

Wood preservatives — Determination of the effectiveness against soft rotting micro-fungi and other soil inhabiting micro-organisms

ICS 71.100.50

National foreword

This Draft for Development is the official English language version of ENV 807:2001. It supersedes DD ENV 807:1993 which is withdrawn.

This publication is not to be regarded as a British Standard.

It is being issued in the Draft for Development series of publications and is of a provisional nature. It should be applied on this provisional basis, so that information and experience of its practical application may be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the European organization responsible for its conversion into a European Standard. A review of this publication will be initiated 2 years after its publication by the European organization so that a decision can be taken on its status at the end of its three-year life. The commencement of the review period will be notified by an announcement in *Update Standards*.

According to the replies received by the end of the review period, the responsible BSI committee will decide whether to support the conversion into a European Standard, to extend the life of the prestandard or to withdraw it. Comments should be sent in writing to the Secretary of BSI Subcommittee B/515/4, Assessment of preservation efficacy, at 389 Chiswick High Road, London W4 4AL, giving the document reference and clause number and proposing, where possible, an appropriate revision of the text.

A list of organizations represented on this committee can be obtained on request to its secretary.

Cross-references

The British Standards which implement international or European publications referred to in this document may be found in the BSI Standards Catalogue under the section entitled "International Standards Correspondence Index", or by using the "Find" facility of the BSI Standards Electronic Catalogue.

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English version

Wood preservatives - Determination of the effectiveness against soft rotting micro-fungi and other soil inhabiting micro-organisms

Produits de préservation du bois - Détermination de l'efficacité vis-à-vis des micro-organismes de pourriture molle et d'autres micro-organismes du sol

Holzschutzmittel - Prüfverfahren für die Bestimmung der Grenze der Wirksamkeit gegen Moderfäule und andere erdbewohnende Mikroorganismen

This European Prestandard (ENV) was approved by CEN on 1 March 2001 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.

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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Foreword

This European Prestandard 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 Prestandard supersedes ENV 807:1993.

The annexes A, B, C, D, E and F are informative.

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

Introduction

This European Prestandard specifies a laboratory method of test which gives a basis for assessing the effectiveness of a wood preservative against micro-fungi (ascomycetes and fungi imperfecti) which cause soft rot of wood in service. The infection source is the natural micro-flora of the soil which may also contain other micro-organisms, such as bacteria and other fungi, such as moulds and basidiomycetes. This laboratory method provides one criterion by which the value of a wood preservative product can be assessed. This information has to be supplemented by data from other relevant tests and from practical experience.

1 Scope

This European Prestandard specifies a method of test for determining the toxic effectiveness of a wood preservative, applied to wood by full impregnation, against the micro-fungi which cause soft rot of wood.

The method is applicable to testing of formulated products or of their active ingredients.

NOTE A method suitable for undertaking screening tests of potential active ingredients is given in annex A.

2 Normative references

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 or 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 (including amendments).

EN 84, *Wood preservatives — Accelerated ageing of treated wood prior to biological testing — Leaching procedure*

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

3 Terms and definitions

For the purposes of this European Prestandard, 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

4 Principle

A number of small test specimens (as small stakes) are impregnated with the preservative under test at a minimum of three concentrations ranged about the retention expected to provide protection throughout the test period. The test specimens are exposed to leaching according to EN 84. The specimens are partly buried vertically in a microbially active soil. Sets of test specimens are assessed after 8, 16, 24 and 32 weeks of exposure. The performance of the test preservative is evaluated by comparison with the performance of a reference preservative.

5 Test materials

5.1 Biological materials

5.1.1 Soil

Natural top soil or a fertile loam-based horticultural soil ¹⁾ of pH 6 to pH 8 and not containing added agro-chemicals. It shall have a waterholding capacity (WHC) of between 25 % (*m/m*) and 60 % (*m/m*).

NOTE 1 A suitable method for determining WHC is described in annex B.

¹⁾ A horticultural soil of the John Innes No.2 type and with the following composition has been found to be suitable; seven parts by volume loam, three parts by volume sphagnum peat, two parts by volume sharp sand plus 0,6 g chalk and 6,0 g slow release fertilizer per litre of soil mixture. If the WHC is too high, it can be lowered by modifying the soil with the addition of sand.

If a natural soil is used, it shall have the turf or top 50 mm removed and shall not be taken from a depth below 200 mm from the original surface. It shall be passed through a sieve of nominal aperture size 12,5 mm. If it is necessary to store the soil prior to use, it shall be stored in closed moisture-proof containers. Before use, thoroughly mix the sample of soil.

NOTE 2 The soil should only be collected in a moist condition.

If a horticultural soil is used which is sterilized during its preparation, then 20 % (*m/m*) of a natural soil, prepared as above, shall be added and the soils thoroughly mixed prior to the start of the test.

The soil shall be used only once.

NOTE 3 If assurance of the virulence of the soil is required, the test procedure using cotton cloth described in annex C, or a similar standardized procedure, may be used.

5.2 Products and reagents

5.2.1 Solvents and diluents

Water to grade 3 of EN ISO 3696 and, if appropriate, volatile organic liquids leaving in the wood no residue which would have a toxic effect on the soil inhabiting micro-organisms at the end of the post-treatment conditioning period.

NOTE Information on appropriate solvents and diluents should be provided by the supplier.

5.2.2 Reference preservative

A copper/chromium preservative with a composition equivalent to the following :

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 50,0 % (*m/m*)

$\text{K}_2\text{Cr}_2\text{O}_7$ 48,0 % (*m/m*)

CrO_3 2,0 % (*m/m*)

The preservative shall be prepared from ingredients of at least 95 % (*m/m*) purity.

5.2.3 Xylene

($\text{C}_6\text{H}_4(\text{CH}_3)_2$) mixed isomers, technical grade.

5.3 Apparatus

5.3.1 Conditioning chamber, well ventilated and maintained at (20 ± 2) °C and (65 ± 5) % r.h.

5.3.2 Ventilated drying oven, capable of being maintained at (103 ± 2) °C.

5.3.3 Desiccators, with efficient desiccant (silica gel for example).

5.3.4 Treatment vessels, of a material that does not react with either the preservative or solvents or diluents, for example of glass for organic products and plastics materials for salts containing fluorine.

5.3.5 Weights, of a material that does not react with the preservative solutions under test, to provide ballast for the test specimens.

5.3.6 Plastics mesh, of a material that does not react with the preservative solutions under test, for retaining test specimens during impregnation.

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 kPa.

5.3.9 Drying vessels, provided with a cover and containing supports which will give a minimum of contact with the treated test specimens which are to be placed on them. The vessels and supports shall be of a material that does not react with the test solvent or test preservative, for example glass for organic products or of plastic material for salts containing fluorine.

5.3.10 Culture chamber (incubator or room), dark and maintained at (27 ± 2) °C and (70 ± 5) % r.h.

5.3.11 Vacuum filtration apparatus, comprising vacuum flask, 146 mm diameter Buchner funnel and fitting coarse grade filter papers.

5.3.12 Test containers, made of material which does not have a toxic effect on the soil inhabiting micro-organisms and provided with a ventilated lid. The depth shall be at least 150 mm, so as to provide at least 30 mm below the test specimens when inserted in the soil to a depth of 80 mm and adequate clearance above the top of the protruding parts of the test specimens.

NOTE The exact dimensions are not critical but they determine the number of test specimens in each vessel (which should not be less than 10). An example of a suitable test container is described in annex D.

5.3.13 Safety equipment and protective clothing, appropriate for the test product, test solvent and reference preservative, to ensure the safety of the operator.

5.3.14 Ordinary laboratory equipment, including a balance accurate to 0,001 g.

6 Sample 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 following species shall be used for the test :

- Scots pine (*Pinus sylvestris Linnaeus*) for products intended to be used on softwoods ;
- beech (*Fagus sylvatica Linnaeus*) for products intended to be used on hardwoods.

NOTE Additional tests may be carried out using other species but, if so, this should be stated in the test report.

7.2 Wood quality

The wood shall be free from cracks, stain, decay, insect damage and 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 latewood in the annual rings shall not exceed 30 % of the whole.

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

7.3 Provision of test specimens

Condition the wood to (12 ± 2) % (*m/m*) moisture content. Prepare planed strips having a cross-section of $(10 \pm 0,1)$ mm \times $(5 \pm 0,1)$ mm. The longitudinal faces shall be parallel to the direction of the grain. The annual rings

shall have a contact angle of $(90 \pm 15)^\circ$ to the broad faces. Make transverse cuts, neatly to give sharp edges and a fine-sawn finish to the end-grain surfaces, to give test specimens (100 ± 1) mm long.

The specimens shall originate from a minimum of three trees or shall be taken from a stock of more than 500 specimens and originating from at least five planks.

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

7.4 Dimensions and density of specimens

The dimensions of each test specimen at $(12 \pm 2)\%$ (*m/m*) moisture content shall be (100 ± 1) mm \times $(10 \pm 0,1)$ mm \times $(5 \pm 0,1)$ mm.

