SLOVENSKI STANDARD
SIST EN 117:1996

01-marec-1996

Zaščitna sredstva za les - Določanje toksičnih vrednosti za Reticulitermes santonensis de Feytaud - Laboratorijska metoda

Wood preservatives - Determination of toxic values against Reticulitermes santonensis de Feytaud (Laboratory method)

Holzschutzmittel - Bestimmung der Grenze der Wirksamkeit gegenüber Reticulitermes santonensis de Feytaud (Laboratoriumsverfahren)

Produit de préservation des bois - Détermination du seuil d'efficacité contre Reticulitermes santonensis de Feytaud (Méthode de laboratoire)

Ta slovenski standard je istoveten z: **EN 117:1989**

ICS:

71.100.50 Kemikalije za zaščito lesa Wood-protecting chemicals

SIST EN 117:1996 **en**

EUROPEAN STANDARD — EN 117

NORME EUROPEENNE

EUROPAISCHE NORM — December 1989

UDC 674.048.4:620.193.873:615.91.015.13 — Supersedes EN 117:1981

Key words: Wood preservatives, xylophagous animals, termites, analysis methods, determination, effectiveness limit, test equipment, test specimen, test specimen conditioning, tests, testing conditions, visual inspection, test results, animal husbandry

English version

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CEN

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

Central Secretariat: rue Bréderode 2, B-1000 Brussels

Ref. No. EN 117:1989 E

BRIEF HISTORY

This European Standard was drawn up by the Technical Committee CEN/TC 38 "Durability of wood and wood products" the Secretariat of which is held by AFNOR.

At its plenary meeting of October 1988, CEN/TC 38 adopted resolution n° 4 so that Standard EN 117, which is over 5 years old, be submitted to formal vote in view of its confirmation.

In accordance with 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

Introduction

This European Standard describes a laboratory test method which gives a basis for the assessment of the effectiveness of a wood preservative against *Reticulitermes santonensis*. It allows the determination of the concentration at which the product completely prevents attack by this insect of impregnated wood of a susceptible species.

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 can be applied should be taken into account. It is further recommended that results from this should be supplemented by those from other appropriate tests and, above all, by comparison with practical experience.

1. Scope

This European Standard specifies a method for the determination of the toxic values of a wood preservative against *Reticulitermes santonensis* de Feytaud [1].

2. Field of application

This method is applicable to:

water-insoluble chemicals which are being studied as active insecticides, or

organic water-insoluble formulations as supplied or as prepared in the laboratory by dilution of concentrates, or

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 given progression.

Exposure of these test specimens to specified colonies of *Reticulitermes santonensis* and assessment of the attack suffered after exposure under fixed conditions and over a fixed period. Comparison of these results with those obtained with 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

Reticulitermes santonensis de Feytaud workers, soldiers and nymphs.

The termites used shall be taken from colonies; annex B describes an example of a method of culture.

4.2 Products and reagents

4.2.1 *Solvents and diluents.* Distilled or demineralized water or suitable volatile liquids which leave no residue in the wood which would have a toxic effect on the insect at the end of the conditioning period [2].

4.2.2 *Fumigant* (if necessary) xylene, analytical reagent grade.

4.2.3 *Substrate for establishing the colonies.* A choice of:

4.2.3.1 *Fine white quartz sand* consisting of grains of crystallized silica, very pure (99,5 % silica), and free from any organic substances [3].

4.2.3.2 *An hydrated, laminar, aluminium-iron-magnesium silicate* exfoliated to give particles of 1 mm to 3 mm with an apparent density of 80 kg/m^3 to 90 kg/m^3. Particles of less than 1 mm shall be eliminated by sieving prior to use, to ensure the absence of free water and prevent any significant agglomeration of the particles.

4.2.3.3 *Rigid polyurethane foam* with open pores of mass per unit of volume of 14 kg/m^3 and compressive strength [4]. 0,02 N/mm^2 to 0,03 N/mm^2. It is advisable to cut the foam into 15 mm thick sheets.

4.2.4 *Distilled or demineralized water* to moisten the substrate.

4.3 Apparatus

4.3.1 *Culturing chamber* (incubator or room) with air circulation, controlled at between 26 °C and 28 °C, with a tolerance of ± 1 °C, and at a minimum of 75 % r.h.

4.3.2 *Conditioning chamber*, well ventilated and controlled at 20 ± 2 °C and 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*, protected from light, ventilated and controlled at a temperature between 26 °C and 28 °C with a tolerance of ± 1 °C and at a minimum of 75 % r.h.

