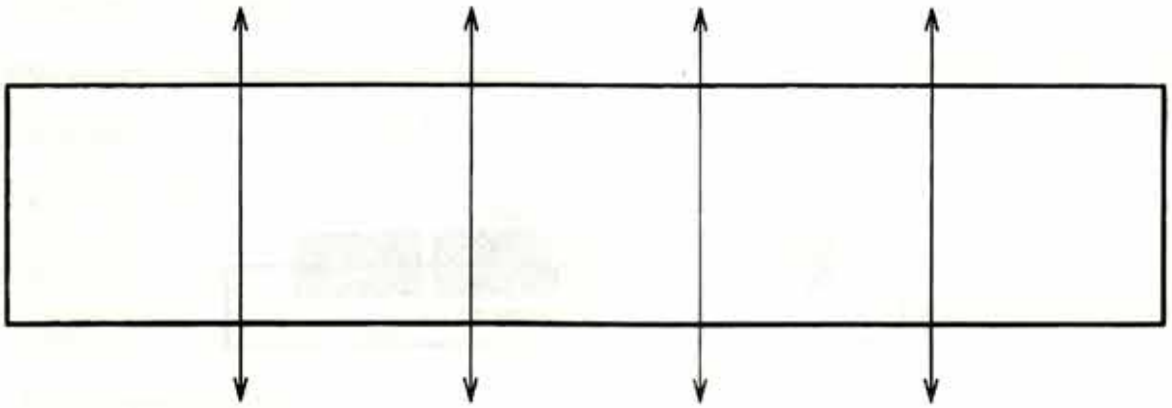
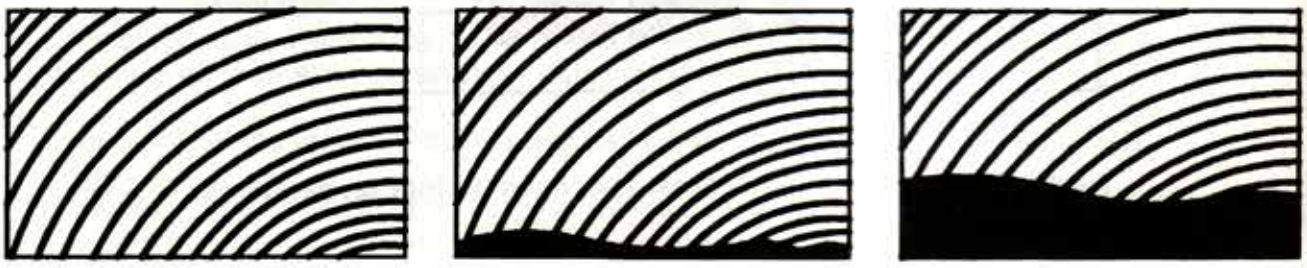


