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BSI Standards Publication

Wood preservatives — Determination of the protective effectiveness against Anobium punctatum (De Geer) by egg-laying and larval survival

Part 1: Application by surface treatment (Laboratory method)

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National foreword

This British Standard is the UK implementation of EN 49-1:2016. It supersedes BS EN 49-1:2005 which is withdrawn.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 protective effectiveness against Anobium punctatum (De Geer) by egg-laying and larval survival - Part 1: Application by surface treatment (Laboratory method)

Produits de préservation du bois - Détermination de l'efficacité protectrice vis-à-vis d'Anobium punctatum (De Geer) par l'observation de la ponte et du taux de survie des larves - Partie 1: Application par traitement de surface (Méthode de laboratoire)

Holzschutzmittel - Bestimmung der vorbeugenden Wirkung gegenüber Anobium punctatum (De Geer) durch Beobachten der Eiablage und des Überlebens von Larven - Teil 1: Oberflächenverfahren (Laboratoriumsverfahren)

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European foreword

This document (EN 49-1:2016) 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of an identical text or by endorsement, at the latest by December 2016, and conflicting national standards shall be withdrawn at the latest by December 2016.

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 49-1:2005.

Significant technical differences between this document and EN 49-1:2005 are as follows:

a) generalization of material for preparing the egg-laying zones;

b) introduction of new harmonized specifications for wood quality.

This document consists of two parts, Part 1 is required to enable effectiveness assessments of wood preservatives that are intended to be applied by surface treatment and Part 2 those that are intended to be applied by impregnation.

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 assessment of the effectiveness of a wood preservative, when applied as a surface treatment, against *Anobium punctatum.* It allows the determination of the concentration at which the product prevents the development of infestation from egg laying. It can also be used with formulations ready for use.

The method simulates conditions that can occur in practice on timber which has been treated some time previously with wood preservative applied by dip, brush or spray and on which eggs of *Anobium punctatum* are laid.

This laboratory method provides one criterion by which the value of a product 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 low concentrations 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 protective effectiveness or the toxic values of a wood preservative against infestation by *Anobium punctatum* (De Geer) when the product is applied as a surface treatment to wood.

This method is applicable to:

— water-insoluble chemicals that 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;

— water-soluble materials, for example salts.

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

2 Normative references

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 835, *Laboratory glassware — Graduated pipettes (ISO 835)*

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

Depending on the test being carried out either:

— on a set of test specimens of a susceptible wood species that is surface treated with a solution of the preservative, or

— if toxic values are to be determined, on several sets of test specimens of a susceptible wood species that are surface treated with a series of solutions in which the concentration of preservative is ranged in a given progression.

The treated test specimens are exposed to gravid females of *Anobium punctatum.* The number of eggs laid, the number of eggs hatched and the numbers of surviving larvae are observed and compared with those in untreated control test specimens. If the preservative has been prepared in the laboratory by dilution of a concentrate or by dissolution of a solid, the resulting attack is also compared to that in solvent or diluent treated control test specimens.

5 Test materials

5.1 Biological material

***Anobium punctatum* (De Geer)**

Adult males and females in good condition.

Adults to be used in the test shall be collected at daily intervals from naturally infested wood or laboratory culture (see Annex C).

Use recently emerged adults which have been recently collected; kept overnight in quarantine (see C.6); and then checked to ensure that they are undamaged, active and free from any infestation by mites. Determine the sex (see Annex B) of the collected and checked adults and place the males and females in separate containers.

NOTE The proportion of males and females varies during the emergence period.

5.2 Products and reagents

5.2.1 Paraffin wax, for sealing the relevant faces of test specimens to be treated with solutions in which water is the continuous phase.

NOTE Paraffin wax with a setting point of 52 °C to 54 °C has been found to be suitable.

5.2.2 Gelatin, for sealing the relevant faces of test specimens to be treated with solutions in which an organic solvent is the continuous phase.