4.3.5 *Treatment vessels* of material that does not react with the preservative under test, for example, of glass for organic products and of plastic 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 7 mbar [7] and *manometer.*

4.3.10 *Instruments* for handling the termites (e.g. forceps).

4.3.11 *Test containers* of glass or transparent plastic, inert to the product under test and having the following dimensions:

1) This working method can be applied not only to other species of *Reticulitermes* but also to other species of the family of the *Rhinotermitidae*, adapting the conditions of temperature and humidity where necessary to the specific requirements of the species concerned.

2) Do not use benzene as a solvent because it poses a health risk to those conducting the test.

3) In France, Fontainebleau sand, of which more than 97 % of the particles are between 75 µm and 300 µm in size, provides these features.

4) Determined in accordance with ISO 844.

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

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

7) 1 mbar = 10^{-1} kPa.

base area	35 cm^2 to 60 cm^2
minimum height	8,5 cm
volume	500 cm^3 to 1000 cm^3

They shall be closed with a perforated cover to permit ventilation.

4.3.12 *Glass rings* 20 mm high, 20 mm in diameter and with a wall thickness of at least 1 mm.

4.3.13 *Ordinary laboratory equipment* including an analytical balance.

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 using other species are permitted but, if so, this shall be stated in the test report.

6.2 **Quality of wood.** Use only sapwood, straight-grained, without knots and with a low resin content.

Average growth rate: 2,5 to 8 annular rings per centimetre.

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

The wood shall have been neither floated nor stored in water nor dried at a temperature higher than 60 °C nor subjected to any chemical treatment.

6.3 **Provision of test specimen.** Cut the test specimens from planed strips having a cross-section of 25 mm × 15 mm, in which the annual growth rings shall form an angle of 45 ± 15 ° with the edge of the longitudinal face; the transverse sections of the test specimens shall be clean cut and have sharp edges.

The specimens required for one test shall be taken at random from a batch of specimens originating from more than one tree.

6.4 **Dimensions of test specimens.** The nominal dimensions of each specimen, measured at 12 % moisture content, shall be as follows:

50 mm × 25 mm × 15 mm

The theoretical volume of each specimen is 18,75 cm^3, but the size of each specimen shall be carefully checked so that the exact volume is known.

6.5 **Number and sub-division of test specimens.** The test specimens shall be divided as follows:

treated test specimens: these are the specimens which are impregnated and subjected to attack by *Reticulitermes santonensis;* use at least 3 test specimens for each concentration of the product;

untreated control test specimens for checking the virulence of the termites taken for the test: these non-impregnated specimens are subjected to attack by *Reticulitermes santonensis*; they are 3 in number;

solvent or diluent treated control test specimens subjected to attack by *Reticulitermes santonensis*; they are 3 in number.

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 (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 (4.2.1).

Prepare a series of at least 5 concentrations, by mass, distributed about the expected toxic values. Include, by way of control, pure solvent or diluent to allow for the preparation of solvent or diluent, treated control test specimens. 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.

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

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

For each concentration weigh each specimen to the nearest 0,05 g, and then stack the specimens in one of the treatment vessels (4.3.5) so that as much of their surface as possible is exposed (e.g. by piling them crosswise). Ballast the stack of specimens with weights (4.3.6) to prevent them from floating later when the liquid is admitted.

Place each treatment vessel in one of the vacuum vessels (4.3.8), and after reducing the pressure to 7 mbar, hold the specimens at this pressure for 15 min [8]. After this period, close the valve to the vacuum pump (4.3.9) and open the other valve to allow the solution of preservative to be drawn into the treatment vessel within the vacuum vessel. Keep the specimens covered completely by the solution.

Next bring the vacuum vessel back to atmospheric pressure and remove the treatment vessel 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 their surfaces by light blotting with absorbent 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 [9].

In the case of water-insoluble formulations, the amount retained is expressed for each block in a corresponding mass and volume of the product in the ready to use condition, in the specified dilution, for concentrates.

Calculate the mass of preservative retained per unit volume of wood.

8) The proper safety measures for vacuum vessels must be observed.

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

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

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

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

In the same way, place the test specimens impregnated with water-insoluble preservatives in a closed vessel for 1 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

7.2.1 *Collecting and selecting the termites.* Pick up the insects individually, using forceps with parallel ends (**4.3.10**) taking hold of each insect by the abdomen and taking care not to press too hard. Make up groups of 250 workers, rejecting insects which are moulting (indicated by the dull white colour of the abdomen) and those which appear to be wounded or remain motionless. To each group made up in this way add a number of soldiers corresponding to the proportion found in the colony from which the workers were taken: add a corresponding proportion of nymphs (1 % to 5 %).