For the purposes of calculating the density of the specimens (8.1.1) and the mass of preservative retained per unit volume of wood (8.1.3), the nominal volume of each test specimen shall be taken as $5,0 \text{ cm}^3$.

In any batch of specimens, the mass of an individual is permitted to differ from the mean value of the batch by $\pm 10(m/m)$.

7.5 Number and distribution of test specimens

The test specimens are divided into :

s_1 treated test specimens.

$s_{1.1}$ test specimens treated with the test preservative: these are impregnated with the solutions of the test preservative (clause 6) and subjected to attack by the micro-organisms in the soil. Use at least six test specimens for each combination of test preservative concentration, species of wood and exposure period (8.3.1).

$s_{1.2}$ test specimens treated with the reference preservative: these specimens are impregnated with the solutions of the reference preservative (5.2.2) and subjected to attack by the micro-organisms in the soil. Use at least six test specimens for each combination of reference preservative concentration, species of wood and exposure period.

s_2 untreated test specimens.

$s_{2.1}$ virulence control specimens : these specimens are not treated, they are of the same species of wood as the treated test specimens, and are subjected to attack by the micro-organisms in the soil. They are used to provide a measure of comparability between tests. Use three virulence control specimens for each test container.

NOTE 1 The virulence control specimens are assessed after 16 weeks exposure.

$s_{2.2}$ moisture monitoring specimens : these specimens are not treated, they are of the same wood species as the treated test specimens and are planted in the soil to assess that the moisture content level established in the test specimens is adequate to support active fungal attack. Use three moisture monitoring specimens for each test container.

NOTE 2 If the test is to be carried out in eight test containers of the type described in annex D (four containers for Scots pine specimens and four containers for beech specimens, (see 8.2.2), each of these containers would require three replicates of the virulence control specimens and three replicates of the moisture monitoring specimens of the appropriate species of wood; this gives a total of 12 virulence control specimens and 12 moisture monitoring specimens per species of wood. With smaller test containers, lower numbers of replicates are acceptable but each test container should contain at least one replicate virulence control specimen and one moisture monitoring specimen.

s_3 treated check test specimens for calculation of the correction values.

$s_{3.1}$ check test specimens treated with the test preservative: these are test specimens treated in exactly the same way as the $s_{1.1}$ test specimens, except that after drying, conditioning and leaching, they are allowed to dry fully and are not planted in the soil. Use at least four specimens for each combination

of tests preservative concentration and species of wood. Variations in the mass of these specimens make it possible to determine the correction value (C_1) for the variations in mass of the treated test specimens $s_{1,1}$, resulting from factors other than attack by the soil inhabiting micro-organisms. At a given treating solution concentration, the correction value C_1 is the mean percentage change in mass of the $s_{3,1}$ test specimens.

- $s_{3,2}$ check test specimens treated with the reference preservative: these are test specimens treated in exactly the same way as the $s_{1,2}$ test specimens, except that after drying, conditioning and leaching, they are allowed to dry fully and are not planted in the soil. Use at least four specimens for each combination of reference preservative concentration and species of wood. Variations in the mass of these specimens make it possible to determine the correction value (C_2) for the variations in mass of the reference preservative treated test specimens $s_{1,2}$ resulting from factors other than attack by the soil inhabiting micro-organisms. At a given treating solution concentration, the correction value C_2 is the mean percentage change in mass of the $s_{3,2}$ test specimens.

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

NOTE 3 It is advisable to treat more specimens than the minimum number required to allow the rejection of specimens having more than the permitted variation in the quantity of product absorbed (8.1.3).

8 Procedures

8.1 Preparation of test specimens

8.1.1 Conditioning of specimens before treatment

Place the numbered test specimens in the oven (5.3.2) and leave them there for 18 h to 24 h²⁾. Cool to room temperature in a desiccator (5.3.3) and weigh to the nearest 0,001 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 nominal volume (see 7.4).

8.1.2 Preparation of treatment solutions

Prepare a series of solutions of at least three concentrations (expressed as % (m/m)) of the test preservative (clause 6) in the appropriate solvent or diluent (5.2.1). A solvent or diluent control, that is treatment at concentration 0, shall also be included.

NOTE 1 It is preferable to use at least five concentrations of the test preservative except when there is prior experience of the performance of the test preservative in the test. It is normal for the treating solution concentrations to be arranged in a geometric or arithmetic progression.

NOTE 2 The selection of the treating solution concentrations (and therefore retentions) of the test preservative should be made giving consideration to the performance of the reference preservative in the test and the method of calculation of the results (see annex E). It is also necessary to include a retention of the test preservative which fails in the test before the reference preservative fails, to be able to calculate accurately the nominal effective retention of the test preservative (see clause 10). With beech test specimens, the retentions in the test specimens will normally be ranged about the likely retention to be effective in practice. However, with Scots pine sapwood test specimens, the retentions in the test specimens should be over a range which is much lower than the likely retention to be effective in practice. This is because of the good performance of wood preservatives in this species of wood in laboratory tests and is exemplified by the selection of the treating solution concentrations for the reference preservative.

Prepare the reference preservative (5.2.2) at the following concentrations in water :

²⁾ In the case of supplementary tests (7.1) using species of wood other than Scots pine sapwood or beech, this drying time may need to be longer than 18 h to 24 h; the drying time should be such that the test specimens achieve constant mass. This can be established by selecting at random from the batch being dried 10 test specimens; after drying and cooling as directed, determine the total mass, return the specimens to the oven and repeat the operation at intervals of not less than 4 h ; constant mass is achieved when the total mass of the selected specimens does not lose more than 0,05 g between weighings.

- Scots pine: 0,1 % - 0,16 % - 0,25 % - 0,4 % (*m/m*) ;
- beech: 1,0 % - 1,6 % - 2,5 % - 4,0 % (*m/m*).

All treatment solutions shall be freshly prepared.

8.1.3 Impregnation

Carry out impregnation of the sets of test specimens with the test preservative solutions in ascending order of concentration starting with the solvent control (concentration = 0). Using clean equipment, impregnate the appropriate sets of test specimens with the reference preservative, again in ascending order of concentration.

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

For each solution place the test specimens, kept dry as described in 8.1.1 and of known mass m_0 , in one of the treatment vessels (5.3.4) so that as much of their surface as possible is exposed (for example, by stacking them crosswise). Ballast the stack of specimens with the weights (5.3.5) using the plastics mesh (5.3.6) if necessary, to prevent them floating when the liquid is admitted.

Place each treatment vessel in one of the vacuum vessels (5.3.7) and after reducing the pressure to 0,7 kPa, using the vacuum pump (5.3.8), hold it at this pressure for 15 min. After this period, close the stopcock to the vacuum pump and open the stopcock to allow the solution of preservative to be drawn into the treatment vessel within the vacuum vessel until it completely covers the test specimens. Keep the specimens covered completely by the solution throughout the remainder of the impregnation process.

Next, admit air slowly to bring the vacuum vessel back to atmospheric pressure, remove the treatment vessel with its submerged specimens from the vacuum vessel. Cover the top of the treatment vessel and leave it for 2 h, adding further solution if necessary to keep the specimens fully covered by the liquid.

After impregnation, remove the test specimens one by one from the treatment vessel and remove excess liquid from them by lightly blotting with absorbent paper. Immediately weigh each to the nearest 0,001 g to ascertain the mass after impregnation (m_1).

In the case of preservatives which are being studied as active ingredients, calculate the mass of preservative retained for each test specimen, from the mass of solution absorbed ($m_1 - m_0$) and its concentration ³⁾.

In the case of formulated wood preservatives, express the retention for each test specimen in terms of the ready-to-use product, and, for products supplied in the form of a concentrate, in terms of the product as supplied.

NOTE If the product has been supplied as a concentrate for dilution prior to use, the nominal effective concentration established (see clause 10) will need to be equivalent to the product as marketed, to provide the data in the correct form for use in EN 599-1.

Calculate the mass of active ingredient or formulation retained per unit volume of wood, in kilograms per cubic metre, for each test specimen from the retention of product and volume of the test specimens (7.4) and the mean value for each simultaneously impregnated group of test specimens.

Reject those test specimens in which the quantity of product absorbed varies by more than 15 % from the mean absorption of the group. Replace them with supplementary specimens and calculate a new mean.

8.1.4 Drying and conditioning of specimens after treatment

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

Keep the test specimens for four weeks in the conditioning chamber (5.3.1). Arrange the test specimens in the drying vessels (5.3.9), resting on their narrow faces on the supports, and placing only specimens treated with the

³⁾ When dealing with preservative formulations whose constituents are absorbed selectively by the wood, it may be necessary to carry out chemical analysis of the solution before and after impregnation. Similarly, analysis is recommended when very dilute solutions are used.

same concentration of the test or reference preservative in the same drying vessel; avoid contact between specimens. Invert the test specimens twice a week at intervals of three or four days.

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

In the case of test specimens impregnated using a volatile organic liquid as the solvent or diluent, keep each vessel covered for one week. Open each vessel gradually during the second week and finally leave them open during the third and fourth weeks.

