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Wood preservatives — Determination of the toxic values against larvae of Hylotrupes bajulus (Linnaeus) — (Laboratory method)

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National foreword

This British Standard is the UK implementation of EN 47:2016. It supersedes BS EN 47:2005 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee B/515, Wood preservation.

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

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Wood preservatives - Determination of the toxic values against larvae of Hylotrupes bajulus (Linnaeus) - (Laboratory method)

Produits de préservation du bois - Détermination du seuil d'efficacité contre les larves d'Hylotrupes bajulus (Linnaeus) - (Méthode de laboratoire)

Holzschutzmittel - Bestimmung der Grenze der Wirksamkeit gegenüber Larven von Hylotrupes bajulus (Linnaeus) - (Laboratoriumsverfahren)

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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European foreword

This document (EN 47:2016) has been prepared by Technical Committee CEN/TC 38 “Durability of wood and wood-based products”, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2017, and conflicting national standards shall be withdrawn at the latest by February 2017.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 47:2005.

The significant technical difference between this document and EN 47:2005 is as follows:

— introduction of new harmonized specifications for wood quality.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This document describes a laboratory method of testing which gives a basis for the general assessment of the effectiveness of a wood preservative against *Hylotrupes bajulus* by determination and comparison with different classes of larvae, of the concentration at which the product prevents their survival in totally impregnated wood of a susceptible species.

In this respect it differs from the method specified in EN 46-1 which is intended to determine whether a preservative applied to the surface is capable of preventing infestation of wood by these larvae.

This laboratory method provides a criterion by which the value of a preservative can be assessed. In making this assessment the methods by which the preservative may be applied should be taken into account. It is further recommended that results from this test should be supplemented by those from other appropriate tests and, above all, by comparison with practical experience.

When products which are very active at very low concentration are used, it is very important to take suitable precautions to isolate and separate, as far as possible, operations involving chemical products, other products, treated wood, laboratory apparatus and clothing. Suitable precautions should include the use of separate rooms, areas within rooms, extraction facilities, conditioning chambers and special training for personnel (see also Annex D for environmental, health and safety precautions).

1 Scope

This European Standard specifies a method for the determination of the toxic values of a wood preservative against the larvae of *Hylotrupes bajulus* (Linnaeus), introduced into wood treated previously by full impregnation.

This method is applicable to:

— water-insoluble chemicals which are being studied as active insecticides;

— organic formulations, as supplied or as prepared in the laboratory by dilution of concentrates;

— organic water-dispersible formulations as supplied or as prepared in the laboratory by dilution of concentrates; and

— water-soluble materials, for example salts.

The method is applicable whether or not the test specimens have been subjected to appropriate ageing procedures.

2 Normative reference

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

3 Terms and definitions

For the purposes of this document, 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

[SOURCE: EN 1001-2:2005, 4.71]

3.2
supplier
sponsor of the test (person or company providing the sample of wood preservative to be tested)

Note 1 to entry: Adapted from EN 1001–2:2005, 4.83.

4 Principle

Impregnation of several sets of test specimens of susceptible wood species with a series of concentrations of the preservative.

Introduction of *Hylotrupes bajulus* larvae of a given category into these test specimens and determination of their survival rate at fixed intervals of time.

Comparison of the results with those obtained with untreated and solvent or diluent-treated control test specimens. Derivation of the toxic values of the product under test for the category of larvae in question.

5 Test materials

5.1 Biological material

5.1.1 *Hylotrupes bajulus* (Linnaeus) larvae

— Category 1 (obligatory test): larvae within a maximum of 3 days of hatching;

— Category 2 (optional additional test): larvae with masses in the range 50 mg to 150 mg.

5.1.2 Source of larvae

The larvae shall preferably be obtained from cultures reared according to the method described in Annex B.

Otherwise larvae in Category 2 can be taken from naturally infested wood, in which case they should be transferred into sapwood of pine and stored for at least 4 weeks under the rearing conditions specified in Annex B.

