Provläsningsexemplar / Preview

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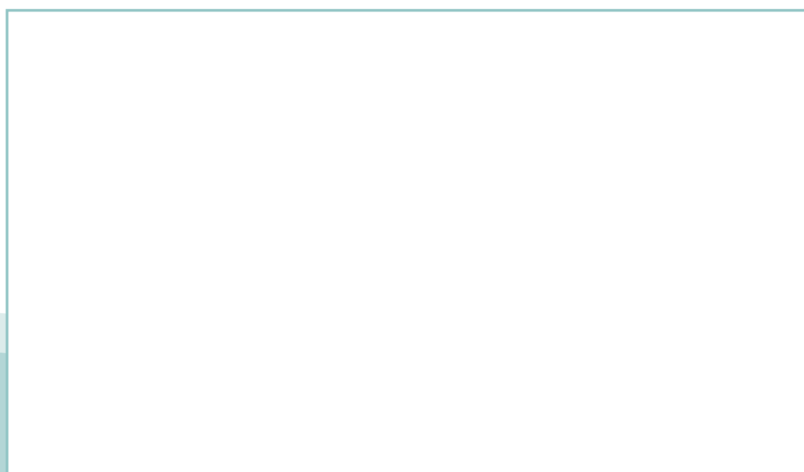
Wood preservatives – Test method for determining the protective effectiveness against wood destroying basiomycetes – Determination of the toxic values



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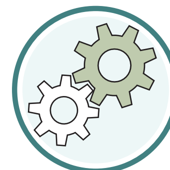

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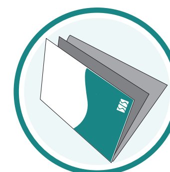

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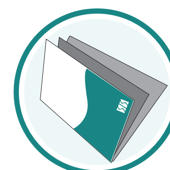

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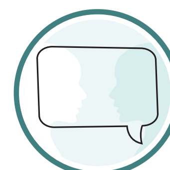

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Denna standard ersätter SS 27218, utgåva 1.

The European Standard EN 113:1996 has the status of a Swedish Standard. This document contains the official English version of EN 113:1996.

This standard supersedes the Swedish Standard SS 27218, edition 1.

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EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 113

September 1996

ICS 71.100.50

Supersedes EN 113 : 1980, EN 113 : 1980/A1 : 1981 and EN 113 : 1980/A2 : 1985

Descriptors: Wood, wood preservatives, pest control, fungi, basidiomycetes, laboratory tests, effectiveness, effectiveness limit

English version

Wood preservatives — Test method for determining the protective effectiveness against wood destroying basidiomycetes — Determination of the toxic values

Produits de préservation du bois — Méthode d'essai pour déterminer l'efficacité protectrice vis-à-vis des champignons basidiomycètes lignivores — Détermination du seuil d'efficacité

Holzschutzmittel — Prüfverfahren zur Bestimmung der vorbeugenden Wirksamkeit gegen holzzerstörende Basidiomyceten — Bestimmung der Grenze der Wirksamkeit

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Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.

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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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Ref. No. EN 113 : 1996 E

Provläsningsexemplar / Preview

Foreword

This European Standard has been prepared by Technical Committee CEN/TC 38, Durability of wood and derived materials, the Secretariat of which is held by AFNOR.

This European Standard supersedes EN 113 : 1980, EN 113 : 1980/A1 : 1981 and EN 113 : 1980/A2 : 1985.

The significant technical differences between this edition and EN 113 : 1980 are as follows:

- application to water-dispersible formulations;
- the use of only one fungus but *Coriolus versicolor* being only used for particular hazards;
- the taking into account of the correction value for the loss in mass;
- new criteria of validity of test and interpretation of results;
- complement to annex regarding test fungi.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 1997, and conflicting national standards shall be withdrawn at the latest by March 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Contents

	Page
Foreword	2
Introduction	3
1 Scope	3
2 Normative reference	3
3 Definitions	3
4 Principle	3
5 Test materials and apparatus	3
6 Sampling of the preservative	5
7 Test specimens	5
8 Procedure	6
9 Statement of results	8
10 Test report	8
Annex A (informative) Example of a test report	9
Annex B (normative) Methods of sterilization	12
Annex C (informative) Culture vessels	13
Annex D (normative) Test fungi	16
Annex E (informative) Recommended but non-comprehensive list of optional fungi	17
Annex F (informative) Bibliography	18

Provläsningsexemplar / Preview

Introduction

This European Standard specifies 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 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 the standard. Any deviation, however small, from the procedures given in this standard can influence the results, so it is important that the procedures given in this method are followed precisely.

To ensure the continued resistance of the test strains to wood preservatives, a requirement is included that laboratories holding the parent cultures test them against reference fungicides periodically.

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.

