BS EN 49-2:2015

BSI Standards Publication

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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National foreword

This British Standard is the UK implementation of EN 49-2:2015. It supersedes BS EN 49-2:2005 which is withdrawn.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 2: Application by impregnation (Laboratory method)

Produits de préservation du bois - Détermination de l'efficacité protectrice vis à vis de Anobium punctatum (De Geer) par l'observation de la ponte et de la survie des larves - Partie 2 : Application par imprégnation (Méthode de laboratoire)

Holzschutzmittel - Bestimmung der vorbeugenden Wirkung gegenüber Anobium punctatum (De Geer) durch Beobachten der Eiablage und des Überlebens von Larven - Teil 2: Anwendung durch Volltränkung (Laboratoriumsverfahren)

This European Standard was approved by CEN on 16 July 2015.

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European foreword

This document (EN 49-2:2015) 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of an identical text or by endorsement, at the latest by February 2016 and conflicting national standards shall be withdrawn at the latest by February 2016.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 49-2:2005.

Significant technical differences between this document and EN 49-2:2005 are as follows:

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

b) introduction of new harmonized specifications for wood quality.

EN 49, *Wood preservatives — Determination of the protective effectiveness against Anobium punctatum (De Geer) by egg-laying and larval survival*, consists of two parts:

— *Part 1: Application by surface treatment (Laboratory method)*;

— *Part 2: Application by impregnation (Laboratory method)*.

EN 49-1 is required to enable effectiveness assessments of wood preservatives which are intended to be applied by surface treatment and EN 49-2 those which 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, against *Anobium punctatum.* It allows the determination of the concentration at which the product prevents the development of infestation from egg laying.

The method simulates conditions which can occur in practice on timber which has been treated some time previously with a deeply penetrating wood preservative 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 *Anobium punctatum* (De Geer) by egg-laying and larval survival in wood which has been 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.

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

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

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

4 Principle

The treated test specimens are exposed to gravid females of *Anobium punctatum.* The numbers of eggs laid, the numbers of eggs hatched, and the numbers of the surviving larvae are 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.

Depending on the test being carried out either

— on a set of test specimens of a susceptible wood species that is impregnated 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 impregnated with a series of solutions in which the concentration of preservative is ranged in a given progression.

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 daily 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.2.2 and 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 end sections of test specimens.

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

5.2.2 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 carboxymethyl cellulose, food grade, has been found to be suitable.

5.2.3 Xylene, technical grade, mixed isomers.

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 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(s), 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 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.

5.3.7 Vacuum vessel(s), fitted with stopcocks, capable of receiving the treatment vessels (5.3.3).

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

5.3.9 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.

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

5.3.11 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.

5.3.12 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 ranges:

— voltage: 10 kV to 50 kV;

— current: 0 mA to 15 mA.

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 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 timber species[2)] 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[3)] and having between two 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 × (15 ± 0,5) mm[4)] 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 test 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

Use:

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

2) The growth of young larvae of *Anobium punctatum* is slow in test specimens from resinous wood. Results from test specimens in resinous wood should be compared with those obtained from oak test specimens.

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

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

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.

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.

8 Procedure

8.1 Preparation of the 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 solution

8.1.2.1.1 Solid preservatives

Water-soluble preservatives:

— dissolve the preservative in the water (5.2.4) to the required concentration, or to 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 to a series of concentrations if toxic values are to be determined.

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 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.

8.1.2.1.3 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.

8.1.2.2 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 (5.3.3) so that as much of their face as possible is exposed (e.g. by piling them crosswise). Ballast the stack of test specimens with the weights (5.3.9) to prevent them floating later when the liquid is admitted.

Place each vessel in one of the vacuum vessels (5.3.7), attach the vacuum pump (5.3.8) and reduce the pressure to 700 Pa. Maintain this vacuum for 15 min. Observe the proper safety measures for vacuum vessels. After this period, close the stopcock to the vacuum pump (5.3.8) and open the other stopcock to allow the solution of 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 the excess liquid from their surfaces by lightly blotting with filter paper (5.2.6) 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[5)].

In the case of organic 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.

Calculate the mean mass of preservative retained per unit volume of wood for each set of five test specimens.

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 (5.3.6). 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 (5.2.3) in the drying vessel (5.3.6).

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 under test and on the solvent or diluent used. For slow drying products, it may be necessary to extend the conditioning process.

5) 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.

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

Coat the transverse faces of each test specimen with the paraffin wax (5.2.1) applied as a single brush coat at 70 °C to 90 °C, and allow to dry for 24 h.