Figure 2 : The positioning of test specimen on four feeder blocks



Cut lines every 20 mm

Figure 3 : The position of cutting for evaluation



0 1 2

Figure 4 : The rating of fungal colonization in exposed specimens

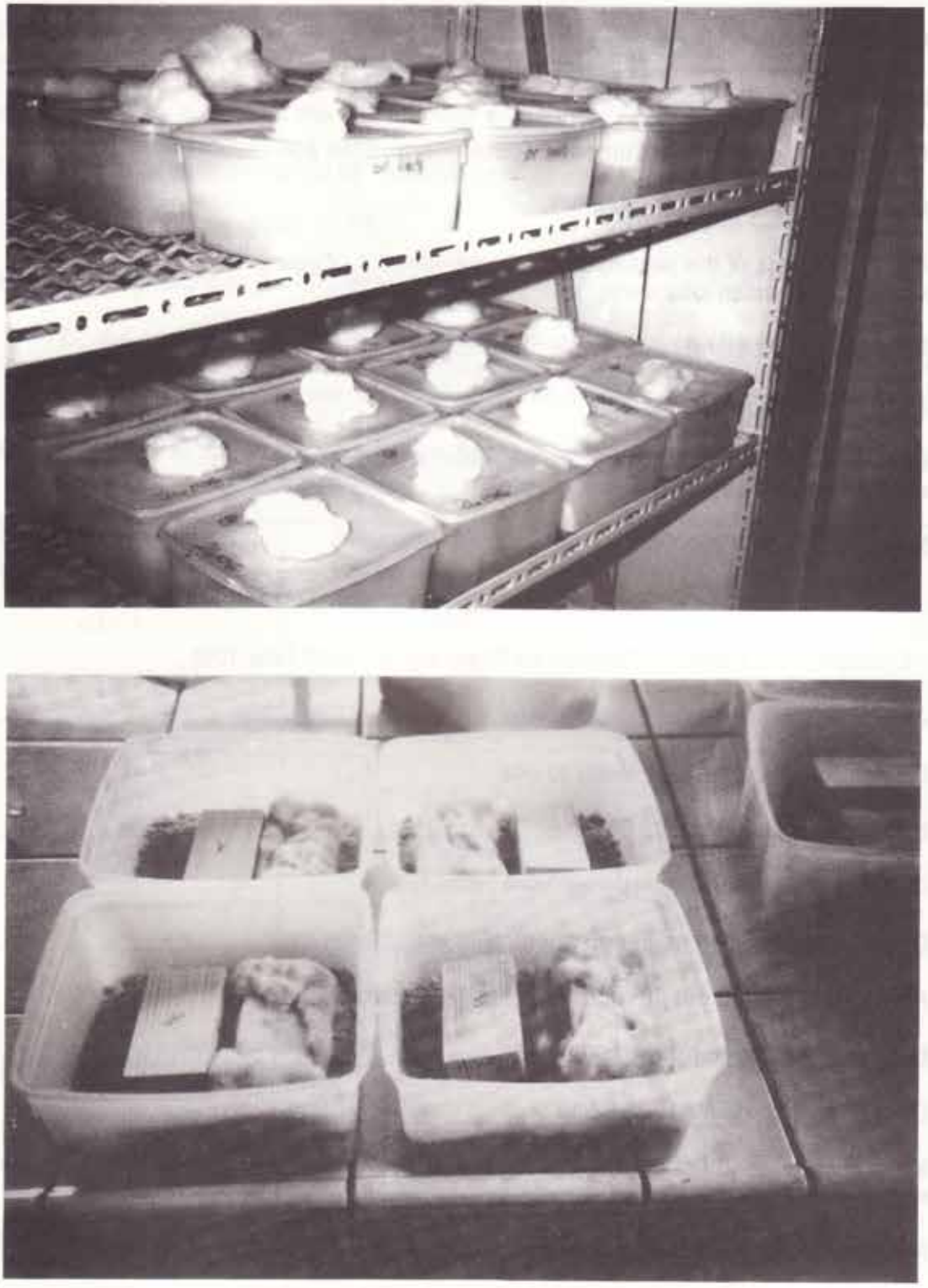


Figure 5 : Example of convenient containers for the test, containing two specimens on the test substrate (the lid had been removed)

Annex A (informative)

Example of a test report

- Number and date of this European Prestandard : ENV 839 :1993.

- Name of the supplier : Company A

- Name and type of the preservative : Preservative X - Organic solution, the full formulation of which was declared.

- Name and concentration of active fungicide : Fungicide Y - 1,5 % (*m/m*).

- Solvent or diluent : none.

- Species of wood used : Scots pine sapwood (*Pinus sylvestris* L.), and the density 485 kg/m^3.

- Species of fungi used :

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

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

  - *Poria polacenta* (Fries) Cooke sensu J. Eriksson (FPRL 280).

- Concentration (s) tested : ready to use.

- Date of application : 1992.09.16.

- Quantity of preservative retained : see table A.1.

- Duration of conditioning period after treatment : 10 days.

- Ageing test carried out : Evaporative ageing according to EN 73.

- Date of exposure to fungi : 1992.09.26.

- Date of examination : 1992.12.19.

- Method of assessment : Dipping in fungicidal solution followed by incubation for one week in a culture chamber.

- Assessment rating of fungal development on each cross section : see table A.1.

- Notional mean value for each fungus : see table A.2.

- Mean percentage of the cross-section colonized by the test fungi for control test specimens :

- e2 : 80 % of the surface of the (4 x 4) cross-sections.

- This report has been prepared by : laboratory B.

- Location and date : C 1992.12.28.

- 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 preservative X on Scots pine sapwood against *Coniophora puteana* (BAM Ebw.15)

Number of specimens	Absorption of solution (g/m^2)	Rating on each cross-section
e1 - 1	154	0 0 1 0
e1 - 2	147	0 0 1 1
e1 - 3	149	0 0 0 0
e1 - 4	148	1 0 0 1
NOTE : Notional mean rating 0,31.		