Each group is intended to form a colony; the number of groups is determined by the number of colonies required for the test.

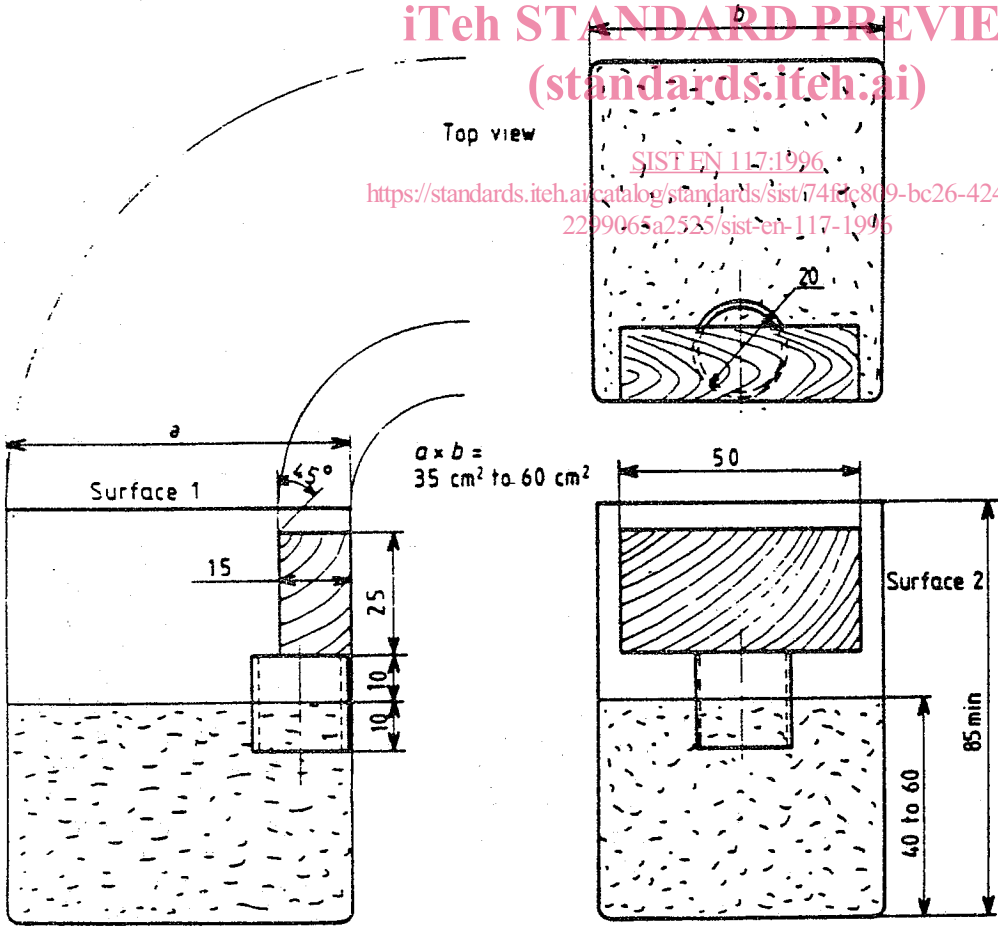
The number of colonies to be prepared as indicated above is equal to the number of test specimens to be subjected to attack by the termites. It is, however, recommended that a few additional ones are prepared so as to ensure that a sufficient number of colonies in perfect condition is available to receive the test specimens.

If the required number of termites is more than that in a single culture, the control series and test series shall contain the same number of groups from each colony. Termites from different colonies shall not be mixed in a single group.

7.2.2 *Constitution of the colonies* (see figure 1)

7.2.1.1 *With sand.* In each container (**4.3.11**), form a layer of remoistened, non-compacted sand (**4.2.3.1**) 4 cm to 6 cm thick. To do this, first introduce the distilled or demineralized water (**4.2.4**) and then the sand, in the proportions of 1 volume of water to 4 volumes of sand.

At the (approximate) centre of the container, place some wood from the original culture (approximately 0,5 g) and push it down to the bottom of the container.



Dimensions in mm.

Figure 1. Example showing exposure of the test specimens to the colonies

10) 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.

In each container, place a glass ring (4.3.12) against one of the vertical walls of the container and in the middle of this wall, place it in the substrate so that it penetrates below the surface by about 10 mm. Distribute a group of termites made up as indicated in 7.2.1 in each container, spreading them carefully over the entire substrate.

Close each container by means of its lid and place it in the testing chamber (4.3.4).