8.1.5 Leaching procedure

Subject all treated test specimens and treated check test specimens ($s_{1,1}$, $s_{1,2}$, $s_{3,1}$ and $s_{3,2}$) to the procedure described in EN 84.

Stop the drying stage of EN 84 when all the test specimens have reached a moisture content of $(50 \pm 5) \% (m/m)$. If the test specimens become too dry, they shall be rewetted by a short soak in water.

NOTE The moisture content should be checked by periodic weighing of a minimum of 10 specimens taken at random during the drying period and comparing their mass with their initial dry mass (m_0), making an allowance for the mass of preservative retained. Drying to 50 % moisture content is likely to occur within 24 h.

8.1.6 Wetting of untreated specimens

Impregnate the virulence control specimens ($s_{2,1}$) and the moisture monitoring specimens ($s_{2,2}$) with water, using the method described in EN 84. Allow to soak for 2 h, then lay to dry. Continue with the drying stage of EN 84, stopping this when the test specimens have reached a moisture content $(50 \pm 5) \% (m/m)$.

NOTE This impregnation procedure should be timed to coincide with the end of the leaching period of the treated specimens (8.1.5) in order that drying of all the test specimens is undertaken at the same time.

8.2 Exposure of the test specimens to soil inhabiting micro-organisms

8.2.1 Preparation of test containers

Determine the mass of soil required to provide at least 120 mm depth of soil in a selected test container. Determine the moisture content and water holding capacity (WHC) of the soil (5.1.1). Calculate the amount of water required to bring the soil in the fully charged container to 95 % of its WHC.

NOTE A suitable procedure for determining WHC and the quantity of water required to wet up the soil is described in annex B.

Add the required volume of soil to each test container and add the calculated amount of water slowly whilst thoroughly mixing to ensure an even distribution of moisture.

8.2.2 Planting the test specimens

Plant the Scots pine and beech test specimens in different containers. Plant the treated and untreated test specimens s_1 and s_2 vertically with 20 mm of their length protruding above the surface of the soil and with a minimum of 20 mm between adjacent specimens and from the sides of the container. Assign the correct number of virulence control specimens $s_{2,1}$ and moisture monitoring specimens $s_{2,2}$ (7.5.2) to each test container and distribute them at random. Assign the positions of the test specimens treated with the test preservative $s_{1,1}$ and the reference preservative $s_{1,2}$ at random among all the test vessels being used for the appropriate species of wood. During planting and subsequent handling ensure the exact location of each specimen is recorded to guard against loss of identity if the numbering is obscured. Apply a ventilated lid to each charged test container.

NOTE The test specimens of each species of wood are separated to reduce the number of untreated specimens (s_2) that are required and to allow separate adjustment to the moisture content of the test specimens (8.2.4) which may vary between wood species.

8.2.3 Culture conditions and duration of test

Transfer the charged test containers to the culture chamber (5.3.10) and incubate for up to 32 weeks (see 8.3.1).

8.2.4 Monitoring initial moisture content

After 5 days incubation remove the moisture monitoring specimens from each container, cleanse them of adhering soil particles and weigh to the nearest 0,001 g (m_2). Calculate the moisture content of each specimen by expressing its water content ($m_2 - m_0$) as a percentage of the initial dry mass (m_0).

NOTE 1 The optimal conditions for decay are achieved if the initial moisture content of the test specimens is between $(50 \pm 5) \% (m/m)$ and $(80 \pm 5) \% (m/m)$.

If the mean moisture content of the specimens from any container is below 45 % (m/m), add a volume of water to that container not greater than 10 % (m/m) of that originally added (8.2.1) distributing it evenly over the surface of the soil. Replant the moisture monitoring specimens, incubate for 2 days and repeat the weighing and calculation. Add further water and repeat if necessary.

If the mean moisture content of the specimens from any container is greater than 85 % (m/m), remove the lid of that container and leave for several days to allow some drying. Replant the moisture monitoring specimens, replace the lid and continue incubation; recheck the moisture content of the specimens after 5 days as previously.

NOTE 2 If a continuous indication of soil moisture content is required or as an alternative to the procedure described in 8.2.5, insert sets of new moisture monitoring specimens at suitable intervals during incubation and check as above.

8.2.5 Monitoring and maintenance of soil moisture

Prior to the start of incubation, weigh each charged test vessel to the nearest 5 g and record the initial mass. After 4 weeks incubation, reweigh each test container. Make good any loss in mass, allowing for the mass of moisture monitoring specimens removed, by addition of water distributed evenly over the soil surface. Repeat the operation at 4 weeks intervals.

If the test containers are too large to be weighed, use the procedure described in 8.2.4.

NOTE If the culture chamber is a sealed incubator fitted with a water tray, the moisture content may be assumed to remain constant during incubation and no monitoring of moisture content is required. Similarly, if previous experience has shown that there is no moisture loss under the incubation conditions used, no monitoring of moisture content is required.

8.2.6 Control test

Complete drying of the treated check test specimens ($s_{3.1}$ and $s_{3.2}$) as described in EN 84. Transfer the specimens to the drying oven (5.3.2). Dry the specimens for 18 h to 24 h, cool to room temperature in desiccators (5.3.3), weigh each specimen to the nearest 0,001 g and record the final dry mass (m_3).

NOTE 1 It is convenient to oven dry these specimens at the same time as the first set of replicates exposed to fungal attack. After air drying has been completed, wrap the specimens in polyethylene and store in the conditioning chamber (5.3.1) until the end of the first exposure period.

Calculate the change in mass of each test specimen by expressing the change in mass ($m_0 - m_3$) as a percentage of the initial dry mass (m_0). Calculate the correction value (C_1) for the check test specimens treated with each concentration of the test preservative ($s_{3.1}$) and the correction value (C_2) for the check test specimens treated with each concentration of the reference preservative ($s_{3.2}$). At a given treating solution concentration, the correction value is the mean percentage change in mass of the check test specimens.

NOTE 2 These correction values are used to correct the mass losses of the treated test specimens for changes in mass other than those caused by fungal attack (8.3.2).

8.3 Assessment of test

8.3.1 Examination of the test specimens

After each exposure period (8, 16, 24 and 32 weeks after the test specimens have been planted in the soil (see 8.2.2)), remove one replicate set of test specimens treated with the test preservative and the reference preservative from the soil. After 16 weeks of exposure, remove the set of virulence control specimens. Cleanse the specimens of adhering soil particles. Make good any obscured numbering, note outstanding features of their condition including evidence of waterlogging and weigh each specimen to the nearest 0,001 g (m_2).

NOTE The presence of decay types in addition to soft rot (for example white rot, brown rot and bacterial attack) can be useful in the interpretation of the test data.

Transfer the specimens to the drying oven (5.3.2). Dry the specimens for 18 h to 24 h, cool to room temperature in desiccators (5.3.3), weigh each specimen to the nearest 0,001 g and record the final dry mass (m_3). Calculate the moisture content of each specimen at the end of the test by expressing the water content ($m_2 - m_3$) as a percentage of the final dry mass (m_3).

8.3.2 Assessment of test specimens

Calculate the loss in mass of each test specimen by expressing the loss in mass ($m_0 - m_3$) as a percentage of the initial dry mass (m_0). Use the appropriate correction value (8.2.6) to correct the loss in mass of each treated test specimen after all the exposure periods.

Reject any test specimen having a corrected loss in mass of less than 3 % (m/m) which appears abnormal in relation to moisture content, that is having a final moisture content of less than 40 % (m/m) or greater than 150 % (m/m).

Calculate the mean corrected loss in mass for each set of replicate test specimens. If any test specimen shows an increase in corrected mass this increase shall be recorded as such but taken as zero in these calculations.

9 Validity of test

The test shall be accepted as valid if, among the test specimens treated with the reference preservative, the mean corrected mass loss of specimens treated with the lowest concentration is greater than 3 % (m/m) after 32 weeks exposure.

10 Expression of results

The performance of the test preservative in a given species of wood shall be evaluated by comparison with the performance of the highest concentration of the reference preservative which showed a mean corrected loss in mass greater than 3 % (m/m) after 32 weeks exposure using the same species of wood.

For this concentration of the reference preservative, the mean retention is equivalent to the nominal retention of the reference preservative (*n.r.R.*).

For each concentration of the test preservative, determine the mean corrected loss in mass of the test specimens which would have been achieved at the same time as 3 % (m/m) loss in mass was achieved by the specimens treated with the concentration of the reference preservative selected above.

From the mean loss in mass of the test specimens treated with each concentration of the test preservative, determine the retention of the preservative that would equate to a mean loss in mass of 3 % (m/m). This retention is equivalent to the nominal retention of the test preservative (*n.r.P.*).

NOTE An example of these calculations is given in annex E.

Calculate the nominal effective retention (*n.e.r.*) of the test preservative as follows recording the calculated value to the nearest 0,1 kg/m³:

$$n.e.r. = \frac{n.r.P. \text{ at } 3\% (m/m) \text{ mass loss}}{n.r.R. \text{ at } 3\% (m/m) \text{ mass loss}} \times \text{target retention of the reference preservative}$$

where

n.r.P. is the nominal retention of the test preservative ;

n.r.R. is the nominal retention of the reference preservative.

