BRITISH STANDARD

BS 5218:1989
EN 21:1988

Wood preservatives — Determination of the toxic values against — *Anobium punctatum* (De Geer) by larval transfer (laboratory method)

This European Standard EN 21 has the status of a British Standard.

UDC 674.048.4:620.193.87

Cooperating organizations

The European Committee for Standardization, under whose supervision this European Standard was prepared, comprises the national standards organizations of the following Western European countries.

Austria	Oesterreichisches Normungsinstitut
Belgium	Institut belge de normalisation
Denmark	Dansk Standardiseringsraad
Finland	Suomen Standardisoimisliito, r.y.
France	Association française de normalisation
Germany	Deutsches Institut für Normung e.V.
Greece	Hellenic Organization for Standardization
Iceland	Icelandic Council for Standardization
Ireland	National Standards Authority of Ireland
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Switzerland	Association suisse de normalisation
United Kingdom	British Standards Institution

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Contents

National foreword

This British Standard has been published under the direction of the Wood Preservation Standards Committee.

BS 5218 was first published in 1975. This revision supersedes the 1975 edition which is withdrawn.

BS 5218 is identical with EN 21:1988 "*Wood preservatives. Determination of the toxic values against Anobium punctatum (De Geer) by larval transfer (Laboratory method)*", published by the European Committee for Standardization (CEN). EN 21:1988 was prepared as a result of discussion in CEN Technical Committee 38 "Methods of test of wood preservatives", in which the UK participated.

National appendix A gives the constitution of the committee responsible for the UK participation in the preparation of this standard.

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Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN title page, pages 2 to 14, an inside back cover and a back cover.

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

EUROPEAN STANDARD

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EUROPÄISCHE NORM

EN 21

November 1988

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Supersedes EN 21, September 1974

Key words: Wood, wood preservatives, pesticides, insecticides, protection against the pest, laboratory tests, determination, effectiveness limit, Anobiidae.

English version

Wood preservatives; Determination of the toxic values against *Anobium punctatum* (De Geer) by larval transfer (laboratory method)

Produits de préservation des bois; Détermination du seuil d'efficacité contre *Anobium punctatum* (De Geer) par transfert de larves (Méthode de laboratoire)

Holzschutzmittel; Bestimmung des Giftwerkes gegenüber *Anobium punctatum* (De Geer) durch Umsetzen von Larven (Laboratoriumsverfahren)

This European Standard was accepted by CEN on 4 December 1987. CEN members are bound to comply with the requirements of the CEN/CENELEC Rules which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN Central Secretariat or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to CEN Central Secretariat has the same status as the official versions.

CEN members are the national standards organizations of Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxemburg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and United Kingdom.

CEN

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

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

Brief history

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.

According to the Common CEN/CENELEC Rules, the following countries are bound to implement this European Standard:

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 describes a laboratory method of test which gives a basis for the assessment of the effectiveness of a wood preservative against *Anobium punctatum*. It allows the determination of the concentration at which the product completely prevents the survival of *Anobium punctatum* larvae in impregnated wood of a susceptible species.

Toxic values determined by larval transfer appear to be somewhat higher than those derived from the test of egg-laying and larval survival[1)] and therefore give a safety margin.

Although an infestation normally starts from egg-laying, a larval transfer test is applicable when considering the situation of treated wood being put in contact, during repair work, with wood that might be infested.

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 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 *Anobium punctatum* (de Geer), introduced into wood which has been treated previously by full impregnation.

2 Field of application

This method is applicable to:

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

— organic water-insoluble 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.

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 with solutions in which the concentrations of preservatives are ranged in a given progression.

Introduction of *Anobium punctatum* larvae into these specimens, and determination of their survival rate. Comparison of the results at the different concentrations and, taking into account the survival in the untreated and solvent or diluent treated control specimens, derivation of the toxic values of the product under test.

4 Test materials

4.1 Biological material

Anobium punctatum (de Geer) larvae

4.1.1 *Origin of larvae.* Obtain the larvae from cultures reared as described in Annex C.

Cut up this wood and extract the larvae in an area separate from the test environments (see **4.3.1** to **4.3.4**), so as to avoid the risk of introducing mites.

[1)] EN 49.