Do not use the larvae in the test if they have not fed normally during this storage period.

5.1.3 Provision of larvae

Collect larvae in Category 1 from eggs laid by different females.

Carefully cut out the larvae in Category 2 from the culture blocks and keep them separated from one another for 2 days to 3 days in the culturing chamber (5.3.1) to check that they are healthy.

5.1.4 Choice of larvae in Category 2

Use only healthy larvae in the test.

NOTE A healthy larva can be recognized by its ivory-white colour, its firm consistency and rounded appearance, and by the absence of wounds or bites which show up as dark marks. Healthy larvae react to the touch by vigorous movement and attempts to bite.

Reject any larvae which are shrunken or aged, which have recently moulted, or which are in a pre-pupal stage.

5.1.5 Number of larvae

The number of larvae per treated and control test specimen shall be six of Category 1 or one of Category 2.

Sort the larvae retained in Category 2 mentioned above.

Do not use larvae weighing more than 150 mg as they may pupate and therefore interfere with the test.

For a single test, use a mixed batch of larvae of Category 1 and for Category 2, as far as possible, use larvae of similar masses. The number of larvae necessary is given in Table 1.

Table 1 — Number of larvae and test specimens

Type of test specimen	Concentrations of preservatives Mass fraction	Larvae in Category 1		Larvae in Category 2			
				Without radiography		With radiography [a]	
		Number of test specimens	Number of larvae	Number of test specimens	Number of larvae	Number of test specimens	Number of larvae
Treated test specimens	1	5	30	10	10	7	7
_	2	5	30	10	10	7	7
_	3	5	30	10	10	7	7
_	4	5	30	10	10	7	7
_	5	5	30	10	10	7	7
etc.							
Untreated control test specimens	0	5	30	10	10	7	7
Solvent or diluent control test specimens (including water)	0	5	30	10	10	7	7
Total for 5 concentrations		35	210	70	70	49	49

[a] The use of radiography is only recommended in the case of tests with larvae in Category 2.

5.2 Products and reagents

5.2.1 Xylene, technical grade, mixed isomers.

5.2.2 Water, complying with grade 3 of EN ISO 3696.

5.2.3 Solvent or diluent, a volatile liquid that will dissolve or dilute the preservative but does not leave a residue in the wood at the end of the post-treatment conditioning period that has a toxic effect on the insects.

CAUTION — Do not use benzene or other solvents which pose a health risk.

5.2.4 Cellulose or absorbent cotton wool and filter paper

5.3 Apparatus

5.3.1 Culturing chamber, with air circulation, and controlled at (28 ± 2) °C and at relative humidity (70 ± 5) %.

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

NOTE The conditioning of test specimens can be carried out in the laboratory work area (see 5.3.3) provided that this has the conditions specified for the conditioning chamber (see 5.3.2).

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

CAUTION — It is essential to follow safety procedures for handling flammable and toxic materials. Avoid excessive exposure of operators to solvents or their vapours.

5.3.4 Testing chamber, ventilated and air-conditioned, controlled at (22 ± 2) °C and at a relative humidity of (70 ± 5) %.

5.3.5 Treatment vessels, of a material that does not react with the preservative under test, for example of glass for organic products and of polyethylene for salts containing fluorine.

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

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

5.3.8 Vacuum vessel(s), fitted with stopcocks, capable of receiving the treatment vessels (5.3.5).

5.3.9 Vacuum pump, fitted with a pressure gauge and capable of maintaining a pressure of 700 Pa[1)].

5.3.10 Drying vessel(s), capable of holding sets of five test specimens (7.5), provided with a close-fitting cover and containing supports that will give minimum contact with treated test specimens to be placed on them. The vessels and supports shall be of a material that does not react with the preservative under test, for example glass for organic compounds and polyethylene for products containing fluorine.