This method is applicable to products which are capable of achieving uniform and complete penetration of the test specimens including:

- water-insoluble chemicals which are being studied as active ingredients; or
- organic water-insoluble 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 which are capable of achieving uniform and complete penetration of test specimens (see clause **7**) by the procedure described in **8.2.2**; or
- water-soluble products, for example salts.

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

2 Normative reference

This European Standard 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 or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revisions. 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

Impregnation of several series of test specimens of a susceptible wood species with 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.

5 Test materials and apparatus

5.1 Biological material

The test fungi to be used as follows:

5.1.1 ***Obligatory fungus in all cases*** (see also annex D)

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

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

5.1.2 ***Obligatory fungus for particular hazards*** (see also annex D)

- *Coriolus versicolor* (Linnaeus) Quélet (CTB 863 A) on hardwood and/or on softwood, as appropriate.

  Loss in mass in percentage in 16 weeks of beech specimens: minimum 20 % (*m/m*); of Scots pine sapwood specimens: minimum 15 % (*m/m*).

Provläsningsexemplar / Preview

5.1.3 ***Two species to be used compulsorily on the basis of the nature of the product*** (see also annex D)

For creosotes and similar products:

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

Loss in mass in percentage in 16 weeks of Scots pine sapwood specimens: minimum 20 % (*m/m*);

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

Loss in mass in percentage in 16 weeks of beech specimens: minimum 20 % (*m/m*).

For all other products:

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

Loss in mass in percentage in 16 weeks of Scots pine sapwood specimens: minimum 20 % (*m/m*);

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

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

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

5.1.5 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 **D.2**). The parent strain shall be maintained in the laboratory of its origin so as to conserve and to assure its vigour.

If tests are not undertaken regularly or if a strain shows signs of degeneration a new standard culture of the strain should be obtained from the laboratory of its origin for each test (annex **D.2**). When new strains are received the virulence shall be tested to ensure it is within the range given in annex **D.2**.

5.2 Products and reagents

5.2.1 *Culture medium*

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

– malt extract

in concentrated form: (50 ± 0,5) g;

in powder form: (40 ± 0,5) g.

– agar causing no inhibition of growth of fungi: (20 ± 0,5) g to (30 ± 0,5) g;

– water conforming to grade 3 of ISO 3696; 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 minimum depth of 3 mm to 4 mm when in its in-use position. Close the vessels as specified in **5.3.9** and sterilize in the autoclave at 121 °C for 20 min. Let the vessels cool in their in-use position.

NOTE. It is essential that the nitrogen content of the culture medium is within the given limits.

5.2.2 *Solvents and diluents*

For water soluble preservatives:

– water conforming to grade 3 of ISO 3696.

For preservatives to be diluted or dissolved in an organic solvent:

– suitably volatile liquids that leave no residue in the wood that would have a toxic effect on the fungi at the end of the post-treatment conditioning period.

NOTE. Toluene and xylene of recognized analytical grade have been found suitable.

5.2.3 *Fumigant* (if necessary)

Xylene technical grade.

5.3 Apparatus

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

5.3.2 *Culture chamber*, (incubator or room), dark and capable of being controlled at (22 ± 2) °C and (70 ± 5) % relative humidity.

5.3.3 *Drying oven*, capable of being controlled at (103 ± 2) °C.

5.3.4 *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.

5.3.5 *Weights*, to provide ballast for the test specimens. The weights shall not react with any materials with which they come into contact during the test.

5.3.6 *Safety equipment and protective clothing*, appropriate for the test product reference preservative and the test solvents, to ensure the safety of the operator.

5.3.7 *Vacuum vessels*, fitted with stopcocks.

5.3.8 *Vacuum pump*, fitted with a pressure gauge and capable of maintaining a pressure of (0,7 ± 0,1) kPa[2].

5.3.9 *Kolle flasks or equivalent culture vessels*, with a capacity of between 400 ml and 650 ml, providing a flat surface area of between 85 cm^2 and 120 cm^2 for the medium (see figures 1, 2 and 3 in annex C) and allowing air exchange.

NOTE. Kolle flasks are usually plugged with a wad of cotton wool. Other culture vessels are usually fitted with leakproof lids, the centres of which are to be pierced with a round hole of up to 15 mm diameter and plugged with a wad of cotton wool.

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[1] See annex E for a recommended but non-comprehensive list of optional fungi.
[2] 100 Pa = 1 bar.

Provläsningsexemplar / Preview

5.3.10 *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.

NOTE. If abnormally high moisture contents are experienced consistently, use of specimen supports of approximately 5 mm thick may help to control the problem. If thicker specimen supports are used, this should be recorded in the test report.

