

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

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National foreword

This Draft for Development has been prepared under the direction of the Technical Sector Board for Civil Engineering and is the English language version of ENV 807:1993 *Wood preservatives — Determination of the toxic effectiveness against soft rotting micro-fungi and other soil inhabiting micro-organisms*, published by the European Committee for Standardization (CEN). ENV 807 was produced as a result of international discussion in which the United Kingdom took an active part.

An ENV is made available for provisional application but it does not have the status of a European Standard. The aim is to use the experience gained and, if necessary, to modify the ENV so that it can be adopted as a European Standard.

CAUTION. Attention is drawn to the Health and Safety at Work etc. Act 1974, and the need for ensuring that the method specified in this Draft for Development is carried out with suitable precautions. In connection with the use of epoxyethane-based sterilization in **E.2**, attention is drawn to the Control of Substances Hazardous to Health Regulations 1988 and the Health and Safety Commission's Approved Code of Practice COP30 Control of Substances Hazardous to Health in Fumigation Operations.

The procedures described in this Draft for Development are intended to be carried out by appropriately qualified and experienced persons or other suitably trained and/or supervised personnel. Normal safety precautions should be used throughout the use of the method. Attention is drawn to the precautions given in **5.2.1.1**, **8.1.3** and **E.2**.

The future of this Draft for Development is bound to that of ENV 807 and the Draft for Development will not be reviewed or developed separately.

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 because there is at present insufficient experience in the use of the test method. It should be applied on this provisional basis, so that information and experience of its practical application may be obtained and reported to the responsible CEN Technical Committee. Users of this Draft for Development are invited to comment on its technical content, ease of use and any ambiguities or anomalies. These comments will be taken into account when preparing the UK national response to CEN on the question of whether the ENV can be converted to an EN. Comments should be sent in writing to BSI, 2 Park Street, London W1A 2BS, quoting this Draft for Development, the relevant clause and any proposed revision, within 2 years of the issue of this Draft for Development.

Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the ENV title page, pages 2 to 26, an inside back cover and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

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Descriptors: Wood, wood preservatives, pest control, micro-organisms, fungi, bioassay, laboratory tests, effectiveness

English version

Wood preservatives — Determination of the toxic 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-champignons de pourriture molle et
d'autres micro-organismes du sol

Holzschutzmittel — Bestimmung der Wirkung
gegenüber Moderfäule und Mikroorganismen

This European Prestandard (ENV) was approved by CEN on 1992-10-10 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 an European Standard (EN).

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

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CEN

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

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

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Foreword

This European prestandard has been drawn up by the Technical Committee CEN/TC 38 “*Durability of wood and derived materials*”, the Secretariat of which is held by AFNOR.

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

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Introduction

This European Prestandard specifies a laboratory method of test in two parts, 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. In Test I a mixture of six such fungi is used whereas in Test II, the infection source is the natural micro-flora of the soil substrate which may also contain other organisms, such as bacteria, and other fungi, such as *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 in order to provide a more complete assessment of the product.

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 preservative products or of their active components.

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 Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

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

EN 84:1989, *Wood preservatives — Accelerated ageing of treated wood prior to biological testing — Leaching procedure*.

3 Definitions

For the purpose of this standard, the following definitions apply.

3.1

representative sample

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

3.2

supplier

the sponsor of the test

4 Principle

The method employs two tests carried out in parallel.

- Test I — impregnation of a series of test specimens of a susceptible wood species with solutions in which the concentration of the preservative is ranged in a given progression.
- exposure of the test specimens to leaching conditions according to EN 84.
 - installation of the test specimens in a vermiculite medium under sterile conditions.
 - inoculation with a mixed population of pure cultures of specific soft rot micro-fungi and establishment of the toxic values for the preservative under test.
- Test II — impregnation of a number of test specimens (as small stakes) with the preservative under test at a minimum of three concentrations ranged about the toxic values that are expected to be determined in Test I.
- exposure of the test specimens to leaching conditions according to EN 84.
 - partial burial of the specimens vertically in a microbially active soil.