The target retentions of the reference preservative are as follows :

- Scots pine 8 kg/m³ ;
- beech 20 kg/m³.

11 Test report

The report shall include at least the following (see also annex F 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 or unique code or number of the preservative tested, with an indication of whether or not the composition has been declared ;
- d) the name and concentration of active ingredient(s) ;
- e) the solvents or diluents used ;
- f) the reference preservative used ;
- g) the species of wood used ;
- h) the mean density of test specimens of each wood species used for the test ;
- i) the concentrations, as percentages by mass, of the preservative tested and the reference preservative ;
- j) the quantity of solution, expressed in grams, absorbed by each test specimen and the quantity of preservative, expressed in kilograms per cubic metre, retained by each specimen ;
- k) the conditioning period after impregnation ;
- l) the date when the test specimens were planted in the test containers ;
- m) the type of soil used and its water holding capacity ;
- n) the dates when the test specimens were removed from the test containers and the exposure periods ;
- o) for each specimen, the uncorrected loss in mass expressed as a percentage of the initial dry mass ;
- p) the percentage loss in mass of each virulence control, the mean loss in mass for each species of wood after the 16 week exposure ;
- q) the correction value (C_1) or (C_2) for each concentration studied ;
- r) the corrected percentage loss in mass of each test specimen treated with the reference preservative and, for each combination of preservative concentration, exposure time and species of wood and the mean corrected loss in mass together with a statement as to whether or not these validate the test ;

- s) the corrected percentage loss in mass of each test specimen treated with the test preservative and, for each combination of preservative concentration, exposure time and species of wood, the mean corrected loss in mass ;
- t) the nominal effective retention of the test preservative for each species of wood and the concentration of the reference preservative used for the calculations ;
- u) the name of the organization responsible for the report and the date of issue ;
- v) the name(s) and signature(s) of the officer(s) in charge ;
- w) the following note:

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

The report shall also list any variation from the specified test method as well as any factors which may have influenced the results.

Annex A (informative)

Optional screening test

A.1 Introduction

To provide information on the effectiveness of active ingredients or wood preservatives to prevent decay of the soft rot type, it may be appropriate to carry out screening tests using the method described in this annex. The method uses a mixture of five fungi, which are all capable of causing soft rot, to challenge treated test specimens thus providing basic information on activity against this group of fungi. This method does not expose the test specimens to the wide range of organisms found in the soil used in the main prestandard and which are known to influence significantly the overall performance of a wood preservative in the ground contact service situation.

The annex refers to the main text where requirements are similar.

A.2 Principle

A number of small test specimens are impregnated with the preservative under test at a minimum of five concentrations. The test specimens are exposed to leaching according to EN 84. The specimens are buried in a test substrate and inoculated with a mixed spore suspension of five fungi capable of causing soft rot. The test specimens are assessed after a minimum period of exposure of 12 weeks for beech and 16 weeks for Scots pine. The performance of the test preservative is evaluated by comparison with the performance of a reference preservative.

A.3 Test materials

A.3.1 Biological materials

A.3.1.1 Test fungi

The following fungi should be included in every test :

Species	Strain No.
<i>Chaetomium globosum</i> Kunze	ATCC 6205
<i>Humicola grisea</i> Traaen	MG 28
<i>Petriella setifera</i> (Alf. Schmidt) Curzi	MG 50
<i>Lecythophora mutabilis</i> (van Beyma) W. Gams and Mc Ginnis	S 24-E
<i>Trichurus spiralis</i> Hasselbr.	MG 31

Other fungal species or strains may be relevant to specific circumstances. These should be obtained from a recognized culture collection and used in parallel tests. Pure cultures recently isolated from naturally infected material are also relevant. They should be identified and deposited in a recognized collection.

A.3.1.2 Maintenance and treatment of strains

The laboratory holding the parent strains should maintain them so as to retain both their ability to cause soft rot decay of wood and to produce spores in large numbers. Laboratories who run tests regularly may maintain the strains themselves, but if a strain shows signs of degeneration, for example a reduced level of sporing, it should no longer be used and the laboratory should obtain a new standard culture of the strain.

The laboratory supplying test cultures should provide a description of the growth features characteristic of each fungus to the recipient.

NOTE Test strains should be available from the Bundesanstalt für Materialforschung und -prüfung, D-12200 Berlin, Germany.

Maintain stock cultures on test tube slopes of a medium containing 2 %(*m/m*) agar and 5 %(*m/m*) malt extract at a temperature of 6 °C to 10 °C. Subculture at intervals not exceeding six months.

A.3.1.3 Preparation of spore suspension

Subculture the test fungi, separately, on test tube slopes of malt agar medium containing 40 g/l malt extract (powder) or 50 g/l of malt extract (concentrate) and 25 g/l agar; incubate at a temperature of between 26 °C and 28 °C. Cultures should be used for preparing spore suspensions when they are between 14 days and 28 days old, preferably between 14 days and 21 days old. Cultures from previously unopened tubes should be used to make up each batch of suspension.

Select the number of subcultures of each test fungus necessary to produce one fifth of the required quantity of spore suspension (A.5.2.2). Taking each culture in turn, gently add 10 ml of the wetting agent solution (A.3.2.1) that has been sterilized in an autoclave at 121 °C for 20 min. Sterilize a platinum or nichrome wire by heating to red heat in a flame and allowing to cool. Otherwise use a sterile plastics loop. Use the wire or loop to scrape gently the surface of the culture to liberate the spores. Agitate the liquid slightly to disperse the spores without detaching mycelial fragments. Gently decant the spore suspension from each test fungus into a separate sterile container. Determine the number of spores in a sample of the spore suspension prepared from each fungus, using a haemocytometer counting chamber or particle counter (A.3.3.1), and use only spore suspensions containing at least 10^5 spores/ml. Mix together the spore suspensions of all the test fungi.

Use the suspension on the day on which it is prepared and do not store it for future use.

A.3.2 Products and reagents

A.3.2.1 Wetting agent solution

Water containing 0,5 g/l of dioctyl sodium sulfosuccinate.

A.3.2.2 Test substrate

An hydrated, laminar, aluminium-iron-magnesium silicate ⁴⁾ exfoliated to yield particles up to 3 mm diameter. Particles less than 2 mm diameter should be removed by sieving. Before use, mix the sample of the test substrate well.

The test substrate should be used only once.

⁴⁾ Vermiculite is suitable.

A.3.2.3 Nutrient solution for spore suspension

The nutrient solution is a salts solution with the following composition :

3,00 g	NH ₄ NO ₃
2,56 g	K ₂ HPO ₄
1,02 g	MgSO ₄ ·7H ₂ O
0,25 g	KCl
0,005 g	NaCl
0,001 g	FeSO ₄
0,001 g	MnSO ₄
0,001 g	ZnSO ₄
1,00 l	water

NOTE 1 In practice, the minor constituents may be prepared as a stock solution, and the quantity of water used to prepare the nutrient solution reduced to compensate for the amount of stock solution added.

Dissolve the salts in the water and, using the pH meter (A.3.3.2), adjust the pH to 6,2 by dropwise addition of 0,25 mol/l sulfuric acid solution (H₂SO₄).

NOTE 2 If there is visible precipitation of salts after autoclaving (A.5.2.2), the manganese sulphate should be prepared separately in some of the water, sterilized and the appropriate quantity added to each conical flask after sterilization.

A.3.3 Apparatus

A.3.3.1 Haemocytometer counting chamber or particle counter

To count the number of spores.

A.3.3.2 pH meter

Capable of determining pH to the nearest 0,1 units.

A.3.3.3 Test containers

The test containers should be 500 ml in capacity, made of a material that does not have a toxic effect on the test fungi and provided with a lid. The containers should be approximately 65 mm in depth.

NOTE An example of a suitable test container is shown in Figure A.1.

A.4 Test specimens

A.4.1 Species of wood

The following species should be used for the test :

- Scots pine (*Pinus sylvestris* Linnaeus) ;
- beech (*Fagus sylvatica* Linnaeus).

NOTE 1 Beech may be preferred as the test species of wood in this screening test because results are generally obtained after shorter incubation periods than when using Scots pine. However, the performance of a test product in beech may not give a true indication of its performance in Scots pine.

NOTE 2 Additional tests can be carried out using other species but, if so, this should be stated in the test report.

A.4.2 Wood quality

The wood quality should be as described in clause 7.2.

A.4.3 Provision of test specimens

Condition the wood to $(12 \pm 2) \% (m/m)$ moisture content. Prepare planed strips having a cross section of $(15 \pm 0,1) \text{ mm} \times (5 \pm 0,1) \text{ mm}$. The longitudinal faces should be parallel to the direction of the grain. The annual rings should have a contact angle of $(90 \pm 15)^\circ$ to the broad faces. Make transverse cuts, neatly to give sharp edges and a fine-sawn finish to the end-grain surfaces, to give test specimens $(40 \pm 0,5) \text{ mm}$ long.