Prepare the storage blocks from Scots pine sapwood, of dimensions 50 mm × 25 mm × 15 mm each with 10 evenly spaced holes (see **7.2**) drilled into one of the wide faces with the drill (see **4.3.10**).

Before inserting the larvae into the storage blocks, keep them overnight in small glass receptacles.

Then sort the larvae into small, medium and large sizes.

The large larvae, with a mass greater than 5 mg, are not used for this test[2)].

Examine all the other larvae under a binocular microscope and destroy those that are damaged or infested with mites, keeping only those that are in perfect condition.

Insert the small and medium larvae into separate sets of storage blocks, placing each larva head first into a drilled hole.

Keep these filled storage blocks holes uppermost, in the test containers (**4.3.11**) closed with filter paper fixed with emulsion adhesive.

Keep the larvae in the storage blocks in the culturing chamber (see **4.3.1**) for not less than two months before using them in a test.

4.1.2 *Provision of larvae*. Carefully cut out the larvae from the storage blocks and examine them under a binocular microscope. Destroy any larvae that show injury or mite infestation, or that do not respond by movement when touched. It is particularly important to avoid including mite-infested larvae (see Annex B).

Keep those larvae that are between 2 mg and 5 mg [2)] in mass and in perfect condition overnight, separate from one another, in clean receptacles in the culturing chamber (see **4.3.1**).

Then re-examine them, and again reject any which do not show normal movements.

4.1.3 *Choice of larvae*. The larvae used in the test shall be between 2 mg and 5 mg in mass, and the 10 larvae placed in each test specimen shall have a mean mass of approximately 3.5 mg [3)].

The numbers of larvae required are indicated in the following table.

Table 1 — Number of larvae and test specimens

Type of test specimen	Concentration of preservative	Number of test specimens		Number of larvae	
		Without radiography available	With radiography available	Without radiography available	With radiography available
Treated specimens	1	10	5	100	50
Treated specimens	2	10	5	100	50
Treated specimens	3	10	5	100	50
Treated specimens	4	10	5	100	50
Treated specimens	5	10	5	100	50
etc.	—	—	—	—	—
Untreated control specimens	0	10	5	100	50
Solvent or diluent treated control specimens (including water)	0	5	5	50	50
Total for five concentrations				650	350

4.2 Products and reagents

4.2.1 *Solvents and diluents*

— for water-soluble preservatives:

distilled or deionized water.

— for preservatives that will be dissolved in or diluted with organic solvents:

a suitable volatile liquid[4)]. This shall not leave a residue in the wood at the end of the post-treatment conditioning period which would have a toxic effect on the insects.

4.2.2 *Xylene*

[2)] Experienced operators can judge the sizes well enough by eye.
[3)] The mass may be judged by eye by comparison with larvae of known mass.
[4)] Do not use benzene as a solvent because it poses a health risk to those conducting the test.

4.3 Apparatus

4.3.1 *Culturing chamber* (incubator or room), with air circulation, and controlled at between 20 °C and 22 °C with a tolerance of ± 1 °C, and at between 80 % r.h. and 85 % r.h. with a tolerance of ± 5 % r.h.

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

4.3.3 *Laboratory work area*, well ventilated, where treatment of the test specimens is carried out[6)].

4.3.4 *Testing chamber* (incubator or room), with conditions identical to those of the culturing chamber (see **4.3.1**).

4.3.5 *Treatment vessels* (beakers), of 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 blocks.

4.3.7 *Protective gloves*

4.3.8 *Vacuum vessels*, fitted with stopcocks.

4.3.9 *Vacuum pump*, fitted with a pressure gauge, and capable of maintaining a pressure of 700 Pa[7)].

4.3.10 *Drill*, provided with a bit capable of drilling cylindrical or conical holes, as specified in **7.2**.

4.3.11 *Test containers* (jars), with *rests*, suitable for holding the test specimens.

4.3.12 *Ordinary laboratory equipment* including two analytical balances.

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

The sample shall be representative of the product under test.

6 Test specimens

6.1 Species of wood

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

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 about 30 % of the whole.

Take the test blocks from trees felled during their dormant period (generally from October to May in temperate climates) and converted within 4 weeks of felling. They may await conversion for longer periods in cold climates, e.g. Scandinavia.

