SLOVENSKI STANDARD
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Zaščitna sredstva za les - Določanje toksičnih vrednosti za ličinke hišnega kozlička Hylotrupes bajulus (Linnaeus) - Laboratorijska metoda

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

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

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

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English version

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

Holzschutzmittel; Bestimmung der Giftwerte gegenüber Larven von Hylotrupes bajulus (Linnaeus) (Laboratoriumsverfahren)

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CEN

European Committee for Standardization
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B R I E F H I S T O R Y

This European Standard was drawn up by the Technical Committee CEN/TC 38 "Methods of test for wood preservatives", the Secretariat of which is held by AFNOR.

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Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxemburg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

Contents

0 Introduction

This European Standard specifies a laboratory method of test 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 'Determination of the preventive action against recently hatched larvae of *Hylotrupes bajulus* (Linnaeus) (laboratory method)', 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 the product can be assessed. This criterion should be used to assess the value of the preservative taking into account the methods by which the preservative may be applied. It is further recommended that the results should be supplemented by those from other appropriate tests and, above all, by practical experience.

When using products which are very active at very low concentration, it is of great importance that suitable precautions be taken to isolate and separate as far as possible operations involving chemical products, other products, treated wood, all clothing and laboratory apparatus. Suitable precautions shall include the use of separate rooms, areas within the rooms, extraction facilities, conditioning chambers and special personnel training.

1 Object

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

2 Field of application

This method is applicable to:

- water-insoluble chemicals which are being studied as active insecticides; or
- organic water-insoluble formulations, as supplied or as prepared in the laboratory by dilution of concentrates; or
- organic water dispersible formulations as supplied or as prepared in the laboratory by dilution of concentrates; or
- water-soluble materials, for example salts.

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

3 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 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 specimens.

Derivation of the toxic values of the product under test for the category of larvae in question.

4 Test materials

4.1 Biological material

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.

4.1.1 *Source of larvae.* The larvae should preferably be obtained from cultures reared according to the method described in annex B.

Otherwise larvae in category 2 may 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.

4.1.2 *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 to 3 days in the culturing chamber (4.3.1) to check that they are healthy.

4.1.3 *Choice of larvae in category 2.* Use only healthy larvae in the test. 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.

4.1.4 *Number of larvae.* 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 per treated and control specimen shall be six of category 1 or one of category 2.

The number of larvae necessary is given in table 1.

4.2 Products and reagents

4.2.1 *Solvents and diluents*

For water-soluble preservatives:

distilled or deionized water.

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

suitable volatile liquids which leave no residue which would have a toxic effect on the insect[1] in the wood at the end of the post-treatment conditioning period.

4.2.2 *Xylene.*

4.2.3 *Cellulose or absorbent cotton wool* and *filter paper.*

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[1] Do not use benzene as a solvent as it poses a health risk for those conducting the test.

4.3 Apparatus

4.3.1 *Culturing chamber*, with air circulation, and controlled at between 27 °C and 29 °C with a tolerance of ± 1 °C and at 85 ± 5 % r.h.

4.3.2 *Conditioning chamber*, well ventilated and controlled at 20 ± 2 °C and 65 ± 5 % r.h.[2].

4.3.3 *Laboratory work area*, well ventilated, where treatment of the test specimens is carried out[3].

4.3.4 *Testing chamber*, ventilated and air-conditioned, controlled at between 21 °C and 23 °C with a tolerance of ± 1 °C and at between 70 % and 75 % r.h. with a tolerance of ± 5 % r.h.

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

4.3.6 *Weights*, chemically inert, for ballasting the test specimens.

4.3.7 *Protective gloves*.

4.3.8 *Vacuum vessels*, fitted with stopcocks.

4.3.9 *Pneumatic pump*, fitted with a *pressure gauge* and capable of maintaining a pressure of 700 Pa[4].

4.3.10 *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.

4.3.11 *Ordinary laboratory equipment*, including an analytical balance.