5.2.3 Paste, for securing filter paper. The paste shall be starch-free, non-toxic to *Anobium punctatum* and insoluble in the product under test.

NOTE Sodium carboxy methyl cellulose, food grade, has been found to be suitable.

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

5.2.5 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.

5.2.6 Filter paper, ordinary quality, medium-fast grade.

5.2.7 Fine cloth of a suitable material with a mesh aperture of 0,3 mm to 0,6 mm for the preparation of the egg-laying zones.

NOTE Cotton, linen and polyamide-gauze have been proven as suitable.

5.3 Apparatus

5.3.1 Culturing chamber, with air circulation, controlled at (21 ± 2) °C, and at relative humidity (80 ± 5) %.

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

The conditioning of test specimens may be carried out in the laboratory work area (see 5.3.4) provided that this has the conditions specified for the conditioning chamber (see 5.3.2).

5.3.3 Treatment vessel, 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.

5.3.4 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.

5.3.5 Testing chamber, with conditions identical to those of the culturing chamber (see 5.3.1).

5.3.6 Pipette, of type specified in EN ISO 835, Class B: graduated pipette with no waiting time. Capacity 1 ml with an accuracy of ± 0,01 ml.

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 Test containers, suitable for holding the test specimens and of material resistant to the solvents used, and fitted with perforated covers to provide a good exchange of air.

NOTE Jars of approximately 60 mm diameter and 100 mm height have been found to be suitable.

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

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

— voltage: 10 kV to 50 kV;

— current: 0 mA to 15 mA.

5.3.11 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.

7 Test specimens

7.1 Species of wood

The reference species is European oak. This shall be either sessile oak (*Quercus petraea* (Mattuschka) Lieblin) or pedunculate oak (*Quercus robur* Linnaeus).

Additional tests may be carried out using other species[1)] 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 be stored for more than five years.

The wood shall be exclusively sapwood[2)] and having between 2 annual rings per 10 mm and 10 annual rings per 10 mm.

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 x (15 ± 0,5) mm[3)] removing a minimum of 2 mm from any surfaces exposed during drying. The longitudinal faces shall be parallel to the direction of the grain. The annual rings shall be parallel to the broad faces (contact angle of less than 5°). 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 x (25 ± 0,5) mm x (15 ± 0,5) mm.

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

7.5 Number of test specimens

Use:

a) five test specimens (see 7.4) for each preservative and each concentration;

b) five untreated control test specimens (see 7.4) for a complete test of any given preservative;

c) five control test specimens (7.4) treated with that solvent or diluent (5.2.4 or 5.2.5) if a solvent or diluent (water included) is used.

When dipping is to be used (8.1.3.2.1) 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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1) The growth of young larvae of *Anobium punctatum* is slow in specimens from resinous wood. Results from test specimens in resinous wood should be compared with those obtained from oak specimens.

2) It is not essential in this test for the starch content of the wood to be high.

3) These test specimens may be taken from the trunk of the tree or the large branches.

8 Procedure

8.1 Preparation of the test specimens

8.1.1 Conditioning of test specimens prior to sealing

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

8.1.2 Sealing

8.1.2.1 General

Sealing of the narrower longitudinal and the transverse faces and one of the large faces of the test specimens.

Seal these faces as follows:

8.1.2.2 For tests with solutions in which water is the continuous phase, apply three coats of the paraffin wax (5.2.1) at about 90 °C so that the first coat adheres closely to the wood and the successive coatings bond to one another. Condition the sealed test specimens in the conditioning chamber (5.3.2) for at least one day.

8.1.2.3 For tests with preservative solutions in which the continuous phase is an organic solvent that dissolves paraffin wax, use the gelatine (5.2.2): apply the first coat as an aqueous solution of 200 g/l at 40 °C, then after a minimum of 8 h of drying, apply two further coats of an aqueous solution of 300 g/l at 50 °C. Condition the sealed test specimens in the conditioning chamber (5.3.2) for at least one day.