Table A. 2 : Results for 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 (normative)

Informations on obligatory test fungi

B.1 General information on maintaining and sending of test strains

The laboratory holding the parent strain shall reisolate 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 shall be obtained from the laboratory of a recognized culture collection. At least every 2 years, test strains shall be re-isolated from untreated wood which is actively being attacked. Sterilize 2 feeder blocks and expose them to attack as described in 8.3, for a period of 6 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 shall test the virulence once a year.

When sending cultures special care has to be taken to avoid any harmful effects during transit, for example by freezing during air-transport. Against X-rays in airport controls it is advisable to pack strains in aluminium containers or paper.

> NOTE : International Regulations exist concerning the transport of cultures. Information on these can be obtained from the Information Centre for European Culture Collections, Mascheroder Weg 1b, 2D-3300 Braunschweig, Germany.

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

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

Strain : BAM Ebw. 15 (Bundesanstalt für Material-prüfung - Unter den Eichen 87 - D 1000 - BERLIN 45)

Activity :

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

Simple laboratory culture, rapid growth on nutrient 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 6 °C to 10 °C.

Subculturing shall be carried out every 6 months. Every 2 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 Material-prüfung - Unter den Eichen 87 - D - 1OOO - BERLIN 45)

Activity :

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

Cultivation in well-ventilated conditions, rapid growth on malt agar nutrient 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 6° C to 1O° C. Subculture every 6 months.

B.4 *Poria* placenta (Fries) Cooke sensu J. Eriksson (Synonym : *Poria* monticola Murrill)

Strain : FPRL 28O : (BRE - Timber Division - Garston - WATFORD WD2 7JR - U.K.)

Activity :

Fungus causing a brown, cubical rot of softwood.
Activity check carried out at BRE at least 3 times per year.

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 laboratory temperature, keep reserve cultures on 5 % (*m/m*)malt agar medium under mineral oil and subculture every 4 years.

Keep stock cultures on 5 % (*m/m*) malt agar medium and subculture every 3 months. Every 2 years, or more frequently if necessary, inoculate the subcultures on wood or sawdust and subculture on malt agar after 6 to 12 weeks of developmen: on this substrate.

B.5 *Coriolus versicolor* (Linnaeus) Quélet
(Synonym *Polyporus versicolor* Linnaeus ex Fries).

Strain : CTB 863 A (Centre Technique du Bois et de l'Ameublement - 10 avenue de St Mandé - 75012 Paris).

Activity : Fungus causing a white, fibrous rot of hardwood.

Simple laboratory culture, rapid growth on nutrient malt agar medium.

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

Maintenance and treatment : Sub-culture every 6 weeks on agar medium.

Annex C (normative)

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 specimens individually in low density polyethylene envelopes (between 30 µm and 90 µm thick) and seal by hot iron welding.

Place the 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 specimens for 48 h 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 specimens individually in polyethylene envelopes (at least 90 µm thick) and seal them by hot iron welding.

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

To minimize possible influence from ozone, reduce the oxygen content in the envelope, before, sealing, by blowing nitrogen into the envelope.

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

Subject the envelopes to radiation to a minimum level of 1,5 Mrad [4]. The maximum dose shall not exceed 2,5 Mrad when using radioisotopes (e.g. ^{60}Co sources) or 5 Mrad when using electron-accelerators. 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.

NOTE 2 : After irradiation, the envelopes may 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.

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4) 1 Mrad = 10^4 J/kg = 10^4 Gy

Annex D (normative)

Determination of water holding capacity (WHC)

NOTE : The calculation of water holding capacity in this European Prestandard is different from that given in 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 Buchner 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 (m_1) of the wet sample plus the wet filter paper. Determine the mass of a wet filter paper after subjecting it to suction in the Buchner funnel (m_2). 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.

Combine the ingredients of the medium and sterilize in an autoclave at 121 °C for 20 min. Dispense under aseptic conditions into suitable containers, 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 exposed to the test fungus, leaving (5 ± 0,1) mm of wood beyond the end of each hole (see figure E.1). Space the hole 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 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 dishes to retain the identity of the test specimen, the location of the assessment hole and the total period of incubation of the test speciments.

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 de petri dishes containing the baits in the culture chamber (5.4.2) ; observe the baits every 3 to 4 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.