Next prepare the egg-laying zones by attaching a piece of the fine cloth (5.2.7) measuring approximately 45 mm × 20 mm on each large face of the test specimen. Use the paste (5.2.2) to attach the cloth and smooth this out so that the mash 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 (5.3.11) 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 the week.

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[6)].

Cut up all the test specimens and count the larvae, noting their general condition.

NOTE Although certain preservatives give good results if the final evaluation is carried out after 26 weeks, experience has shown that a 52-week period is necessary for a large number of stomach poisons.

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 (5.3.13), if available.

6) 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.

9 Validity of test

The results shall be accepted as valid provided that

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

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

NOTE A lower number of live larvae may be acceptable if the control test specimens show extensive tunnelling.

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 of preservative retained per unit volume 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 of preservative per unit volume 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 in kilograms of preservative per cubic metre of treated wood, 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 part of this European Standard;

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) 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;

   1) number of eggs laid on each test specimen;

   2) number of eggs hatched on each test specimen;

   3) number of test specimens containing live larvae; and also

   4) 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) the 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 European Standard	EN 49-2:2015
Name of supplier	company S
Name and type of preservative	X-preservative in the form of an organic solvent, formulation declared.
Name and concentration of active ingredient	W mass fraction 0,10 %
Solvent or diluent used	toluene
Species of wood used	European oak (*Quercus petraea* (Mattuschka) Lieblein)
Concentration of the preservative tested(mass fraction)	0,5 % - 1,0 % - 2,0 % - 4,0 % - 8,0 %
Date of impregnation	2014-12-12
Mass of solution absorbed and retention of preservative	see Table A.1
Method of drying	as specified for organic solvent formulations
Ageing test applied	none
Date of exposure to beetles	2014-12-12
Radiographic examination	None
Date of examination of the test specimens	2014-12-12
Results	see Table A.1
Toxic values	12 kg/m^3 and 27 kg/m^3
This report has been prepared by	Laboratory L
Location and date	Y 2015-02-12
Name and signature of the officer(s) in charge	Mr 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.

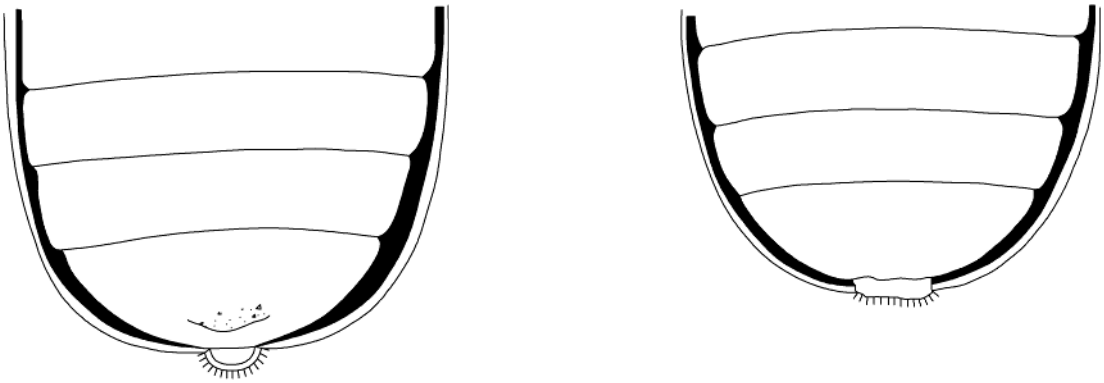
Table A.1 — Results

Concentration of preservative tested	Absorption				Examination of test specimens			
	Mass of solution absorbed per test specimen			Mean retention of the preservative tested	Total number of eggs		Number of test specimens containing live larvae	Number of live larvae retrieved
	Min	Mean 5 test specimens	Max		Laid	Hatched		
Mass fraction in %	g	g	g	kg/m^3				
0 (toluene alone)	5,4	5,7	6,0	0	291	273	5	205
0,5	5,5	6,0	6,3	1,6	274	253	5	184
1,0	5,7	5,9	6,1	3,1	281	268	5	110
2,0	5,1	5,5	5,8	6,0	268	249	4	61
4,0	5,0	5,2	5,5	12,0	274	256	2	15
8,0	5,1	5,3	5,6	27,0	263	250	0	0
Untreated control test specimens		–		–	295	289	5	233
NOTE The toxic values of product x, as tested by egg-laying and larval survival of *Anobium punctatum*, are 12 kg/m^3 to 27 kg/m^3, corresponding to mass fractions of 4,0 % and 8,0 % respectively for the impregnation solutions.								

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)*.

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 may be utilized with sawn and split surfaces only or with muslin mesh of 0,3 mm to 0,6 mm fixed on to one end grain surface using the paste (5.2.2). 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 d and 80 d. 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. Reinfestation 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

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

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