8.1.3 Treatment of the test specimens

8.1.3.1 Preparation of treatment solutions

8.1.3.1.1 Solid preservatives

— Water-soluble preservatives: dissolve the preservative in the water (5.2.4) to the required concentration, or in a series of concentrations if toxic values are to be determined;

— Non-water-soluble preservatives: dissolve the preservative in an appropriate solvent (5.2.5) to the required concentration, or in a series of concentrations if toxic values are to be determined.

All treatment solutions shall be freshly prepared.

8.1.3.1.2 Liquid preservatives

If appropriate, use the preservative without further preparation other than any necessary stirring. If it is a concentrate or if toxic values are to be determined, 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.

8.1.3.1.3 Toxic values

If toxic values are to be determined, 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.

8.1.3.2 Application of the treatment solution

8.1.3.2.1 Treatment by dipping

Weigh to the nearest 0,05 g each sealed test specimen, to obtain its initial mass.

Treat each test specimen in the treatment vessel (5.3.3) as follows:

— immerse completely in the solution for 1 min;

— remove and lightly blot test specimens on absorbent paper to remove free fluid from the faces;

— immediately weigh to the nearest 0,05 g.

In the case of water-soluble chemicals, for example salts, and water-insoluble chemicals which are being studied as active insecticides, calculate the mass of chemical retained for each test specimen from the mass of solution absorbed and its concentration.

In the case of organic formulations or organic water-dispersible formulations, the retention is expressed for each test 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 area of unsealed wood surface.

8.1.3.2.2 Treatment by pipette application

Determine the actual area of the unsealed face to be treated taking into account any possible encroachment of the sealing compound.

NOTE 1 The area to be treated is theoretically 12,5 cm^2.

Determine the volumes or masses of the treatment solution (8.1.3.1) to be applied to the unsealed face to give the application rate specified by the supplier.

The quantity of treatment solution to be applied should be realistic in view of the field of application and the manufacturer's instructions. Normally the quantity should not exceed 100 g/m^2.

In the laboratory work area (5.3.4), using the pipette (5.3.6) apply the calculated volume or mass of the treatment solution (8.1.3.1) to the unsealed faces as uniformly as possible. Apply the treatment solution to each unsealed face while keeping that face in a horizontal and upward facing position. Allow any surface liquid to be absorbed, make a mark to indicate this face for further operations.

NOTE 2 If the required quantity cannot be applied in one application, the treatment solution may be applied in successive applications at appropriately close intervals so as to avoid solidification of any substances hindering the penetration of the subsequent applications.

From the quantity of treatment solution applied to the unsealed face of each treated test specimen, determine and record the application rate in grams per square metre or millilitres per square metre of the treated test specimens.

Treat the control test specimens (7.5 c)) in an identical manner using solvent or diluent (5.2.4 or 5.2.5) if solvent or diluent are used in the preparation of the treatment solution.

8.1.4 Drying and conditioning of the test specimens after treatment

If the sealing has been damaged before or after treatment, reject the test specimens concerned from the tests.

After treatment, condition the test specimens for four weeks in the environment specified for the conditioning chamber (5.3.2). Arrange the test specimens on their narrow faces, resting on glass rods, not touching one another. Invert the test specimens twice a week.

NOTE The drying and conditioning of the test specimens depend on the nature of the product under test 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

Prepare the egg-laying zones by attaching a piece of the fine cloth (5.2.7) measuring approximately 45 mm × 20 mm to the unsealed face of the test specimen. Use the paste (5.2.3) to attach the cloth and smooth this out so that the mesh openings are not twisted.

Immediately prior to exposure to egg-laying, condition all the test specimens for one week in the testing chamber (5.3.5).

Place each test specimen in one of the test containers (5.3.8) and add five female insects and at least five male insects. Cover the container with a disc of filter paper (5.2.6). Keep this in place with the cover.