4.3.12 *X-ray apparatus* (if desired) 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 Sample of the preservative

The sample shall be representative of the product under test.

Table 1. Number of larvae and test specimens

Type of test specimen	Concentrations of preservatives	Larvae in category 1		Larvae in category 2			
				Without radiography		With radiography[5]	
		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 specimens	0	5	30	10	10	7	7
Solvent or diluent control specimens (including water)	0	5	30	10	10	7	7
Total for 5 concentrations		35	210	70	70	49	49

[2] The conditioning of specimens after treatment may be carried out in the laboratory work area (**4.3.3**) provided that this meets the conditions specified for the conditioning chamber (**4.3.2**).

[3] Proper safety measures for handling flammable or toxic materials shall be followed. Avoid excessive exposure to solvents or their vapours.

[4] 100 Pa = 1 mbar.

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

6 Test specimens

6.1 Species of wood

The reference species is Scots pine, *Pinus sylvestris* Linnaeus[6].

Additional tests may be made using other species but, if so, this shall be stated in the test report.

6.2 Quality of wood

Use only sound sapwood, straight-grained, without knots and with a low resin content.

Cut the specimens from wood of average growth rate (2.5 to 8 annual rings per centimetre).

The proportion of summer wood in the annual rings shall not exceed 30 % of the whole.

The wood shall neither have been floated nor subjected to chemical or heat treatment[7]. It shall be air-dried and shall not have been stored for more than 5 years.

6.3 Provision of test specimens

Cut the test specimens from planed strips having a cross section 25 mm x 15 mm and with the longitudinal edge parallel to the grain of the wood.

In the case of X-ray examination for larvae in category 2, the large longitudinal surfaces shall be tangential.

The transverse cross sections shall be cut cleanly and have sharp edges.

Avoid using test specimens from the butt or crown of the tree. Take the specimens required for one test at random from a batch of specimens originating from at least three trees.

6.4 Dimensions of test specimens

The nominal dimensions of each specimen measured at 12% (*m/m*) moisture content shall be 50 mm x 25 mm x 15 mm.

The volume of each test specimen is theoretically 18.75 cm^3 but the size of each block shall be checked so that the exact volume is known.

6.5 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 specimens so that, after weighing, any specimens with abnormally high or low retentions can be rejected from the batch.

7 Procedure

7.1 Preparation of the test specimens

7.1.1 *Conditioning of specimens before treatment.* Allow the specimens to reach equilibrium in the conditioning chamber (4.3.2).

7.1.2 *Treatment of specimens*

7.1.2.1 *Preparation of the treatment.* Prepare a series of at least five concentrations, by mass, distributed 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 the first test and a more closely spaced geometric or arithmetic progression for subsequent tests.

All treatment solutions shall be freshly prepared.

7.1.2.2 *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 specimen, to the nearest 0.05 g, and then stack the specimens in one of the treatment vessels (4.3.5) so that as much of their surface as possible is exposed (e.g. by piling them crosswise). Ballast the stack of specimens with the weights (4.3.6) to prevent them from floating later when the liquid is admitted.

Place each beaker in one of the vacuum vessels (4.3.8) and, after reducing the pressure to 700 Pa, hold the specimens at this pressure for 15 min[8]. After this period, close the stopcock to the pneumatic pump (4.3.9) and open the other stopcock to allow the solution of the preservative to be drawn into the treatment vessel. Keep the specimens completely covered by the solution throughout the remainder of the impregnation process.

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

After this impregnation treatment, remove the test specimens one by one, remove excess liquid from their surfaces by lightly blotting with filter paper, and weigh each immediately, to the nearest 0.05 g.

In the case of water-soluble preservatives, for example salts and water-insoluble chemicals which are being studied as active substances, calculate the mass of preservative retained by each specimen from the mass of solution absorbed and its concentration[9].

In the case of organic water-insoluble formulations, the retention is expressed for each specimen in terms of the corresponding mass of the formulation ready for use 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.