The wood shall have been neither floated nor subjected to any chemical or heat treatment[8)]. It shall be air-dried and shall not have been stored for more than 5 years.

6.3 Provision of test specimens

Cut the specimens from planed strips having a cross section 25 mm × 15 mm and with the wide longitudinal faces orientated tangentially. The individual specimens shall be cut cleanly and shall have sharp edges.

Do not take specimens from the butt or the crown of the tree.

Take the specimens required for a test at random from a stock originating from at least three trees.

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

[6)] It is essential to follow proper safety measures for handling flammable and toxic materials. Avoid excessive exposure to solvents or their vapours.

[7)] 100 Pa = 1 mbar.

[8)] Gentle artificial drying at a temperature below 60 °C is, however, permissible.

6.4 Dimensions of test specimens

The nominal dimensions of each specimen, measured at 12 % moisture content, are 50 mm × 25 mm × 15 mm.

The theoretical volume of each block is 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 specimens required is shown in the table in **4.1.3**.

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.

7 Procedure

7.1 Preparation of test specimens

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

7.1.2 ***Treatment of test specimens***

7.1.2.1 *Preparation of treatment solutions*. Prepare a series of concentrations (by mass) of the preservative in the appropriate solvent or diluent (see **4.2.1**).

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 a first test and a more closely spaced geometric or arithmetic progression for subsequent tests.

All treatment solutions shall be prepared freshly.

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 beakers (see **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 weights (see **4.3.6**) to prevent them from floating later when the liquid is admitted.

Place each beaker in one of the vacuum vessels (see **4.3.8**) and, after reducing the pressure to 700 Pa, hold the specimens at this pressure for 15 min[9)].

After this period, close the valve to the vacuum pump (see **4.3.9**) and open the other valve to allow the solution of preservative to be drawn into the beaker within the vacuum vessel. Keep the specimens covered completely by the solution throughout the remainder of the impregnation process.

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

After this impregnation treatment, remove the test specimens one by one, remove the excess liquid from each by light 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 insecticides, calculate the mass of preservative retained for each test specimen, from the mass of solution absorbed and its concentration[10)].

In the case of water-insoluble 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 volume of wood.

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9) Observe the proper safety measures for vacuum vessels.

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

7.1.3 *Drying and conditioning of the test specimens after treatment*[11]. After impregnation, dry the test specimens for at least 4 weeks in the conditioning chamber (see **4.3.2**).

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

The specimens thus arranged and impregnated with water-soluble preservatives are placed 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 xylene (see **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 treated with water-insoluble preservatives, keep the vessel covered for one week and then open it gradually throughout 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

All the test blocks shall be stored in the testing chamber (see **4.3.4**) for at least 1 week before holes are drilled for the insertion of larvae. With the bit (see **4.3.10**) drill[12] a pattern of 10 holes about 5 mm deep, or 10 conical holes about 7.5 mm deep, in one of the wide faces of each test specimen (see Figure 1).

Determine the diameter of the holes according to the mass of the larvae, from the following table.

Table 2 — Diameter of holes

Mass of larvae	Approximate diameter of hole
mg	mm
2 to 2.9	1.2
3 to 3.9	1.4
4 to 5.0	1.6

Then without delay insert 10 larvae, as specified in **4.1.3**, into each test specimen following the procedure already described for the storage blocks (see **4.1.1**).

Place each specimen, resting on a stand in its own test container (**4.3.11**) with the holes uppermost. Close the containers with filter paper fixed with emulsion adhesive.

7.3 Conditions and duration of test

Keep the jars containing the specimens, with their inserted larvae, in the testing chamber (see **4.3.4**). The total duration of the test, during which examinations and observations are carried out in accordance with **7.4**, is 52 weeks.

7.4 Examination of the test specimens

7.4.1 *Examination without radiography*. 26 weeks after the start of the test, cut up the five control specimens treated with the solvent or the diluent as well as five untreated control specimens and, for each concentration, five treated test specimens, starting with the highest concentration.

If live larvae are present at any concentration, store the remaining five specimens treated at that concentration and the remaining sets of five specimens treated at lower concentrations in the test room for a further 26 weeks and then cut them up.

If no live larvae are found at a particular concentration, cut up immediately the remaining five specimens treated at that concentration. In the event of any live larvae being found in these remaining specimens, those at lower concentrations, not already cut up, shall be kept until the end of the full 52 weeks.