5.3.11 *Drying vessel(s)*, provided with a close-fitting cover and containing supports that will give minimum contact with the treated test specimens to be placed on them. The vessels and supports shall be of materials that do not react with the test solvent or test preservative, for example glass for organic products or plastics material for salts containing fluorine.

5.3.12 *Equipment for chemical gas or steam sterilization or access to a rediation source* (see annex B).

5.3.13 *Ordinary laboratory equipment*, including a balance capable of weighing to an accuracy of 0,01 g and a dessicator with an efficient desiccant (for example, silica gel).

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 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 species of wood to be used shall be susceptible to attack by fungi and shall be readily impregnated by liquids.

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.

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

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

The beech shall be even-grained wood free from tyloses, discoloration and red heart. It shall have between two annual growth rings and six annual growth rings per 10 mm.

7.3 Provision of test specimens

Cut the specimens from planed strips having a cross-section of (25 × 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 cuts shall be made neatly to give sharp edges.

The specimens shall originate from a minimum of three trees or shall be taken at random from a stock originally of more that 5000 specimens and originating from at least 20 planks.

7.4 Dimensions and density of specimens

The nominal dimensions of each specimen, measured at 12 % (*m/m*) moisture content, shall be:

(50 ± 0,5) mm × (25 ± 0,5) mm × (15 ± 0,5) mm.

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

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 is permitted to differ from the mean value by ± 10 %. This tolerance is increased to ± 20 % for the untreated specimens. The mean density of the specimens used for the test shall be recorded in the test report.

7.5 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 four treated test specimens for each preservative concentration (including a solvent or diluent control (concentration = 0)), for each fungus and for each timber species.

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 of the same wood species which are placed one in each culture vessel with the treated specimens.

$e_{2.2}$ virulence control specimens: six of these non-impregnated specimens of the appropriate timber species are subjected to attack by each wood destroying fungus.

Provläsningsexemplar / Preview

e_3 Treated check test specimens for calculation of the correction value:

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 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 percentage change in mass of the e_3 test specimens.

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

NOTE. It is advisable to treat more specimens than the minimum number required to select those having the uptake nearest to the target.

8 Procedure

8.1 Preparation of test specimens

8.1.1 *Conditioning of test specimens before treatment*

Place the numbered test specimens in the oven (**5.3.3**) and leave them there for 18 h[3]. Cool to room temperature 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.

Calculate the mean density of the specimens of each species using the mean mass and the specimen volume.

8.2 Treatment of test specimens

8.2.1 *Preparation of treatment solutions/dilutions*

Prepare a series of concentrations (by mass) of the preservative in the appropriate solvent or diluent (**5.2.2**).

For solid products prepare a sufficient quantity of the solution of a concentration equal to, or greater than, the highest concentration to be tested. Dilute, on a mass by mass basis, appropriate quantities of this solution to provide a series of at least five concentrations distributed about the expected toxic value.

For liquid products, dilute, on a mass by mass basis, appropriate quantities of the product to provide a series of at least five concentrations distributed about the expected toxic value.

A solvent or diluent control, i.e. treatment at concentration O shall also be included in each test series.

If the approximate toxic values are unknown, the concentrations shall form a widely spaced geometric progression for a first test, and a more closely spaced geometric or arithmetic progression for subsequent tests.

All treatment solutions shall be freshly prepared.

8.2.2 *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.

- For each concentration place the test specimens, kept dry as described in **8.1.1** and of known dry mass (m_0) in one of the treatment vessels (see **5.3.4**) 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 (see **5.3.5**) to prevent them from floating when the liquid is admitted.
- Place each treatment vessel in one of the vacuum vessels (see **5.3.7**) attach the vacuum pump (see **5.3.8**) and reduce the pressure to (0,7 ± 0,1) kPa. Maintain this vacuum for 15 min. Observe the proper safety measures for vacuum vessels. After this period, close the stopcock to the vacuum pump 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 the impregnation process.
- Next, admit air to bring the vacuum vessel back to atmospheric pressure, remove the treatment vessel with its submerged specimens from the vacuum vessel, 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 and immediately weigh each to the nearest 0,01 g to ascertain the mass after impregnation (m_1).

NOTE. Usually the uptake of treatment solution per test specimen will be in the range of 12 ml to 16 ml in Scots pine sapwood and 10 ml to 14 ml in beech, depending on the type of preservative and solvent.

In the case of preservatives, which are being studied as active substances, calculate the mass of preservative retained for each test specimen, from the mass of solution absorbed ($m_1 - m_0$) and its concentration[4].

In the case of organic formulations the retention is expressed for each test specimen in terms of the corresponding mass may be as a product ready for use, may be as concentrated product, if the product under test is supplied in the form of a concentrate.

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[3] In the case of supplementary tests (**7.1**) using species of wood other than beech and pine sapwood, this drying time may need to be longer than 18 h; the drying time should be such that the test specimens reach constant mass.