7.1.3 *Drying and conditioning of the test specimens after treatment*[10]. After impregnation, dry the test specimens for at least 4 weeks in the conditioning chamber (4.3.2).

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[6] 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 standard has been demonstrated in all aspects (development of larvae, resistance to impregnation, etc.).

[7] Gentle artificial drying at below 60 °C is, however, permissible.

[8] Observe the proper safety measure for vacuum vessels.

[9] 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.

[10] Drying and conditioning of the specimens depend on the nature of the preservative under test and on the solvent or diluent used. It may be necessary to modify the conditioning process but, if so, this should be stated in the test report.

Arrange the specimens on their narrow faces, resting on two glass rods, not touching one another. Invert the specimens twice a week.

Place the specimens thus arranged and impregnated with water-soluble preservatives for 2 weeks in a covered vessel 100 mm to 200 mm high. To prevent mould growth, also place in the vessel a small dish containing the xylene (4.2.2). During the third week, uncover the vessel progressively each day to allow the specimens to dry steadily. From the beginning of the fourth week, leave the vessel fully open.

For specimens impregnated with water-insoluble preservatives, keep the vessel covered for 1 week and open it gradually during the second week. From the beginning of the third week, leave the vessel fully open.

7.2 Exposure of the test specimens to the insects

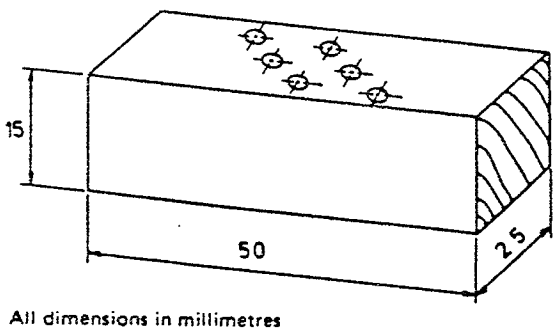
7.2.1 *Use of larvae in category 1.* Make a regular pattern of 6 holes approximately 3 mm deep in one of the wide longitudinal faces of each test specimen (see figure 1). Carefully insert the larvae head first and keep the holes upwards.

7.2.2 *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

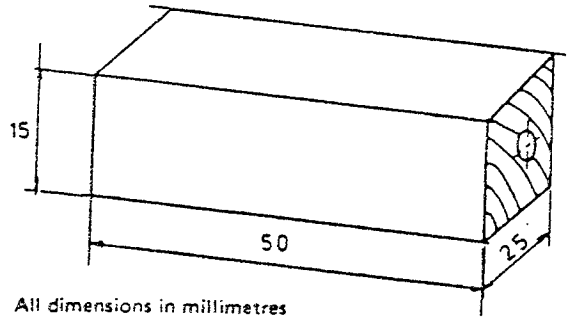
Mass of larvae	Approximate diameter of holes
mg	mm
from 50 to 60	3.0
from 60 to 90	3.5
from 90 to 130	4.0
from 130 to 150	4.5

Carefully insert each larva head first and block the opening of the hole with a wad of the cellulose or cotton wool (4.2.3), so that a space is left between the wad and the larva equal to a quarter of the length of the larva.



All dimensions in millimetres

Figure 1. Holes bored in a longitudinal face for larvae in category 1



All dimensions in millimetres

Figure 2. Hole bored in a transverse face for a larva in category 2

7.3 Conditions and duration of the test

Place all the test specimens in the testing chamber (4.3.4), keeping the different sets of specimens, i.e. untreated control specimens, solvent- or diluent-treated control 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 7.4, is:

12, possibly 24, weeks for larvae in category 1;

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

7.4 Examination of the test specimens

7.4.1 *Examination without radiography.* After 4 weeks (for larvae in category 1) or 12 weeks (for larvae in category 2), cut up the test specimens at 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 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 specimens.

7.4.2 *Examination with radiography, (larvae in category 2).* Using the X-ray apparatus (4.3.12), radiograph all the test specimens after 12 weeks, cut up those 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.