The specimens should originate from a minimum of three trees or should be taken from a stock of more than 500 specimens and originating from at least five planks.

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

A.4.4 Dimension and density of specimens

The dimensions of each test specimen at $(12 \pm 2) \% (m/m)$ moisture content should be $(40 \pm 0,5) \text{ mm} \times (15 \pm 0,1) \text{ mm} \times (5 \pm 0,1) \text{ mm}$.

For the purpose of calculating the density of the specimens (A.5.1.1) and the mass of preservative retained per unit volume of wood (A.5.1.3), the nominal volume of each specimen should be taken as $3,0 \text{ cm}^3$.

In a batch of specimens, the mass of an individual is permitted to differ from the mean value of the batch by $\pm 10 \% (m/m)$.

A.4.5 Number and distribution of test specimens

The test specimens are divided into :

v_1 treated test specimens.

$v_{1,1}$ test specimens treated with the test preservative: these are impregnated with the solutions of the test preservative (clause 6) and subjected to attack by the soft rot fungi in the test substrate. Use at least six treated test specimens for each combination of test preservative concentration and species of wood.

$v_{1,2}$ test specimens treated with the reference preservative: these specimens are impregnated with the solutions of the reference preservative (5.2.2) and subjected to attack by the soft rot fungi in the test substrate. Use at least six test specimens for each combination of reference preservative concentration and species of wood.

v_2 untreated test specimens for virulence control of the fungal mixture: these are untreated test specimens subjected to attack by the mixture of soft rot fungi. Use at least six specimens for each species of wood.

NOTE Where there is no previous experience with the method, or where previous experience has shown that the mass loss required to validate the test may not be achieved in the normal test period, prepare several sets of six replicates. Sets can then be assessed after the minimum incubation period (see A.5.3.1) and at 4 weekly intervals thereafter. The main series of test specimens should be assessed when the virulence requirement has been achieved.

v_3 treated check test specimens for calculation of the correction values.

$v_{3,1}$ check test specimens treated with the test preservative: these are test specimens treated in exactly the same way as the $v_{1,1}$ test specimens, which are placed, after drying, conditioning and leaching, in test vessels which are maintained in a sterile condition (A.5.2.4). Use at least six specimens for each combination of test preservative concentration and species of wood. Variations in the mass of these specimens make it possible to determine the correction value (K_1) for the variations in mass of the treated test specimens $v_{1,1}$, resulting from factors other than attack by the test fungi. At a given treating solution concentration, the correction value K_1 is the mean percentage change in mass of the $v_{3,1}$ test specimens.

$v_{3,2}$ check test specimens treated with the reference preservative: these are test specimens treated in exactly the same way as the $v_{1,2}$ test specimens, which are placed, after drying, conditioning and leaching, in test vessels which are maintained in a sterile condition (A.5.2.4). Use at least six specimens for each combination of reference preservative concentration and species of wood. Variations in the mass of these specimens make it possible to determine the correction value (K_2) for the variations in mass of the treated test specimens $v_{1,2}$, resulting from factors other than attack by the test fungi. At a given treating solution concentration, the correction value K_2 is the mean percentage change in mass of the $v_{3,2}$ test specimens.

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

A.5 Procedures

A.5.1 Preparation of test specimens

A.5.1.1 Conditioning of specimens before treatment

Condition the specimens as described in 8.1.1. Calculate the mean density of the specimens of each species using the mean mass and the nominal volume (A.4.4).

A.5.1.2 Preparation of treatment solutions

Prepare a series of solutions of at least five concentrations (expressed as % (m/m)) of the test preservative (clause 6) in the appropriate solvent or diluent (5.2.1). If the approximate toxic values are unknown, the selected concentrations should form a widely spaced geometric progression for a first test and a more closely spaced geometric or arithmetic progression for subsequent tests. A solvent or diluent control, that is treatment at concentration 0 should also be used.

Prepare the reference preservative (5.2.2) at the following concentrations in water :

- Scots pine : 0,4 % - 0,63 % - 1,0 % - 1,6 % (m/m) ;
- beech 1,6 % - 2,5 % - 4,0 % - 6,3 % (m/m).

All treatment solutions should be freshly prepared.

A.5.1.3 Impregnation

Impregnate the test specimens and calculate the retention of the test preservative and the reference preservative using the procedures described in 8.1.3.

A.5.1.4 Drying and conditioning of specimens after treatment

Dry and condition the specimens after treatment as described in 8.1.4.

A.5.1.5 Leaching procedure

Leach the test specimens and dry them to $(50 \pm 5) \%$ (*m/m*) moisture content as described in 8.1.5.

A.5.1.6 Wetting of untreated specimens

Wet the untreated specimens as described in 8.1.6.

A.5.2 Exposure of the test specimens to fungi

A.5.2.1 Preparation of test containers

Determine the moisture content and the water holding capacity (WHC) of the test substrate (A.3.2.2).

NOTE A suitable method for determining WHC is described in annex B.

In each test vessel (A.3.3.3) form a layer of test substrate (A.3.2.2) using (125 ± 5) ml. Place three test specimens treated with the same concentration of the test preservative ($v_{1,1}$) or the reference preservative ($v_{1,2}$) or three untreated test specimens (v_2) onto the substrate layer, orientating the specimens with the annual rings at 90° to the substrate surface and spacing the specimens approximately equidistant from each other and the sides of the vessel. Subsequently cover the specimens with a further (125 ± 5) ml of the test substrate. Sterilize the test vessels and their lids with epoxyethane, steam or by ionizing radiation as described in annex B of EN 113.

Alternatively, sterilize the test vessels, the test substrate and test blocks separately, in accordance with annex B of EN 113 and assemble under sterile conditions.

NOTE Glass test vessels and test substrate can be sterilized by autoclaving.

A.5.2.2 Inoculation

From the determination of the WHC (A.5.2.1) calculate the quantity of fluid (X ml) required to wet up 250 ml of the test substrate (A.3.2.2) to 95 % of its WHC. Dispense quantities of X ml minus $(3 \pm 0,1)$ ml of nutrient solution for spore suspension (A.3.2.3) into the required number of 500 ml conical flasks. Close the conical flasks and sterilize them in an autoclave at 121°C for 20 min; allow to cool to room temperature.

Inoculate each flask with $(3 \pm 0,1)$ ml of the mixed spore suspension (A.3.1.3). Under sterile conditions, evenly distribute the inoculated nutrient solution on to the test substrate surface in the test vessels.

A.5.2.3 Culture conditions and duration of test

Close the inoculated test vessels with the corresponding lids and place them in the culture chamber (5.3.10) for a minimum period of 12 weeks for beech and 16 weeks for Scots pine (see A.5.3.1).

A.5.2.4 Control test

Place the treated check test specimens $v_{3,1}$ and $v_{3,2}$ in sterile test vessels with test substrate as described in A.5.2.1. Add the required amount of nutrient solution (see A.5.2.2) to which has been added $(3 \pm 0,1)$ ml of sterile water in place of the spore suspension. Incubate together with the inoculated test specimens.

A.5.2.5 Maintenance of substrate moisture

Prior to the start of incubation, weigh each charged culture vessel to the nearest 0,1 g and record the initial mass.

At intervals of not more than four weeks, reweigh each culture vessel. Under sterile conditions, make good any loss in mass by adding sterile water around the edge of the culture vessel. Return the culture vessels to the culture chamber.

NOTE If experience has shown that only small losses in mass occur under the particular culture chamber conditions used (for example, a loss of not greater than 5 ml in 12 weeks) it is not necessary to monitor moisture content. This should be recorded in the test report.

A.5.3 Assessment of test

A.5.3.1 Determination of incubation period

After 12 weeks for beech and 16 weeks for Scots pine, remove one set of untreated test specimens (v_2) from the test vessels, removing any adhering mycelium and test substrate. Make good any obscured numbering and weigh each specimen to the nearest 0,001 g (m_2). Dry the specimens for 18 h to 24 h in the drying oven, cool to room temperature in a desiccator, weigh each specimen to the nearest 0,001 g and record the final dry mass (m_3). Calculate the moisture content of each specimen at the end of test by expressing the water content ($m_2 - m_3$) as a percentage of the final dry mass (m_3). Calculate the loss in mass of each specimen by expressing the loss in mass ($m_0 - m_3$) as a percentage of the initial dry mass (m_0). Calculate the mean loss in mass for each species of wood.

If the mean loss in mass of beech test specimens exceeds 20 % (m/m) or Scots pine specimens exceeds 15 % (m/m), then assess the associated treated test specimens. If the mean loss in mass does not exceed these levels which are necessary to validate the test (see A.6), continue incubation of the wood species concerned. Assess another set of untreated test specimens (v_2) after a further 4 week period of incubation. If the necessary levels of decay have been achieved, assess the treated test specimens. Otherwise repeat the procedure after a further period of incubation.

NOTE The losses in mass required to validate the test should be achieved within a period of 24 weeks incubation.