[4] 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. Similarly, analysis is recommended when very dilute solutions are used.

Provläsningsexemplar / Preview

Calculate the mass of preservative retained per unit volume of wood in kilograms per cubic metre, for each specimen.

If, in a series of simultaneously impregnated test pieces, the quantity of product absorbed by some 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.

8.3 Drying and conditioning of test specimens after treatment

The procedures described below are usually applicable, but if the nature of the preservative is such that alternative procedures are required details of the procedure used shall be included in the test report.

Keep the specimens for four weeks in the conditioning chamber (see **5.3.1**). Arrange the test specimens in the drying vessels (see **5.3.11**), resting on their narrow faces on the supports, and placing only specimens treated with the same concentration of the test preservative in the same drying vessel; avoid contact between specimens. Invert the test specimens twice a week.

In the case of test specimens impregnated using water-soluble products keep the vessel covered for two weeks. To prevent mould growth, also place in the vessel a small dish containing xylene (see **5.2.3**). During the third week, uncover the vessel progressively each day to allow the specimens to dry steadily. From the beginning of the fourth week, leave the vessel completely open.

In the case of specimens impregnated with water-insoluble products in an organic solvent, keep the vessel covered for one week. Open it gradually during the second week and finally leave it fully open for the third and fourth weeks.

NOTE. The drying and conditioning of the specimens depends on the nature of the product under test and on the solvent or diluent used.

If the test specimens are to be subject to an ageing procedure, this shall be carried out after this drying procedure.

8.4 Exposure to fungi

Inoculate the culture medium (see **5.2.1**) in the culture vessels (see **5.3.9**) not more than seven days after sterilization of the medium. The inocula shall be obtained from cultures which are less than four weeks old and which are still actively growing across the growth medium, or have fully covered it for less than one week.

The exposure to the fungi shall take place as soon as the mycelium completely covers the surface of the culture medium. This corresponds to the active phase of development; in no case should this period exceed four weeks. The fungi shall be free from contamination by other organisms.

Into each culture vessel introduce aseptically two previously sterilized test specimen supports (see **5.3.10**) and on each place one specimen previously sterilized by one of the procedures given in annex B.

Place one treated specimen (e_1) and one non-impregnated control specimen ($e_{2.1}$) in each inoculated culture vessel.

Place the non-impregnated specimens for virulence control ($e_{2.2}$) two per inoculated culture vessel.

NOTE. If several tests are running in parallel, only one set of virulence specimens is required.

Place two treated check test specimens (e_3) in each uninoculated culture vessel as indicated in **7.5** to establish the correction factor (C).

8.5 Culture conditions and duration of test

After introducing the test specimens, place the culture vessels in the culture chamber (see **5.3.2**) and leave them there for 16 weeks.

8.6 Assessment of test

8.6.1 *Examination of the test specimens*

At the end of the test, withdraw the test specimens from the culture vessels, removing any adhering mycelium. Record evidence of waterlogging or of inhibition of growth of the test fungus caused by volatile components of the preservative or contaminating organisms.

Weigh each specimen to the nearest 0,01 g at the end of the test, (m_2). After oven (see **5.3.3**) drying to constant mass, weigh each specimen again to the nearest 0,01 g, (m_3). Calculate the moisture content of each specimen at the end of the test by expressing its water content ($m_2 - m_3$) as a percentage of its final dry mass (m_3).

8.6.2 *Loss in mass caused by fungal attack*

Calculate the loss in mass of each treated test specimen (e_1) by expressing the loss in mass ($m_o - m_3$) as a percentage of the initial dry mass (m_o). Calculate the correction factor (C) which is the mean percentage loss in mass of the treated check test specimens (e_3) of each treating solution concentration. Subtract the appropriate value of C from the percentage mass loss of each treated test specimen (e_1) to determine the corrected loss in mass.

Calculate the mean corrected loss in mass of the treated test specimens (e_1), and the mean loss in mass of the virulence control specimens ($e_{2.2}$).

NOTE 1. Due to the uptake of the preservative, the correction values for specimens in higher concentrations of the preservative may be negative, indicating an increase of the mass. The correction values should be used strictly mathematically.

NOTE 2. With some preservatives, especially creosotes, the increases in mass of the treated check test specimens (e_3) may be very large for the higher concentrations of the preservative. Because of this fact, the use of the correction values of these concentrations may produce erroneous theoretical losses in mass of the treated test specimens (e_1).

To avoid incorrect assessments, the treated test specimens (e_1) of these concentrations of the preservative should be carefully investigated for any visible surface and/or internal attack by the fungus. If there is a calculated loss in mass of any of those test specimens, but no visible fungal attack, it should be mentioned in the test report.