5.3.11 Drill and twist drills, approximately 3,0 mm to 4,5 mm in diameter, and a fine awl. In all cases, the number of bits shall be sufficient to drill holes to the size of the larvae available; in the case of larvae of Category 1, use a steel awl.

5.3.12 Ordinary laboratory equipment, including a balance capable of weighing to an accuracy of 0,01 g.

5.3.13 X-ray apparatus (optional) with tungsten target and beryllium window, with voltage and current continuously variable in the following ranges:

— voltages: 10 kV to 50 kV;

— current: 0 mA to 15 mA.

5.3.14 Protective gloves

6 Sampling

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

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

1) 100 Pa = 1 mbar.

7 Test specimens

7.1 Species of wood

The reference species is Scots pine (*Pinus sylvestris* Linnaeus)[2)].

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 visible cracks, stain, decay, insect damage and other defects. The wood shall not have been water-stored, floated, chemically treated or steamed. The wood shall originate from trees preferably felled in winter. The trees shall be cut immediately after felling and the timber rapidly air-dried or kiln dried at temperatures below 60 °C. The wood shall not have been stored for more than five years.

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

It is recommended to use test specimens of similar growth rate within a single test.

7.3 Provision of test specimens

Prepare planed strips having a cross-section of (25 ± 0,5) mm × (15 ± 0,5) mm removing a minimum of 2 mm from any faces exposed during drying. The longitudinal faces shall be parallel to the direction of the grain. The annual rings shall have a contact angle of greater than 10° to the faces of the test specimens. Make transverse cuts, neatly to give sharp edges and a fine-sawn finish to the end-grain surfaces, to give test specimens (50 ± 0,5) mm long.

The specimens shall originate from a minimum of three trees or shall be taken at random from a stock originally of more than 500 test specimens.

7.4 Dimensions of test specimens

The dimensions of each test specimen after reaching equilibrium in the conditioning chamber (5.3.2) shall be (50 ± 0,5) mm × (25 ± 0,5) mm × (15 ± 0,5) mm.

For the purposes of calculating the mass of preservative retained per unit volume of wood (8.1.2.2) the nominal volume of each test specimen shall be taken as 18,75 cm^3.

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

7.5 Number of test specimens

The number of test specimens required is given in Table 1.

It is advisable to treat more than the specified number of test specimens so that, after weighing, any test specimens with abnormally high or low retentions can be rejected from the batch.

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2) In southern European countries the species of pine most frequently infested by *Hylotrupes bajulus* may be used as an alternative, provided that the suitability of the species for use in the tests specified in this document has been demonstrated in all aspects (development of larvae, resistance to impregnation, etc.).

8 Procedure

8.1 Preparation of test specimens

8.1.1 Conditioning of test specimens before treatment

Allow the test specimens to condition in the conditioning chamber (5.3.2), for a minimum of two weeks.

8.1.2 Treatment of the test specimens

8.1.2.1 Preparation of the treatment solutions

8.1.2.1.1 Solid preservatives

Water-soluble preservatives:

— dissolve the preservative in the water (5.2.2) in a series of concentrations.

Non-water-soluble preservatives:

— dissolve the preservative in an appropriate solvent (5.2.3) in a series of concentrations.

8.1.2.1.2 Liquid preservatives

If appropriate, use the preservative without further preparation other than any necessary stirring. If it is a concentrate, dilute the preservative with the diluent to the required working concentration, using the procedure specified by the manufacturer.

All treatment solutions shall be freshly prepared.

8.1.2.1.3 Preparation

Prepare a series of at least five concentrations by mass, distributed evenly about the expected toxic values. A solvent or diluent control, i.e. treatment at concentration = 0, shall also be used. If the approximate toxic values are unknown, the concentrations shall form a widely spaced geometric progression for a first test and a more closely spaced geometric or arithmetic progression for subsequent tests.

All treatment solutions shall be freshly prepared.