8.3 Conditions and duration of the test

Place the containers containing the test specimens and the insects in the testing chamber (5.3.5) for approximately one week. Count the eggs on each test specimen and, if there are fewer than 50, add another group of insects to the container and count the eggs again at the end of a further week in the testing chamber (5.3.5).

Each control test specimen should have at least 50 eggs for the test to be valid.

NOTE It may be necessary to add further insects in order to obtain an adequate number of eggs on all the test specimens. However, premature mortality of the insects on the treatment test specimens alone may be due to the action of the preservative.

When premature mortality of the insects occurs, this shall be mentioned in the test report. If 50 eggs have not been laid on treated test specimens after four groups of five pairs of insects have been added, continue without adding further insects and note this in the test report. When, at the end of several weeks, all the insects are dead, they shall be removed and the test specimens left in the containers in the testing chamber (5.3.5). Examine the test specimens 26 weeks or 52 weeks after introducing the last insects, depending on the expected mode of action of the test product.

8.4 Examination of the test specimens

52 weeks after introducing the last insects (26 weeks respectively), count as accurately as possible the number of eggs laid on each test specimen and the number of eggs that have hatched[4)]. Cut up all the test specimens and count the larvae, noting their general condition.

NOTE Evaluation of the presence and size of larvae in the test specimens may be carried out at intervals during the test using the X-ray apparatus (5.3.10), if available.

9 Validity of test

The results shall be accepted as valid provided that the following conditions are met:

a) for each set of control test specimens, a total of more than 50 live larvae are recovered; and

4) Because, in the case of oak, eggs can be laid in the vessels of the wood, it is not always possible to carry out an exact count.

b) live larvae are present in all control test specimens.

10 Expression of results

10.1 Assessment of the protective effectiveness

The protective effectiveness shall be expressed in terms of:

a) number of eggs laid on each test specimen;

b) number of eggs hatched on each test specimen; and

c) number of live larvae retrieved from each test specimen at the end of the test.

10.2 Toxic values

If a range of concentrations of product are tested, the results shall be expressed as toxic values.

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

— mean mass or volume of preservative retained per unit area in the set of test specimens treated with the lowest concentration of the product in the series in which all larvae are dead in all of the test specimens at the end of the test;

— mean mass or volume of preservative per unit area in the set of test specimens treated with the next lowest concentration of the product in the series in which live larvae are found in any of the test specimens at the end of the test.

Express the toxic values as grams or millilitres of preservative per square metre of treated wood surface and also state the corresponding concentrations of the preservative in the solvent or the diluent.

11 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 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 composition has been declared;

d) name and concentration of active ingredient;

e) if relevant the solvent or diluent used;

f) species of wood used;

g) method of application and, if applicable, the concentration of preservatives tested, expressed as mass fraction;

h) date of the application of the preservative;

i) for each test specimen treated:

   — mass of solution absorbed, in grams;

— corresponding quantity, in grams or millilitres per square metre, of the preservative under test, per unit surface area;

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) date when the test specimens were exposed to beetles;

m) date(s) of examination of the test specimens;

n) results obtained, both on treated test specimens and control test specimens:

— number of eggs laid on each test specimen;

— number of eggs hatched on each test specimen;

— number of test specimens containing live larvae, and also;

— total number of live larvae retrieved at the end of the test;

o) if determined, the toxic values;

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

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

r) 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.

It may include any optional observations made, for example X-ray examination (8.4).

Annex A
(informative)

Example of a test report

Number and date of this document	:	EN 49–1:2016
Name of supplier	:	company S
Name and type of preservative	:	X-preservative in the form of an organic solvent, ready for use, composition declared.
Name and concentration of active ingredient (mass fraction)	:	W 0,25 %
Solvent or diluent used	:	none
Species of wood used	:	European oak (*Quercus robur* L)
Date of application of the preservative	:	2015–05–21
Concentration of the preservative tested	:	preservative used undiluted
Type of treatment	:	applied using a pipette
Quantity of preservative applied to each test specimen	:	100 g/m^2
Method of drying	:	as specified in the document
Ageing test applied	:	by evaporation for 12 weeks in accordance with EN 73
Date of exposure to beetles	:	2015–06–02
Date of examination of the test specimens	:	2016–06–07
Results	:	see Table A.1
This report has been prepared by the institute	:	FPL
Location and date	:	Y 2016–06–20
Name and signature of the officer(s) in charge	:	Mrs Z

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.