5 Test materials

5.1 Biological material

5.1.1 Test I

5.1.1.1 *Obligatory test fungi*. The following fungi shall be included in every test:

Species	Strain No.
<i>Chaetomium globosum</i> Kunze	ATCC 6205
<i>Glenospora graphii</i> Vuill.	MG 55
<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

5.1.1.2 *Optional test fungi*. If other fungal species or strains are relevant to specific circumstances, these shall be obtained from a recognized culture collection. They shall be used in a parallel test.

NOTE Pure cultures recently isolated from naturally infected material are also relevant. They should be identified and deposited in a recognized collection.

5.1.1.3 Maintenance and treatment of strains. The strains shall be maintained and handled (frequency of subculturing etc.) in accordance with Annex A.

5.1.1.4 Preparation of spore suspension. Subculture the test fungi, separately, on test tube slopes of malt agar medium containing 40 g/l of malt extract (powder) or 50 g/l of malt extract (concentrate), and incubate at a temperature of between 26 °C and 28 °C. Cultures shall 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 shall be used to make each batch of suspension.

Select the number of subcultures of each test fungus necessary to produce one sixth of the required quantity of spore suspension (5.2.1.2). Taking each culture in turn, gently add 10 ml of the wetting agent solution (5.2.2.3) 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. Use this wire 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 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 (5.3.2.4), and use only spore suspensions containing at least 10⁵ 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.

5.1.2 Test II

5.1.2.1 Soil substrate. Natural top soil or a fertile loam-based horticultural soil¹⁾ of pH 6 to pH 8 and not containing added agro-chemicals. It shall have a water holding capacity (WHC) of between 25 % (m/m) and 100 % (m/m); the WHC shall be determined by the method described in Annex B.

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. Prior to use the soil shall be stored in closed moisture-proof containers. Before use, thoroughly mix the sample of soil.

NOTE 1 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 substrate shall be used only once.

NOTE 2 If reassurance of the virulence of the substrate 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 Common to both tests

5.2.1.1 Solvents and diluents. Water to grade 3 of ISO 3696:1987 and, if appropriate, volatile organic liquids leaving in the wood no residue which would have a toxic effect on the micro-fungi at the end of the post-treatment conditioning period.

CAUTION. Do not use benzene or other solvents which pose a health risk to those conducting the test.

5.2.1.2 Xylene, (C₆H₄(CH₃)₂) mixed isomers, technical grade.

5.2.1.3 Reference preservatives. One of the following shall be used in each test.

A copper/chromium/arsenic preservative of the following composition:

CuSO ₄ · 5H ₂ O	35,0 % (m/m)
K ₂ Cr ₂ O ₇	45,0 % (m/m)
As ₂ O ₅ · 2H ₂ O	20,0 % (m/m)

A copper/chromium preservative of the following composition:

CuSO ₄ · 5H ₂ O	50,0 % (m/m)
K ₂ Cr ₂ O ₇	48,0 % (m/m)
CrO ₃	2,0 % (m/m)

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

5.2.2 Additional items for Test I

5.2.2.1 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

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.

NOTE 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 (5.3.2.1), adjust the pH to 6,2 by dropwise addition of 0,25 mol/l sulfuric acid (H_2SO_4).

5.2.2.2 Test substrate. Anhydrated, laminar, aluminium-iron-magnesium silicate²⁾ exfoliated to yield particles up to 3 mm diameter. Particles less than 2 mm shall be removed by sieving. Before use, mix the sample of test substrate well.

The test substrate shall be used only once.

5.2.2.3 Wetting agent solution. Water containing 0,5 g/l of dioctyl sodium sulfosuccinate.

5.3 Apparatus

5.3.1 Common to both tests

5.3.1.1 Conditioning chamber, well ventilated and maintained at $(20 \pm 2)^\circ C$ and $(65 \pm 5) \% r.h.$

5.3.1.2 Culture chamber (incubator or room), dark and maintained at $(27 \pm 2)^\circ C$ and $(70 \pm 5) \% r.h.$

5.3.1.3 Ventilated drying oven, maintained at $(103 \pm 2)^\circ C$.

5.3.1.4 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.