8.1.2.2 Impregnation

Carry out impregnation in ascending order of concentration, starting with the solvent control (concentration = 0).

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

For each concentration weigh each test specimen, to the nearest 0,05 g, and then stack the test specimens in one of the treatment vessels (5.3.5) so that as much of their surface as possible is exposed (e.g. by piling them crosswise). Ballast the stack of test specimens with the weights (5.3.6) to prevent them from floating later when the liquid is admitted.

Place each beaker in one of the vacuum vessels (5.3.8), attach the vacuum pump (5.3.9) and reduce the pressure to 700 Pa. Maintain it for 15 min. Observe the proper safety measures for vacuum vessels. After this period, close the stopcock to the vacuum pump (5.3.9) and open the other stopcock to allow the solution of the preservative to be drawn into the treatment vessel. Keep the test specimens covered completely by the solution throughout the remainder of the impregnation process.

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

After this impregnation treatment, remove the test specimens one by one, remove excess liquid from their faces by lightly blotting with filter paper (5.2.4), and immediately weigh each to the nearest 0,05 g.

In the case of water-soluble preservatives, for example salts and organic chemicals which are being studied as active substances, calculate the mass of active matter retained by each test specimen from the mass of solution absorbed and its concentration[3)].

In the case of organic water-insoluble formulations and organic water-dispersible formulations the retention is expressed for each test specimen in terms of the corresponding mass of the formulation retained but, if a concentrate is supplied, the retention is expressed in terms of the solution prepared ready for use as specified by the manufacturer.

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

8.1.3 Drying and conditioning of the test specimens after treatment

Arrange the impregnated test specimens treated with each preservative concentration on their narrow faces, resting on two glass rods, not touching each other in the drying vessel (5.3.10). Place the cover on the drying vessel. Place the drying vessel in the conditioning chamber (5.3.2). Invert the test specimens twice each week during the subsequent drying period, temporarily removing the cover to perform these operations.

To prevent mould growth on test specimens treated with water-diluted preservatives, place a small dish containing the xylene (5.2.1) in the drying vessel (5.3.10).

During the first week retain the cover on the drying vessel.

During the second week uncover the drying vessel progressively each day.

From the beginning of the third week leave the drying vessel fully open. Drying shall be complete at the end of the fourth week.

NOTE The drying and conditioning of the test specimens depend on the nature of the product undergoing testing and on the solvent or diluent used. For slow drying products it may be necessary to extend the conditioning process.

If in the case of slow drying products, the conditioning period is extended, the extended conditioning period shall be stated in the test report.

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

8.2 Exposure of the test specimens to the insects

8.2.1 Use of larvae in Category 1

Make a regular pattern of 6 holes approximately 3 mm deep in one of the wide longitudinal faces of each test specimen (see Figure 1). Carefully insert the larvae headfirst and keep the holes upwards.

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

8.2.2 Use of larvae in Category 2

Drill a hole perpendicularly in the centre of one of the transverse faces of each test specimen (see Figure 2) to a depth of about one and a half times the length of the larva to be inserted and with a diameter approximately corresponding to the diameter of the prothorax of the larva (see Table 2).

Table 2 — Diameter of holes

Mass of larvae mg	**Approximate diameter of holes** mm
from 50 to 60	3,0
from 60 to 90	3,5
from 90 to 130	4,0
from 130 to 150	4,5

Carefully insert each larva headfirst and block the opening of the hole with a wad of the cellulose or cotton wool (5.2.4), so that a space is left between the wad and the larva equal to a quarter of the length of the larva.