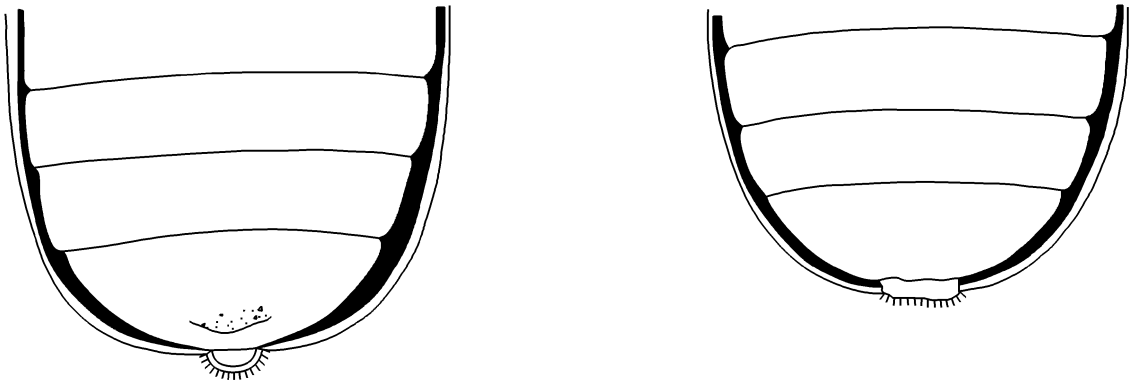
Table A.1 — Results

	Test specimens	Amount of preservative applied to test specimens	Loading of preservative		Examination of test specimens		
	Number of identification				Total number of eggs		Number of live larvae retrieved
		ml	ml/m^2	g/m^2	Laid	Hatched	
Treated test specimens	1	0,12	100	80	72	54	0
	2	0,12	100	80	84	72	0
	3	0,12	100	80	62	54	0
	4	0,12	100	80	102	94	0
	5	0,12	100	80	99	73	0
Control test specimens	1	-	-	-	78	72	60
	2	-	-	-	67	56	45
	3	-	-	-	108	87	76
	4	-	-	-	79	75	43
	5	-	-	-	154	138	69

Annex B
(informative)

Identification of sex of test insects *Anobium punctatum*

The shape of the abdominal segments examined from the underside differs between the sexes. In the male, in the last abdominal segment there is a distinct depression running parallel with the margin and the general convex curve of the abdomen is not pronounced. This depression is absent in the female and the whole of the ventral abdomen has a more convex curve. The shape of the end of the genital equipment which protrudes from the last abdominal segment is also characteristic. In the male this is rounded almost semi-circular whereas in the female it is sinuate, with a distinct concavity in the outer margin (see Figure B.1).



Male (♂) Female (♀)

Figure B.1 — Last segment of the abdomen of *Anobium punctatum* for the identification of sex

Annex C
(informative)

Culturing technique for *Anobium punctatum*

C.1 Culture wood

C.1.1 Wood species

Oak (*Quercus* sp.) or hazel (*Corylus avellana*).

NOTE Other European hardwoods may also be used if experience of their suitability is available.

C.1.2 Collection of culture wood

Use only small branchwood felled in the winter and containing a high proportion of sapwood.

C.1.3 Cutting of culture wood

Strip bark from larger stems (30 mm diameter) and cross cut to lengths of approximately 150 mm. Stems may be split lengthwise to facilitate drying.

C.1.4 Drying of culture wood

Dry as rapidly as possible by placing in a stream of air not exceeding 40 °C.