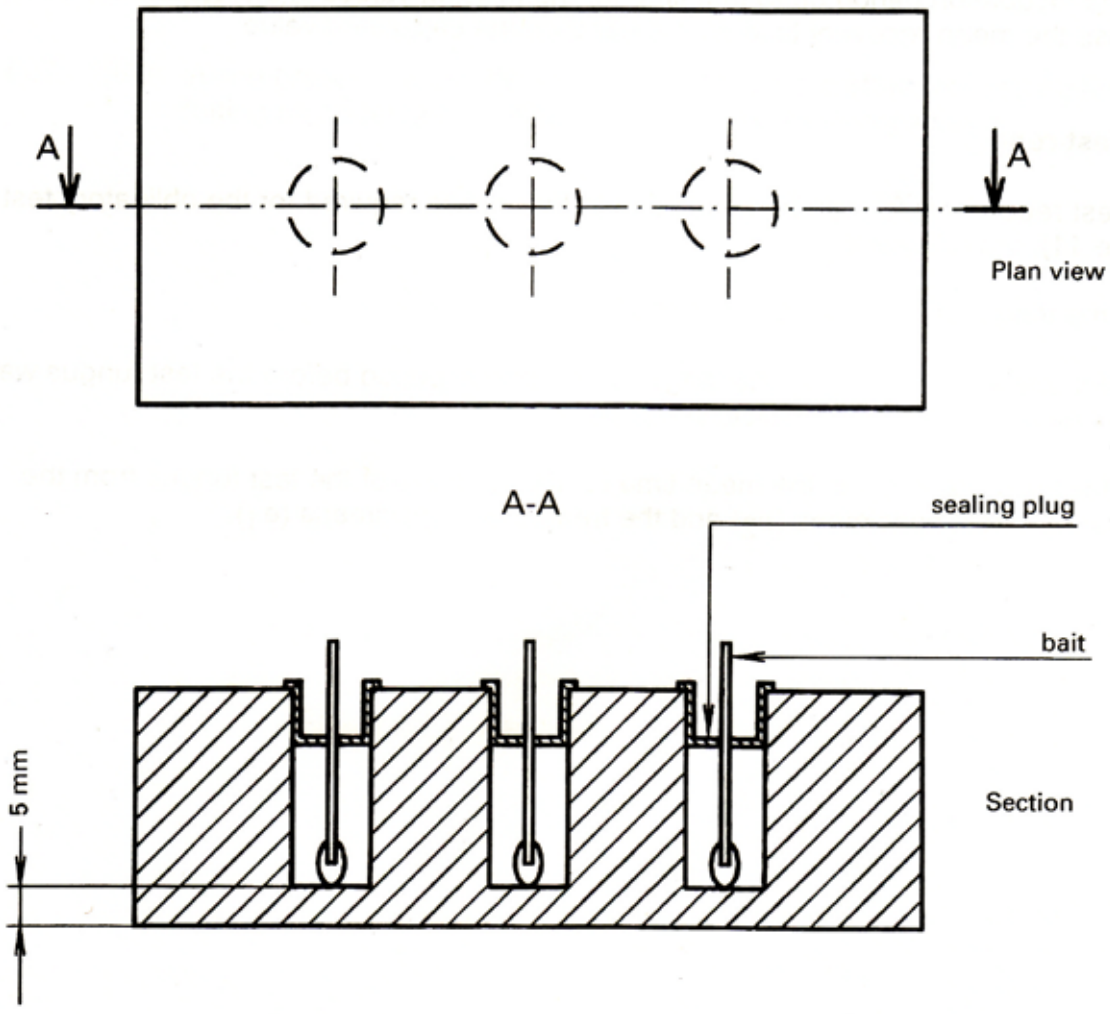

Figure E.1 : Insertion of baits in test specimens - Top location of drilled holes - Bottom positioning of baits

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).

Annex F (informative)

Bibliography

EN 73 : 1988 Wood preservatives - Accelerated ageing tests of treated wood prior to biological testing - Evaporative ageing procedure

EN 113 : 1980 Wood preservatives - Determination of toxic values of wood preservatives against wood destroying basidiomycetes cultured on an agar medium

EN 212 : 1986 Wood preservatives - Guide to sampling and preparation of wood preservatives and treated timber for analysis

ENV 807 : 1993 Wood preservatives - Determination of the toxic effectiveness against soft rotting micro-fungi and other soil inhabiting micro-organisms

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