Dimensions in millimetres

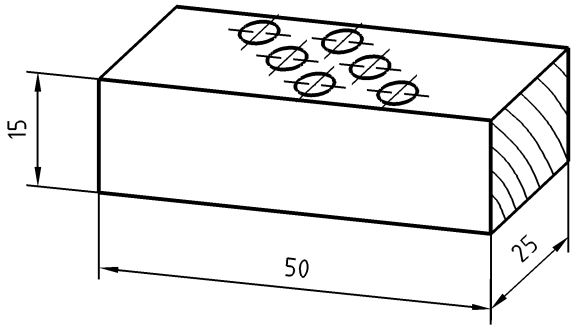


Figure 1 — Holes pricked in a longitudinal face for larvae in Category 1

Dimensions in millimetres

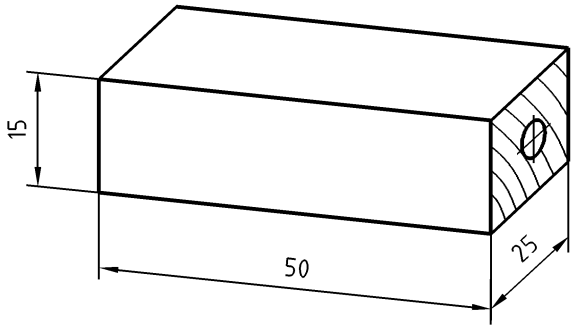


Figure 2 — Hole bored in a transverse face for larvae in Category 2

8.3 Conditions and duration of the test

Place all the test specimens in the testing chamber (5.3.4), keeping the different sets of test specimens, i.e. untreated control test specimens, solvent or diluent-treated control test specimens and different concentrations, separate from one another.

The total duration of the test, during which examinations and observations are carried out as described in 8.4, is:

— 12 weeks, possibly 24 weeks for larvae in Category 1;

— 24 weeks, possibly 48 weeks in some cases, for larvae in Category 2.

8.4 Examination of the test specimens

8.4.1 Examination without radiography

After 4 weeks (for larvae in Category 1) or 12 weeks (for larvae in Category 2), cut up the test specimens in the highest concentration.

If all the larvae are dead, cut up the set of test specimens at the next concentration below in the series and proceed in this way until a concentration is reached at which a live larva is first found in a test specimen. Store the remaining test specimens treated at this concentration as well as those treated at lower concentrations for a further 8 weeks in the case of larvae in Category 1 and a further 12 weeks in the case of larvae in Category 2.

Then resume cutting up the treated test specimens. For treated test specimens containing larvae, if a live larva is found, discontinue the second cutting up and keep the remaining treated test specimens for a further 12 weeks if larvae of Category 1, and 24 weeks if larvae of Category 2, following which cut them all up.

At the end of the test period, cut up all the control test specimens.

8.4.2 Examination with radiography, (larvae in Category 2)

Using the X-ray apparatus (5.3.13), radiograph all the test specimens after 12 weeks, cut up those test specimens containing larvae presumed dead in order to check their actual state and store those containing live larvae for a further 12 weeks before a further X-ray examination. Resume the examination, keeping those test specimens containing live larvae for a further 24 weeks, following which the test specimens shall be cut up.

8.4.3 Verification of the state of the larvae

If there is any doubt about the state of the live larvae in Category 2 at the end of the test, an additional test shall be carried out with these larvae in untreated test specimens for 4 weeks. Report their ability to bore. Those not able to bore normally shall be considered as moribund and counted as dead.

8.4.4 Validity of the test

The test shall be considered valid if at least 70 % of the larvae inserted into all of the untreated control test specimens, and at least 70 % of those inserted into all of the control test specimens treated with the solvent or the diluent alone, survive. Adult insects shall be included in this percentage. Otherwise, repeat the test.

9 Expression of results

Report the number of larvae that have bored into the treated and control test specimens and the number of surviving larvae for each period of exposure.

The toxic values of a preservative are expressed as the following two concentrations:

— the lowest concentration at which no adults emerge and at which, at the end of the test, all larvae are dead;

— the next, lower, concentration in the series at which some adults emerge or at which, at the end of the test, live larvae are found.

Express these values in kilograms of preservative per cubic metre of treated wood, and also state the corresponding concentrations of the preservative in the solvent or diluent.