A.5.3.2 Assessment of treated test specimens

After the incubation period determined for each species of wood according to A.5.3.1, withdraw all the treated test specimens from the test vessels, removing any adhering mycelium and test substrate. Make good any obscured numbering, note their condition (for example evidence of waterlogging) and weigh each specimen to the nearest 0,001 g (m_2). Dry the specimens for 18 h to 24 h in the drying oven, cool to room temperature in a desiccator, weigh each specimen to the nearest 0,001 g and record the final dry mass (m_3).

Calculate the moisture content of each specimen at the end of test by expressing the water content ($m_2 - m_3$) as a percentage of the final dry mass (m_3). Calculate the loss in mass of each specimen by expressing the loss in mass ($m_0 - m_3$) as a percentage of the initial dry mass (m_0). Calculate the correction value (K_1) for the check test specimens treated with each concentration of the test preservative ($v_{3,1}$) and the correction value (K_2) for the check test specimens treated with the reference preservative ($v_{3,2}$). Use the appropriate correction value to correct the loss in mass of each test specimen treated with the test preservative ($v_{1,1}$) and the reference preservative ($v_{1,2}$).

Reject any test specimens having a corrected loss in mass of less than 3 % (m/m) which appear abnormal in relation to moisture content, that is having a final moisture content of less than 40 % (m/m) or greater than 150 % (m/m).

Calculate the mean loss in mass for each set of replicate test specimens, using corrected values for treated test specimens. If any specimen shows an increase in corrected loss in mass, this increase should be recorded as such but taken as zero in these calculations.

A.6 Validity of the test

The results should be accepted as valid if the untreated virulence control specimens (v_2) show a mean loss in mass which is not less than 15 % (m/m) with Scots pine and 20 % (m/m) with beech.

A.7 Expression of results

The toxic values of both the test preservative and the reference preservative should be expressed as the following two concentrations.

- The upper toxic value (*u.t.v.*) corresponding to the lowest concentration of the preservative in the series in which :
 - 1) the mean corrected loss in mass of the specimens is less than 3 % of their initial dry mass ; and
 - 2) not more than one specimen has suffered a corrected loss in mass of greater than 3 % but less than 5 %.
- the lower toxic value corresponding to the next lowest concentration in the series in which the criteria for the upper toxic value are not met.

Express the toxic values in both kilograms of preservative per cubic metre of wood and the concentrations of the preservative in the solvent or diluent for each species of wood.

Calculate the nominal effective retention (*n.e.r.*) of the test preservative as follows :

$$n.e.r. = \frac{u.t.v. \text{ test preservative}}{u.t.v. \text{ reference preservative}} \times \text{target retention reference preservative}$$

The target retentions of the reference preservative are as follows :

- Scots pine 8 kg/m³;
- beech 20 kg/m³.

A.8 Test report

The report should include at least the following :

- a) the number and date of this European Prestandard ;
- b) the name of the supplier of the preservative under test ;
- c) the specific or unique code or number of the preservative tested, with an indication of whether or not the composition has been declared ;
- d) the name and concentration of active ingredient(s) ;
- e) the solvents or diluents used ;
- f) the reference preservative used ;
- g) the species of wood used ;
- h) the mean density of test specimens of each species of wood used for the test ;
- i) the species and strain numbers of the fungi used ;
- j) the concentrations, as percentages by mass, of the preservative tested and the reference preservative ;
- k) the quantity of solution, expressed in grams, absorbed by each test specimen and the quantity of preservative, expressed in kilograms per cubic metre, retained by each specimen ;
- l) the conditioning period after impregnation ;
- m) the means of sterilization used ;

- n) the date when the test vessels were inoculated ;
- o) the dates when the treated test specimens were removed from the test vessels and the exposure period for each species of wood ;
- p) for each specimen, the uncorrected loss in mass expressed as a percentage of the initial dry mass ;
- q) the correction value (K_1) or (K_2) for each concentration studied ;
- r) the corrected percentage loss in mass of each test specimen treated with the reference preservative and, for each combination of preservative concentration and species of wood, the mean corrected loss in mass ;
- s) the percentage loss in mass of the individual virulence control specimens assessed at the same time as the treated test specimens, the mean loss in mass for each species of wood and a statement as to whether or not these validate the test ;
- t) for each wood species, the toxic values of the test preservative and the reference preservative expressed both as retention in kilograms per cubic metre of wood, and as the concentration of the corresponding solutions as a percentage by mass ;
- u) the nominal effective retention of the test preservative and the target retention of the reference preservative used in the calculation ;
- v) the name of the organization responsible for the report and the date of issue ;
- w) the name(s) and signature(s) of the officer(s) in charge ;
- x) the following note:

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

The report shall also list any variation from the specified test method as well as any factors which may have influenced the results.

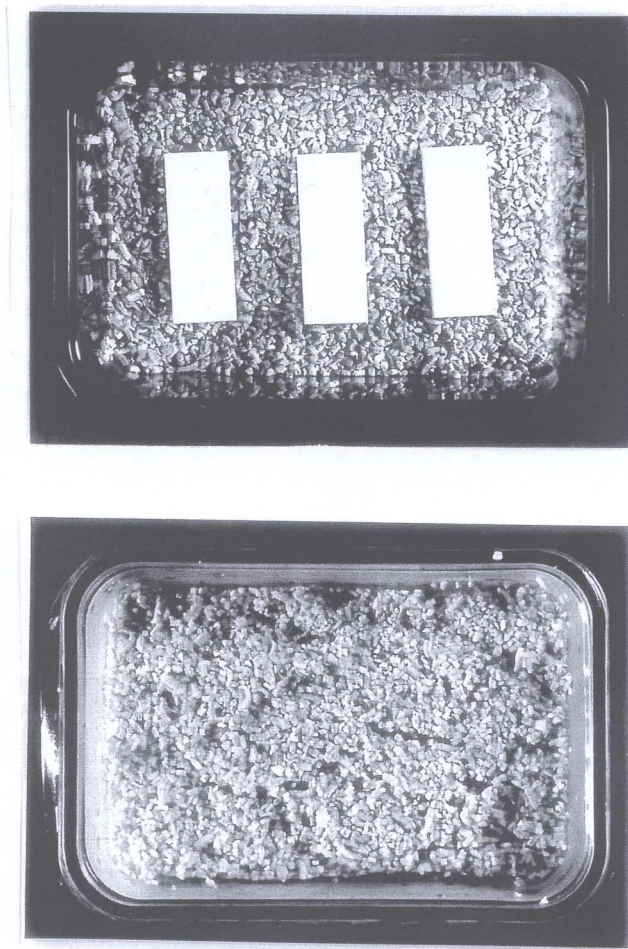


Figure A.1 - Example of a suitable test container

Annex B (informative)

Determination of soil water holding capacity

B.1 Principle

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

NOTE If wood test specimens are sealed in bottles in contact with different soils, each adjusted to its own water holding capacity, then the equilibrium moisture content of all the wood specimens should be approximately equal.

B.2 Preparation of samples

Weigh out three 200 g samples of the soil. Add a small quantity of water to each sample, mix well, and repeat the operation until the soil particles stick to one another (crumb structure). Add a further 25 ml water, mix well, and allow to stand for 1 h to 2 h.

NOTE If determining the WHC of the test substrate used in annex A, use a sample size equivalent to 250 ml of the test substrate.

B.3 Procedure

Place a coarse filter paper in the bottom of a Buchner funnel (5.3.11) and moisten to seal the filter paper to the funnel. Transfer a prepared test sample into the funnel and spread evenly. Apply suction using the vacuum pump (5.3.8) until no more than five drops of water per minute are being withdrawn from the sample, increasing the suction slowly to avoid perforation of the filter paper. Transfer the filter paper plus sample to a container of known mass (m_2) and weigh (m_5). Dry the container in the oven (5.3.2) at $(103 \pm 2)^\circ\text{C}$ and weigh again (m_4). Determine the mass of a wet filter paper after subjecting it to suction in the Buchner funnel (m_6) and also after oven drying at $(103 \pm 2)^\circ\text{C}$ (m_3). Repeat with the other samples.

NOTE The suction phase is normally completed in about 10 min.

B.4 Calculations

B.4.1 Initial moisture content of the test sample

The moisture content, w_1 (in % (m/m)) is given by the following formula :

$$\frac{(m_1 + m_2 + m_3) - m_4}{m_4 - (m_2 + m_3)} \times 100$$

where

m_1 is the mass of sample taken in grams ;

m_2 is the mass of the container in grams ;

m_3 is the mass of the oven dry filter paper in grams ;

m_4 is the mass of the container plus oven dry sample plus filter paper in grams.

Calculate the mean initial moisture content (W_1) from the moisture content of the three samples.

B.4.2 Moisture content of sample at WHC

The moisture content of the sample at its WHC, w_2 (in % (m/m)), is given by the formula :

$$\frac{(m_3 + m_5) - (m_4 + m_6)}{m_4 - (m_2 + m_3)} \times 100$$

where

m_5 is the mass of the container plus wet sample plus wet filter paper in grams ;

m_6 is the mass of wet filter paper in grams.

Calculate the mean moisture content at WHC (W_2) from the moisture content at WHC of the three samples.