7.4.2 *Examination with radiography*. Using the X-ray apparatus (see **4.3.13**), radiograph all the test specimens after storage for 26 weeks. Store those containing live larvae for a further 26 weeks before the final X-ray examination. If the slightest doubt exists on the interpretation of the radiographs, cut up the specimens to confirm the results of the final X-ray examination.

[11] Drying and conditioning of the specimens depend on the nature of the product 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.

[12] Ensure that the holes have smooth walls so as to avoid damage to the larvae.

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

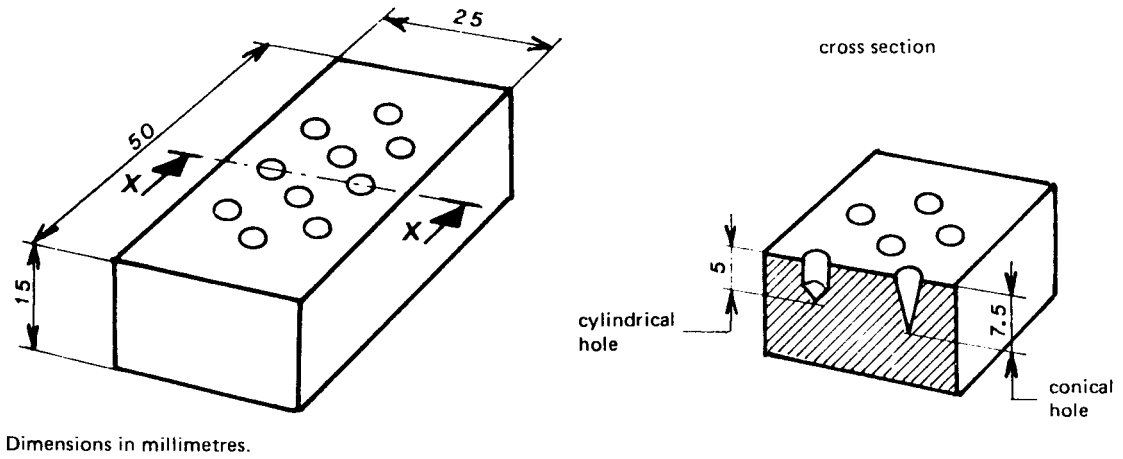

Figure 1 — Pattern and shape of holes

8 Expression of results

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

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

— the lowest concentration at which, at the end of the test, all larvae are dead;

— the next lower concentration in the series, at which live larvae are found.

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

9 Test report

The test report shall show:

a) the number of this European Standard;

b) the name of applicant;

c) the name and type (see clause **2**) of product tested and whether the formulation has been disclosed;

d) the solvent or diluent used;

e) the species of wood used;

f) the concentrations, as percentages by mass, of the preservative tested;

g) the date of impregnation;

h) the 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 actual preservative;

i) the method of drying the specimens;

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

k) the date of insertion of larvae into the specimens;

l) the use or not of radiographic examination;

m) the date of each examination of the specimen;

n) the number of larvae established successfully in each specimen;

o) the results obtained at each examination for both treated and control specimens;

p) the toxic values, expressed in kilograms of preservative per cubic metre of wood, together with the concentrations expressed as percentage by mass of treating solution to which these values correspond;

q) name of organization responsible for the report and the date of issue;

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

s) the following note:

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

The test report shall also list any variation from the described test method, as well as any factors that may have influenced the results.

Annex A Example of a test report

Number of this European Standard	EN 21
Name of the applicant	Company S
Name and type of product	Z water-soluble material, formulation disclosed
Solvent or diluent employed	Water
Wood species	Scots pine (*Pinus sylvestris Linnaeus*)
Concentrations of preservative tested	0.02 %, 0.06 %, 0.18 %, 0.54 %, 1.62 % (*m*/*m*)
Date of impregnation	1984–12–01
Mass of solution absorbed and retention of preservative	See table which follows
Method of drying	As specified for water-soluble preservatives
Ageing procedure previously carried out	None
Date of insertion of larvae	1985–01–12
Radiographic examination	Yes
Dates of examination	1985–07 and 1986–01
Results	See table which follows