10 Test report

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

a) number and date of this part of this document;

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

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

d) name and concentration of active ingredient;

e) if relevant the solvent or diluent used;

f) species of wood used;

g) concentrations of preservatives tested, expressed as mass fraction;

h) date of the impregnation;

i) minimum, maximum and mean masses, in grams, of solution absorbed for each concentration and the corresponding mean mass per unit volume, in kilograms per cubic metre, of the preservative under test;

j) method of drying the test specimens;

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

l) category of larvae used and, where relevant, the mean mass of those larvae used in the optional test;

m) date of insertion of larvae into the test specimens;

n) use or not of radiographic equipment;

o) dates of examination of the test specimens;

p) duration of the test;

q) results obtained at each examination for both treated and control test specimens:

- number of dead larvae not having tunnelled;
- number of dead larvae having tunnelled;
- number of live larvae and their condition;
- number of larvae not retrieved;

r) toxic values, in kilograms of preservative per cubic metre of wood, for the category of larvae under examination, together with the concentrations, as mass fraction, of treating a solution to which these values correspond;

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

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

u) following note:

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

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

Annex A
(informative)

Example of a test report

Number and date of this document	EN 47:2016
Name of supplier	Company D
Name and type of preservative	X water-soluble material, formulation not declared
Wood species	Scots pine *(Pinus sylvestris* Linnaeus)
Concentrations of the preservative tested(in mass fraction)	0,40 % - 0,25 % - 0,16 % - 0,10 % - 0,063 % - 0,040 % - 0,025 %
Date of impregnation	2004–05–05
Mass of solution absorbed and retention of preservative	See Table A.1
Method of drying	As for water-soluble preservatives
Ageing procedure previously carried out	None
Larvae used	Larvae in Category 1 (recently hatched)
Date of insertion of larvae	2004–06–02
Radiographic examination	No
Dates of examination of the test specimens	2004–06–30 and 2004–09–22
Duration of the test	12 weeks
Results	See Table A.1
This report has been prepared by	Laboratory L
Location and date	X 2004–09–27
Name and signature of the officer(s) in charge	Mr. Y

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

Table A.1 — Results

Duration of biological test	Concentration tested Mass fraction	Absorption			Mean retention of preservative	Larvae recovered			Larvae not recovered
		Mass of solution absorbed per test specimen				Dead		Live	
		Minimum	Mean of five specimens	Maximum		Not having tunnelled	Having tunnelled		
weeks		g	g	g	kg/m^3				
4	0,40	14,5	15,0	15,5	3,2	23	7	0	0
	0,25	14,4	15,0	15,5	2,0	20	9	0	1
	0,16	13,5	14,0	14,8	1,2	18	12	0	0
	0,10	15,0	15,4	15,8	0,82	16	14	0	0
	0,063	14,5	14,9	15,3	0,50	3	2	1 [a]	0
12	0,063	14,5	14,9	15,3	0,50	6 [b]	18 [b]	0	0
	0,040	14,4	15,0	15,6	0,32	10	16	3	1
	0,025	14,5	15,0	15,7	0,20	6	12	12	0
	0 (water only)	15,0	15,2	15,4	0	0	0	28	2
	untreated control					0	2	27	1

[a] Cutting up discontinued.

[b] In the remaining 4 test specimens.

At the end of the test (12 weeks) the toxic values of product X against *Hylotrupes bajulus* larvae of Category 1 are 0,32 kg/m^3 and 0,50 kg/m^3, corresponding to treating mass fractions of 0,040 % and 0,063 % respectively.

Annex B
(informative)

Technique for culturing *Hylotrupes bajulus* (Linnaeus)

B.1 General

Before undertaking culturing of *Hylotrupes bajulus,* a basic knowledge of the biology of this insect should be acquired from literature and from official organizations conducting research in wood preservation.