B.4.3 Amount of water required to raise moisture content to a given percentage of the WHC

The amount of water to be added to the soil, as a percentage of the mass of the sample taken, is given by the formula :

$$\frac{\{(W_3 \times W_2) / 100\} - W_1}{100 + W_1} \times 100$$

where

W_3 is the percentage of WHC required.

B.5 Example of calculations

The following are examples of calculations used in B.4.

m_1 is the mass of sample taken = 200 g ;

m_2 is the mass of container = 174 g ;

m_3 is the mass of the oven dry filter paper = 1 g ;

m_4 is the mass of container plus oven dry sample plus filter paper = 367 g

m_5 is the mass of container and wet sample and wet filter paper = 433 g ;

m_6 is the mass of wet filter paper = 3 g ;

W_3 is the percentage of the WHC required = 95 % ;

w_1 is the initial moisture content = $\frac{(200 + 174 + 1) - 367}{367 - (174 + 1)} \times 100 = 4,2 \%(m/m)$;

w_2 moisture content at WHC = $\frac{(1 + 433) - (367 + 3)}{367 - (174 + 1)} \times 100 = 33,3 \%(m/m)$.

Assuming that W_1 is equal to w_1 and that W_2 is equal to w_2 :

$$\text{Amount water to raise sample to 95 \% WHC} = \frac{\{(95 \times 33,3) / 100\} - 4,2}{100 + 4,2} = 26,3\%(m / m) .$$

Annex C (informative)

Rapid soil virulence test

As a quick check on the microbial activity of the soil, expose untreated cotton cloth ⁵⁾ to direct soil contact by partial burial for a period of 14 days. If at the end of this time the cloth has deteriorated to the point where it can be broken by gentle hand pull, the soil may be considered sufficiently active.

A minimum of five test strips of cotton cloth measuring 150 mm × 25 mm folded in half around a blunt knife and inserted vertically to half their length is a satisfactory way of performing the test.

NOTE If there is no previous information on the microbial activity of a particular soil, a test using specimens of Scots pine and/or beech as appropriate should be carried out prior to the main test. Untreated test specimens and test specimens treated with the lowest concentration of the chosen reference preservative should be prepared and tested according to the prestandard to ensure that the activity of the soil meets the validity requirement (see clause 9).

⁵⁾ A cotton cloth of aeric mass of 230 g/m² has been found to be suitable.

Annex D (informative)

Experimental set up of the test containers

D.1 Test container

A plastics tank capacity 15 l, approximately 210 mm high × 200 mm wide × 330 mm long has been found to be suitable (Figure D.1). The tank is closed with a glass lid which rests on draught strip attached to the top edges. Gaps 5 mm long are left in the stripping, at two diagonally opposite corners, to allow some ventilation.

D.2 Introduction and position of the test specimens

Each tank is filled to a depth of approximately 160 mm with moist soil (about 10 kg per tank is needed), and will accommodate up to 60 test and control specimens.

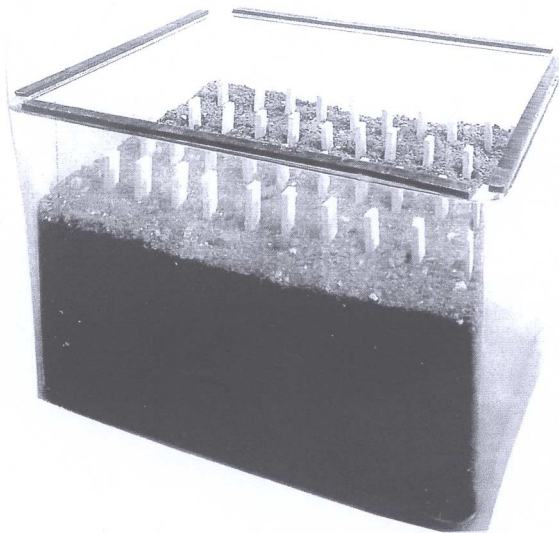


Figure D.1 - A charged test container

Annex E (informative)

Calculation of the nominal effective retention

E.1 Introduction

The performance of a test preservative in a given species of wood is evaluated by comparison with the highest concentration of the reference preservative which showed a loss in mass greater than 3 % (*m/m*) after 32 weeks exposure using the same species of wood. This procedure is carried out in stages which are illustrated below using the data presented in Table E.1.

Table E.1 — Example of test results using beech

Treating solution concentration % (<i>m/m</i>)	Mean preservative retention kg/m ³	Mean corrected per cent loss in mass			
		Exposure period - weeks			
		8	16	24	32
<u>CC ref</u>					
1,0	6,5	5,6	10,4	14,5	20,8
1,6	10,2	1,5	3,2	6,8	10,9
2,5	16,0 (<i>n.r.R.</i>)	0,2	1,2	2,1 (<i>a</i>)	5,2 (<i>b</i>)
4,0	23,8	0	0,3	0,9	2,8
<u>Product X</u>					
0	0	7,5	16,3	24,1	29,5
1,0	6,4	1,8	2,6	5,5 (<i>c</i> ₁)	8,5 (<i>d</i> ₁)
2,0	12,9	0,5	1,0	2,4 (<i>c</i> ₂)	5,0 (<i>d</i> ₂)
3,0	19,6	0	0	1,0 (<i>c</i> ₃)	2,1 (<i>d</i> ₃)

E.2 Calculations

E.2.1 Selection of concentration of reference preservative to be used

The highest concentration of reference preservative which showed a mean corrected loss in mass greater than 3 % (*m/m*) after 32 weeks was 2,5 % (*m/m*). The mean retention of this concentration was 16,0 kg/m³ which is equivalent to the nominal effective retention of the reference preservative (*n.r.R.*).

E.2.2 Determination of losses in mass of test product treated specimens at the time period giving 3 % (*m/m*) loss in mass of specimens treated with the chosen concentration of the reference preservative

The losses in mass for each concentration of the test product (*W*) are derived using the formula :

$$\left[\frac{(3-a)}{(b-a)} \times (d-c) \right] + c$$

where

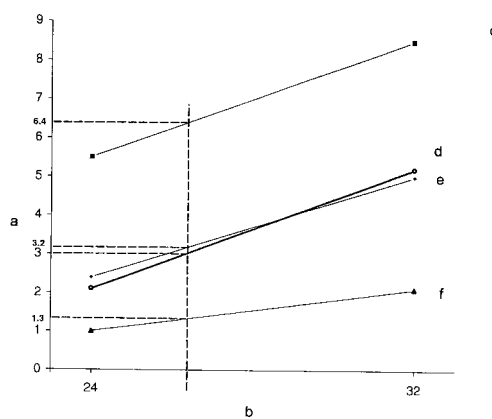
- a* is the loss in mass of the specimens treated with the chosen concentration of the reference preservative after the longest exposure period which gave less than 3 % (*m/m*) loss in mass ;

- b* is the loss in mass of the specimens treated with the chosen concentration of the reference preservative after the shortest exposure period which gave greater than 3 % (*m/m*) loss in mass ;
- c* is the loss in mass of the test product after the exposure period used for *a*, and *c*₁, *c*₂ and *c*₃ denote the different treating solution concentrations of the test product ;
- d* is the in mass of the test product after the exposure period used for *b*, and *d*₁, *d*₂ and *d*₃ denote the different treating solution concentrations of the test product.

Using the data in Table E.1 and the product *X* at 1,0 % (*m/m*) treating solution concentration :

$$\left[\frac{(3 - 2,1)}{(5,2 - 2,1)} \times (8,5 - 5,5) \right] + 5,5 = 6,4 \% (m/m)$$

This procedure is illustrated in Figure E.1.



Key

- a Mean percent loss in mass
- b Exposure period – weeks
- c 1,0 % product *X*
- d 2,5 % CC reference
- e 2,0 % product *X*
- f 3,0 % product *X*

Figure E.1 - Determination of losses in mass of test product at the time period giving 3 % (*m/m*) loss in mass with the chosen concentration of the reference preservative

The values calculated based on the data in Table E.1 are given in Table E.2.

Table E.2 — Summary of calculations using the data in Table E.1

Test product	Treating solution concentration % (m/m)	Product retention kg/m ³	Mean loss in mass		Loss in mass test product = 3 % loss in mass for reference product %	Retention = 3 % loss in mass for test product kg/m ³
			%			
			24 weeks	32 weeks		
CC ref.	2,5	16,0 (n.r.R.)	2,1	5,2	3,0	-
Product X	1,0	6,4	5,5	8,5	6,4	13,6 (n.r.P.)
	2,0	12,9 (R ₁)	2,4	5,0	3,2 (W ₁)	
	3,0	19,6 (R ₂)	1,0	2,1	1,3 (W ₂)	

E.2.3 Determination of the retention of test preservative that equates to a mean loss in mass of 3 % (m/m)

Select the two treating solution concentrations of the test product which have values of W greater than 3 % (m/m) and less than 3 % (m/m).

Using the data in Table E.1, the two concentrations are 2,0 % (m/m) and 3,0 % (m/m).