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

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

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

5.3.1.8 Vacuum vessels, fitted with stopcocks.

5.3.1.9 Vacuum pump, fitted with a pressure gauge and capable of maintaining a pressure of 0,7 kPa.

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

5.3.1.11 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 plastics material for salts containing fluorine.

5.3.1.12 Ordinary laboratory equipment, including a desiccator with efficient desiccant (silica gel for example), an autoclave and a balance accurate to 0,001 g.

5.3.2 Additional items for Test I

5.3.2.1 pH meter, capable of determining pH to the nearest 0,1 unit.

5.3.2.2 Test vessels, 500 ml in capacity, made of material which does not have a toxic effect on the micro-fungi and provided with a lid. The vessels shall be approximately 65 mm in depth.

NOTE An example of a suitable test vessel is described in Annex D.1.

5.3.2.3 Equipment for chemical gas or steam sterilization or access to a radiation service (see Annex E).

5.3.2.4 Haemocytometer counting chamber or particle counter, to count the concentration of fungal spores.

5.3.3 Additional items for Test II

5.3.3.1 Test containers, made of material which does not have a toxic effect on the micro-fungi and provided with a ventilated lid. The depth shall be at least 140 mm so as to provide at least 40 mm below the test specimens when inserted in the substrate to a depth of 80 mm and adequate clearance above the top of the protruding parts of the stakes.

NOTE The exact dimensions are not critical but they determine the number of stakes in each vessel (which should not be less than 10). An example of a suitable test container is described in Annex D.2.

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 procedures 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);

beech (*Fagus sylvatica* Linnaeus)

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

²⁾ Vermiculite is suitable.

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 8 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, free from tyloses, discolouration and red heart. It shall have between 2 annual growth rings per 10 mm and 6 annual growth rings per 10 mm.

7.3 Provision of test specimens

Condition the wood to $(14 \pm 2) \%$ (*m/m*) moisture content. Prepare planed strips having a cross section of $(15 \pm 0,1) \text{ mm} \times (5 \pm 0,1) \text{ mm}$ for test I and $(10 \pm 0,1) \text{ mm} \times (5 \pm 0,1) \text{ mm}$ for test II. The growth rings shall be at $(90 \pm 15)^\circ$ to the broad faces and the longitudinal faces shall be parallel to the direction of the grain. Make transverse cuts, neatly to give sharp edges, to give test specimens $(40 \pm 0,5) \text{ mm}$ long for test I and $(100 \pm 0,5) \text{ mm}$ long for test II.

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 the specimens when conditioned to a moisture content of $(14 \pm 2) \%$ (*m/m*) shall be:

- | | |
|---------|--|
| test I | $(40 \pm 0,5) \text{ mm} \times (15 \pm 0,1) \text{ mm} \times (5 \pm 0,1) \text{ mm};$ |
| test II | $(100 \pm 0,5) \text{ mm} \times (10 \pm 0,1) \text{ mm} \times (5 \pm 0,1) \text{ mm}.$ |

For the purpose 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 volumes of each test specimen shall be taken as 3,0 cm³ and 5,0 cm³ respectively.

In any batch of specimens of the same size, the mass of any individual specimen shall not differ from the mean value of the batch by more than 10 % (*m/m*).

7.5 Number and distribution of test specimens

7.5.1 Test I

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 preservative concentration and species of timber.

$v_{1.2}$ test specimens treated with the reference preservative: these specimens are impregnated with the solutions of one of the reference preservatives (5.2.1.3) and subjected to attack by the soft rot fungi in the test substrate. Use at least six reference specimens for each combination of concentration and species of timber.

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 timber species.

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

$v_{3.1}$ 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 (8.2.1.4). Use at least six specimens for each preservative concentration and each timber species. Variations in the mass of these specimens make it possible to determine the correction factor (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, factor K_1 is the mean percentage change in mass of the $v_{3.1}$ test specimens.