This report has been prepared by Laboratory L
Location X 1986–03–15
Mr.Y

NOTE The interpretation and practical conclusions that can be drawn from a 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 of results

Concentrations tested	Absorption				Number of specimens	Number of larvae established	Results		Remarks
	Mass of solution absorbed per specimen			Mean retention of preservative in specimens			Number of surviving larvae after		
	Minimum	Mean of five specimens	Maximum				26 weeks	52 weeks	
% (*m*/*m*)	g	g	g	kg/m^3					
Water alone	13.3	13.5	14.0	0	5	49	42	40	
0.02	13.1	13.4	13.6	0.14	5	49	36	34	
0.06	13.0	13.4	13.8	0.43	5	47	32	27	
0.18	13.3	13.5	14.0	1.30	5	48	18	0	
0.54	13.0	13.2	13.6	3.80	5	46	0	—	All specimens cut up after 26 weeks
1.62	13.2	13.6	14.0	11.74	5	44	0	—	
Untreated control	—	—	—	—	5	49	47	44	

Toxic values of product Z against *Anobium punctatum*, by larval transfer are 0.43 kg/m^3 to 1.30 kg/m^3 corresponding to treating concentrations of 0.06 % (*m*/*m*) and 0.18 % (*m*/*m*) respectively.

Annex B Precautions against mite infestation

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.

In order to avoid the introduction of mites into the storage blocks, quarantine the small and medium larvae obtained from naturally infested wood singly in suitable containers, each containing a circle of absorbent paper.

Before inserting them into the storage blocks, keep the larvae overnight in these containers in the laboratory and not in the conditioning room or in an incubator or in a test chamber. Larvae that are damaged or that are carrying mites can be detected at the end of this quarantine period by their lack of normal movement, and they should be destroyed.

Annex C 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. Smaller stems may be split otherwise to facilitate drying.

C.1.4 *Drying of culture wood*

Dry as rapidly as possible with the aid of fan heaters at a temperature 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*

Dip large filter paper sheets into 10 g/L solution of naphthol in ethyl alcohol. Dry thoroughly.

Place one 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 hours and then remove the treated filter paper with attached beetles. The attached beetles may be used for culturing. The jar should be sterilised and the remaining beetles destroyed.

C.3 Infestation of culture wood

C.3.1 *Culture vessels*

Large glass jars in which the wood samples are stood vertically.

C.3.2 *Preparation of wood*

The wood samples may be utilised with sawn and split surfaces only or muslin mesh of 0.3–0.5 mm may be fixed on to one end grain surface using sodium carboxy-methylcellulose glue (food quality). Alternatively egg laying sites may be provided by artificially roughening or scoring the surface of the wood.

C.3.3 *Introduction of beetles*

Place wood samples vertically in jars with, where appropriate, muslin-coated ends uppermost. Introduce one pair of adult beetles for every 15–20 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 4 weeks in culturing conditions dead adult beetles may be removed.)

C.4 Culturing conditions

C.4.1 ***Normal environment***

Maintain conditions at an optimum of 20–22 °C and 80–85 % relative humidity.

C.4.2 ***Natural pupation induction***

When the majority of larvae exceed 7 mg, generally 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.

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 60–80 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. Reinfestation to achieve a second generation may be possible in the culture wood. Furthermore material can be used more readily as a source of larvae for test work by crumbling or splitting the infested samples.

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*.

Important precautions are:

prohibit introduction of unsterilised 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 hours;

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

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National appendix A

The United Kingdom participation in the preparation of this European Standard was entrusted by the Wood Preservation Standards Committee (WPC/-) to Technical Committee WPC/10, upon which the following bodies were represented:

Association of Consulting Scientists
British Pest Control Association
British Wood Preserving Association
Chemical Industries Association
Department of the Environment — Building Research Establishment
Timber Research and Development Association

National appendix B

Where reference is made to distilled or deionized water, water complying with the requirements of grade 3 of BS 3978 "*Specification for water for laboratory use*" will be suitable.

National appendix C

The British Standard implementing the European Standard referred to in the text is as follows:

European standard	**British Standard** (content identical)
EN 49	BS 5437 *Wood preservatives. Determination of the toxic values against Anobium punctatum* (De Geer) *by egg-laying and larval survival* (laboratory method)

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