Using the data for these two concentrations, determine the retention of the test preservative that would give a loss in mass of 3,0 % (m/m). This is known as the nominal retention of the test preservative (*n.r.P.*) and is calculated using the following formula :

$$\left[\frac{(W_1 - 3)}{(W_1 - W_2)} \times (R_2 - R_1) \right] + R_1$$

where

W_1 is the loss in mass of the lower treating solution concentration selected ;

W_2 is the loss in mass of the higher treating solution concentration selected ;

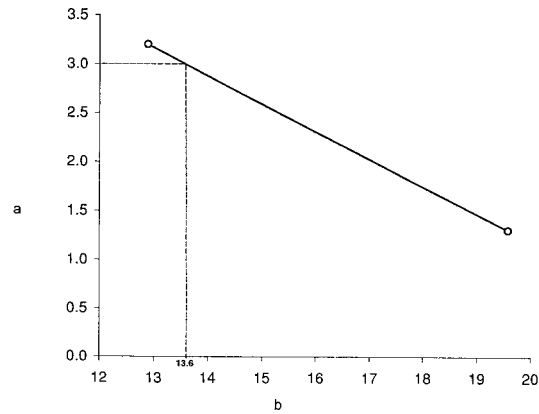
R_1 is the retention of product at the lower treating solution concentration selected ;

R_2 is the retention of product at the higher treating solution concentration selected.

Using the data in Table E.2,

$$n.r.P. = \left[\frac{(3,2 - 3)}{(3,2 - 1,3)} \times (19,6 - 12,9) \right] + 12,9 = 13,6 \text{ kg/m}^3$$

This procedure is illustrated in Figure E.2.



Key

- a Mean percent loss in mass
- b Retention kg/m³

Figure E.2 - Determination of the retention of the test product that equates to a mean loss in mass of 3 % (m/m)

E.2.4 Calculation of nominal effective retention of the test preservative

Calculate the nominal effective retention (*n.e.r.*) of the test preservative using the following formula :

$$\frac{n.r.P. \text{ at } 3 \% (m/m) \text{ mass loss}}{n.r.R. \text{ at } 3 \% (m/m) \text{ mass loss}} \times \text{target retention of reference preservative}$$

where

- n.r.P.* is the nominal effective retention of the test preservative ;
- n.r.R.* is the nominal effective retention of the reference preservative.

The target retention for the CC reference preservative in beech = 20 kg/m³

Using the data presented in Table E.2,

$$n.e.r. = \frac{13,6}{16,0} \times 20 = 17,0 \text{ kg/m}^3$$

Annex F (informative)

Example of a test report

NOTE Detailed results are presented for beech test specimens and the test preservative only.

Number and date of this European Prestandard	: ENV 807:2000
Name of supplier	: Company A
Name and type of preservative	: Product X; a water insoluble powder
Name and concentration of active ingredient(s)	: Z 95 % (m/m)
Solvent or diluent used	: Toluene
Reference preservative	: Copper/chromium/(cc)
Target retentions of the reference preservative	: Scots pine 30 kg/m ³ : Beech 20 kg/m ³
Species of wood used and mean density	: Scots pine sapwood (<i>Pinus sylvestris</i>), density 485 kg/m ³ : Beech (<i>Fagus sylvatica</i>), density 665 kg/m ³
Concentrations of preservative tested	: 0 %, 0,1 %, 0,16 %, 0,25 % (m/m) Scots pine : 0 %, 1,0 %, 2,0 %, 3,0 % (m/m) beech
Concentrations of reference preservative	: 0,16 %, 0, 25 %, 0,4 %, 0, 63 % (m/m) Scots pine : 1,0 %; 1,6 %; 2,5 %; 4,0 % (m/m) beech
Mass of solution absorbed and retention of preservative	: see Tables F.1 and F.2
Conditioning period after impregnation	: 28 days
Test soil and WHC	: John Innes No. 2 Potting Compost, WHC 28,5 % (m/m)
Date of exposure	: 1998.12.11
Dates of removal of specimens	: 1999.02.11, 1999.04.08, 1999.06.03, 1999.07.29
Exposure periods	: 8, 16, 24, 32 weeks
Uncorrected losses in mass	: see Tables F.1 and F.2
Correction values	: see Table F.1
Corrected losses in mass	: see Table F.2
Virulence control specimens losses in mass	: see Table F.3
Highest concentration of reference preservative which failed and the retention (n.r.R.)	: Scots pine 0,16 % (m/m), 1,2 kg/m ³ : Beech; 2,5 % (m/m), 16,0 kg/m ³

Validity of tests	: Scots pine, valid : Beech, valid
Derived mass losses for each concentration of the test preservative at time of failure of reference preservative	: see Table F.4
Nominal retention of the test preservative (<i>n.r.P.</i>)	: 13,6 kg/m ³ (see Table F.4)
Nominal effective retention of the test preservative (<i>n.e.r.</i>)	: 17,0 kg/m ³ (see Table F.4)
This report has been prepared by	: Laboratory B
Location and date	: C 1999.08.12
Name and signature of the officer(s) in charge	: Mr D

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

Table F.1 — Results of product X in beech (treated check test specimens)

Treating solution concentration % (m/m)	Uptake of solution g	Preservative retention kg/m ³	Mass loss %	Correction value (C ₁) %
0	3,22	0	0,3	0,5
	2,97	0	0,9	
	2,99	0	0,7	
	2,95	0	0,1	
	2,96	0	0,3	
	2,88	0	0,5	
mean	3,00	0	0,5	
1,0	3,28	6,6	-0,5	-0,2
	3,08	6,2	0,4	
	3,18	6,4	-0,5	
	3,30	6,6	-0,3	
	3,02	6,1	-0,1	
	3,12	6,2	-0,4	
mean	3,18	6,4	-0,2	
2,0	3,22	12,9	-0,4	-0,6
	3,09	12,4	-0,7	
	3,29	13,2	-0,7	
	3,24	13,0	-0,8	
	3,05	12,2	-0,8	
	3,20	12,8	0,1	
mean	3,18	12,8	-0,6	
3,0	3,32	19,9	-1,2	-1,2
	3,24	19,4	-1,3	
	3,09	18,6	-1,1	
	3,18	19,1	-1,2	
	3,09	18,6	-1,0	
	3,26	19,6	-1,3	
mean	3,20	19,2	-1,2	
- Gain in mass				

Table F.2 — Results of product X in beech (treated test specimens) - 16 weeks

Treating solution concentration % (m/m)	Uptake of solution g	Preservative retention kg/m ³	Mass loss %	Correction value (C ₁) %	Corrected ^a mass loss %
0	3,07	0	14,9	0,5	14,4
	3,08	0	14,2		13,7
	3,10	0	17,5		17,0
	3,14	0	20,9		20,4
	2,76	0	19,7		19,2
	3,12	0	13,8		13,3
mean	3,05	0	16,8		16,3
1,0	3,24	6,5	2,9	-0,2	3,1
	3,11	6,2	0,2		0,4
	3,24	6,5	3,4		3,6
	3,29	6,6	3,5		3,7
	3,10	6,2	2,3		2,5
	3,10	6,2	2,2		2,4
mean	3,18	6,4	2,4		2,6
2,0	3,39	13,6	1,1	-0,6	1,7
	3,16	12,6	-0,5		0,1
	3,16	12,6	-1,0		-0,4
	3,21	12,8	0,3		0,9
	3,28	13,1	1,5		2,1
	3,19	12,8	0,6		1,2
mean	3,23	12,9	0,3		1,0
3,0	3,20	19,2	-1,3	-1,2	-0,1
	3,20	19,2	-1,4		-0,2
	3,22	19,3	-1,3		-0,1
	3,32	20,0	-1,4		-0,2
	3,29	19,7	-1,3		-0,1
	3,31	19,9	-1,2		0
mean	3,26	19,6	-1,3		0
- Gain in mass					
^a Gains in corrected loss in mass are taken as zero in subsequent calculations					

Table F.3 — Virulence controls

Tank No.	Per cent loss in mass - 16 weeks
1	11,3 15,5 14,8
mean	13,9
2	14,4 15,3 13,4
mean	14,4
3	16,8 14,4 14,9
mean	15,4
4	15,6 14,8 15,2
mean	15,2
5	20,7 15,4 16,1
mean	17,4
Mean	15,2

Table F.4 — Calculation of nominal effective retention (*n.e.r.*)

Test product	Treating solution concentration % (<i>m/m</i>)	Product retention kg/m ³	Mean loss in mass		Loss in mass test product = 3 % loss in mass for reference product %	Retention = 3 % loss in mass for test product kg/m ³
			%			
			24 weeks	32 weeks		
CC ref.	2,5	16,0 (<i>n.r.R.</i>)	2,1	5,2	3,0	-
Product X	1,0	6,4	5,5	8,5	6,4	13,6 (<i>n.r.P.</i>)
	2,0	12,9	2,4	5,0	3,2	
	3,0	19,6	1,0	2,1	1,3	
$n.e.r. = \frac{n.r.P.}{n.r.R.} \times \text{target retention} = \frac{13,6}{16,0} \times 20 = 17,0 \text{ kg/m}^3$						

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