B.2 Obtaining parent beetles

A culture can be begun by taking larvae from naturally-infested wood and inserting them headfirst into suitably sized drilled holes in pine sapwood blocks and allowing them to pupate in order to obtain reproductive adults.

It is necessary to ensure that the insects have not been in contact with toxic products or with treated wood at any stage in their development.

Adult beetles of the brown variety should be removed.

B.3 Mating

Place together a male and female adult on a surface, cover them with a Petri dish lid and leave them in daylight, as *Hylotrupes bajulus* is a diurnal insect.

Quite soon, the male will approach the female and mating will take place with the male uppermost. Then separate the two insects as they will bite and damage one another if left confined together.

A male can fertilize two or three females a day.

B.4 Egg-laying

B.4.1 Isolate the females after fertilization and place them for egg-laying, using either of the techniques given in B.4.2 and B.4.3.

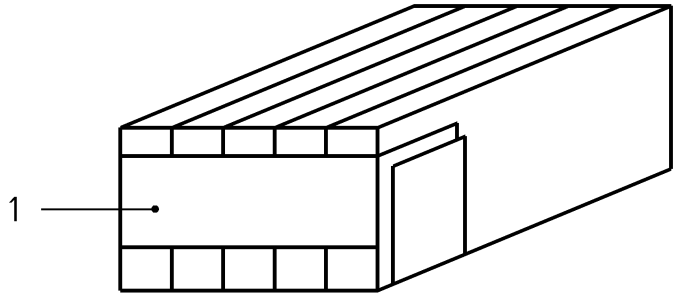
B.4.2 Place the females in glass bottles containing small blocks of pine sapwood, resting on a filter paper disc. The egg-laying will take place between the block and the filter paper on which they may be seen easily.

B.4.3 Place the females on the surface of pine sapwood blocks introduced in an appropriate vessel and which have been previously split into several pieces, and then reassembled with adhesive tape placed at one end, as shown in Figure B.1, so that open cracks remain which diminish in width from the free end to the end bound with the tape. By removal of the tape, the different pieces of the block can be separated easily and any eggs laid in the cracks can be seen. Place the females singly on each composite block and cover with a glass lid.

It should be noted that mating repeated each day or every 2 days stimulates egg-laying.

Once a day check if eggs have been laid. If so, remove the filter paper or the block which carries them and give the female fresh blocks for further egg-Iaying, allowing continued mating in order to activate egg-Iaying.

A favourable temperature for egg-Iaying is about 25 °C.



Key

1 adhesive tape

Figure B.1 — Block with adhesive tape

B.5 Hatching of eggs

Place the surfaces (discs of filter paper or split blocks) on which eggs have been laid in such a manner that, on hatching, the young larvae fall into a glass vessel from which they cannot escape.

The optimum conditions for hatching are as follows:

— temperature about 28 °C;

— relative humidity about 80 %.

B.6 Larval development

Place the newly-hatched larvae in culturing blocks, as described below. They shall not have been deprived of food for more than 3 days before being inserted in the blocks.

Handle the larvae carefully using a soft brush or vacuum tweezers.

As the larvae of *Hylotrupes bajulus* eat one another, they should be kept apart during culturing by having only one larva per block, insert each larva into a hole, pierced with a bradawl at right angles to the grain of the wood, to a depth of 4 mm to 6 mm.

Culturing blocks shall be of pine sapwood. Prior to insertion of the larvae, impregnate the blocks under vacuum with an aqueous solution of 10 g/l peptone and 5 g/l yeast (the yeast may be replaced with 0,01 g/l lactoflavine), then dry them. In this way, the duration of larval development can be shortened to about one-tenth of its natural length.

The size of the blocks is not critical but they may conveniently be of dimensions 50 mm × 25 mm x 15 mm, the length being parallel to the grain of the wood.

The growth of *Hylotrupes bajulus* is most rapid between 28 °C and 30 °C with an optimum relative humidity of 97 % to 98 %, but very high humidities favour the development of moulds. It is therefore preferable to use a relative humidity of (85 ± 5) %.