C.2 Source of beetles

C.2.1 Collection of beetles

Obtain freshly emerged adult beetles of *Anobium punctatum* from naturally infested material. Do not bring naturally infested material into the vicinity of the laboratory or culturing areas.

Moisten naturally infested material occasionally. During the summer emergence period take daily collections of beetles from the surfaces of the infested wood, tapping gently to remove beetles from their exit holes.

C.2.2 Quarantine of beetles

Place one filter paper sheet vertically into a large glass jar and then introduce the collected adult beetles. Place a lid or gauze covering on the jar. Keep the jar remote from the culturing area for 24 h and then remove the filter paper with attached beetles. The attached beetles may be used for culturing. The jar should be sterilized and the remaining beetles destroyed.

C.3 Infestation of culture wood

C.3.1 Culture vessels

Glass jars large enough to contain the pieces of wood (C.3.2) stood in a vertical position.

C.3.2 Preparation of wood

The pieces of wood can be utilized with sawn and split surfaces only or with muslin mesh of (0,3 to 0,5) mm fixed on to one end grain surface using the paste (5.2.3). Alternatively egg-laying sites may be provided by artificially roughening or scoring the surface of the wood.

C.3.3 Introduction of beetles

Place the pieces of wood vertically in jars with, where appropriate, muslin-coated ends uppermost. Introduce one pair of adult beetles for every 15 cm^3 to 20 cm^3 wood (approximately).

Cover the jar tops with an air-permeable material, e.g. muslin (aperture approximately 0,8 mm) or filter paper to prevent escape of beetles.

After four weeks in culturing conditions dead adult beetles may be removed.

C.4 Culturing conditions

C.4.1 Normal environment

The normal culturing conditions are obtained in introducing the culture vessels with the infested wood (C.3.3) into the culturing chamber (5.3.1).

C.4.2 Natural pupation induction

After a minimum of 18 months in conditions as in C.4.1, place the culturing jars in an unheated insectary from mid-November to mid-March, and then return to conditions as in C.4.1. Emergence can be expected after a delay of several months.

After 18 months in the conditions described in C.4.1, the majority of larvae should exceed a mass of 7 mg.

C.4.3 Artificial pupation induction

It is possible to induce pupation and emergence by means of a period of refrigeration of the infested wood at 7 °C for between 60 days and 80 days. However with some sources of insects it has been found necessary to simulate artificially the varying outside temperature conditions for early spring time to achieve adequate emergence. By both means it is also possible to obtain emergence of beetles out-of-season or throughout the year.

C.5 Collection of beetles

Inspect cultures daily and remove adult beetles by tapping the wood samples. Re-infestation to achieve a second generation may be possible in the culture wood.

C.6 General culture hygiene

Special precautions and strict adherence to them is necessary to avoid infestations of parasites, mainly mites of the genus *Pyemotes* or *Hymenoptera* such as *Theocolax formiciformis* or *Spathius exarator.*

The parasitic mites *Pyemotes spp.* and other species can be very troublesome, especially under conditions of incubation. These mites are frequently present in wood with a natural *Anobium* infestation and it is essential not to bring naturally infested wood into the room or incubators where tests are carried out.

Important precautions are:

— prohibit introduction of unsterilized naturally infested wood into laboratory or culturing areas;

— avoid transfer of mites from naturally infested wood by changing clothing before and after working with cultures. After contact with naturally infested material staff should avoid contact with clean cultures for 24 h;

— keep culture jars isolated from each other in shallow trays of water containing a small quantity of detergent;

— keep adult beetles collected for tests or for re-culturing overnight in closed Petri dishes with paper-lined bottoms (10 insects per dish). The following day examine the insects and discard any which seem damaged or inactive.

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 49-2, *Wood preservatives — Determination of the protective effectiveness against Anobium punctatum (De Geer) by egg-laying and larval survival — Part 2: Application by impregnation (Laboratory method)*

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

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

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