When conditions of microclimate and nutrition are perfect, the male adult insects can appear at about 6 months. However, as soon as the larvae reach a size such that the volume of the blocks is insufficient to permit normal growth, it is preferable to transfer them into blocks of larger dimensions that are not impregnated with peptone and yeast, in which larval growth can be continued until pupation.

The larvae of *Hylotrupes bajulus* exhibit a long diapause but pupate more rapidly when they are exposed to low temperature. It is therefore advantageous to place the large blocks outdoors in winter at a temperature of 5 °C to 10 °C. In this way, a mass emergence of insects can be obtained, with a high proportion emerging within a short space of time. This is particularly suitable for establishing new cultures.

B.7 Enemies and parasites

Take care to avoid infestation by hymenopterous parasites and coleopterous predators by closing the rearing vessels with fine mesh grilles.

The insects likely to cause the greatest damage to *Hylotrupes bajulus* cultures are:

— *Rhoptocentruspiceus* Marshall (Braconidae);

— *Scleroderma domesticum* Latreille (Bethylidae).

Experience has shown that special precautions against mites are unnecessary.

Insects collected from the field should undergo a severe quarantine before being introduced into the culturing chamber.

Annex C
(normative)

Differentiation of heartwood and sapwood in *Pinus* species

C.1 Principle

The phenolic compounds in the heartwood of *Pinus* species are detected by the formation of a coloured complex with o-dianisidine.

C.2 Reagents

o-dianisidine diazonium chloride solution, 20 g/l. Dissolve 2 g of o-dianisidine diazonium chloride in 100 ml of water. Prepare freshly as required.

NOTE o-dianisidine diazonium chloride stabilized with zinc chloride may be purchased as 'Fast blue B salt'.

C.3 Apparatus

C.3.1 Spray equipment, capable of producing a fine even spray. Both gas-powered aerosol units and small hand-powered units have been found to be suitable.

C.3.2 Soft paint brush, 5 mm to 50 mm wide, depending on the size of the timber section to be tested.

C.4 Procedure

Prepare the wood sample to give a clean surface and remove any loose sawdust. Brush or spray the solution (C.2) on to the timber section.

CAUTION — It is advisable to wear protective goggles and to work under a fume hood when carrying out spray tests.

The heartwood of most *Pinus* species is coloured magenta and the sapwood is coloured yellow.

Annex D
(informative)

Environmental, health and safety precautions within chemical/biological laboratory

When preparing this document, consideration was given to the minimization of environmental impacts caused by the use of the methods of testing.

It is the users' responsibility to use safe and proper techniques in handling materials in the methods of testing specified in this document.

The following list is not exhaustive but users of this document may use it as a guide to the use of safe and proper techniques. They should:

— investigate if European Directives, transposed European legislation and national laws, regulations and administrative provisions apply;

— consult manufacturers/suppliers for specific details such as material safety data sheets and other recommendations;

— use safety equipment and wear protective clothing, usually goggles and coats, appropriate for the test product and the test chemicals, in all laboratory areas, to ensure the safety of the operator;

— be careful about flammable materials and substances that are toxic and/ or human carcinogens and generally take care during transportation, decanting, diluting and dealing with spillages;

— use a fume cupboard during preparation of organic solvent solutions;

— store, handle and dispose of chemicals in a safe and environmentally satisfactory manner: including chemicals for laboratory test, test specimens, unused solvents and reagents that have to be disposed of.

Bibliography

[1] EN 46-1, *Wood preservatives — Determination of the preventive action against recently hatched larvae of Hylotrupes bajulus (Linnaeus) — Part 1: Application by surface treatment (laboratory method)*

[2] EN 212, *Wood preservatives — General guidance on sampling and preparation for analysis of wood preservatives and treated timber*

[3] EN 1001-1, *Durability of wood and wood-based products — Terminology — Part 1: List of equivalent terms*

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