7.2.2.2 *With aluminium-iron-magnesium silicate.* Prepare enough aluminium-iron-magnesium silicate (4.2.3.2) with a moisture content of about 300 % by mass (for example 300 ml of water to 100 g of substrate) either in bulk or for individual containers. It is essential that there is no free water in the substrate. This quantity is enough to provide a layer 4 cm to 6 cm deep in the test containers, without compacting.

Place some wood from the original culture (approximately 0,5 g) in the centre at the bottom of the container (4.3.11).

In each container, place a glass ring (4.3.12) against one of the vertical walls of the container and in the middle of this wall; place it in the substrate so that it penetrates below the surface by about 10 mm. Distribute a group of termites made up as indicated in 7.2.1 in each container, spreading them carefully over the entire substrate.

Close each container by means of its lid and place it in the testing chamber (4.3.4).

7.2.2.3 *With polyurethane foam.* Place approximately 240 cm^3 of polyurethane foam in each test vessel (4.3.11) by cutting 3 or 4 pieces from the sheets of polyurethane foam (4.2.3.3). If the mouths of the vessels are smaller than the cross-section of these pieces, fragments of polyurethane foam can be used. If so, a sheet of polyurethane foam 13 cm × 13 cm (= 240 cm^3) should be cut or broken into small pieces.

In each test vessel press a glass ring (4.3.12) to a depth of approximately 10 mm in the polyurethane foam and against the walls of the vessel. Place some wood from the original culture (approximately 0,5 g) in the polyurethane foam approximately in the centre at the bottom of the container.

Moisten the polyurethane foam with approximately 50 ml of water (4.2.4) and put in the termites.

Close the vessels and put them in the testing chamber (4.3.4).

7.2.3 *Exposure.* Over a period of 2 to 4 days after setting up the colonies, keep them under observation and confirm that the termites have settled in properly, which is indicated by their being distributed throughout the substrate and by their active movement, which is easily visible through, in particular, the bottom of the container and the lower part of the side walls.

Remove test containers in which the termites cannot be regarded as having established themselves properly, and replace them by test containers housing well-established populations.

Write on each container the number of the single test specimen to be placed in it. Open the container and carefully place the specimen on the glass ring, the side resting on the ring being one of the narrow longitudinal sides, with a wide longitudinal side in contact with the wall of the container (see figure 1). Close the containers.

7.3 Conditions and duration of the test. Place the containers (7.2) in the testing chamber (4.3.4) and leave them there for 8 weeks.

11) Moist sand has a dark colour whereas dry sand is light in colour.

It is recommended that, throughout the duration of the test, each colony be inspected daily, the results of the inspection recorded on a special card and any necessary action taken to maintain the colonies in the best possible condition without disturbing their activity.

These inspections cover the following points in particular:

the presence, location and activity of the termites (tunnelling in the substrate along the visible walls, construction of shafts and movement of the insects);

approach to and enveloping of the test specimen, writing down, should this happen, the date of first contact and subsequent apparent activity of the insects around the test specimen.

Action may be taken:

if the termites are escaping

to maintain the moisture content.

Changes in moisture content of the substrate in which the colonies are established depend on its nature; any action to be taken to maintain an optimum level of moisture content, therefore, varies according to the substrate used.

7.3.1 *Sand.* The sand substrate has to be remoistened periodically. The change in colour due to drying indicates when it is necessary to remoisten [11]. It is better to maintain the moisture content by frequent addition of small quantities of water (4.2.4) with the aid of a pipette rather than by a single large addition which might result in serious damage to the colony, particularly by flooding.

7.3.2 *Aluminium-iron-magnesium silicate.* Add the water (4.2.4) necessary to maintain the appropriate moisture content; changes in the appearance and cohesion of the particles of this substrate indicate the need for re-moistening. A check may also be made by weighing.

7.3.3 *Polyurethane foam.* The requirements for sand also apply to polyurethane foam.

7.4 Examination of the test specimens and colonies. At the end of the test, remove the test specimens from the test containers and carefully free them from all particles of substrate and other substances adhering to their surface. Carry out a visual examination as described below.

In addition, count as carefully as possible the total number of termites still living in each test container and determine the survival level of the workers.

Record, where appropriate, the presence of living soldiers and/or nymphs.

7.4.1 *Visual examination.* Carry out a visual examination of each test specimen and classify any evidence of attack by its locality, its extent and its depth. Express the results of this examination in accordance with the following schedule:

0 – no attack

1 – attempted attack: superficial gnawing or nibbling of insufficient depth to be measured, *or* attack to a depth of 0,5 mm provided this is restricted to 3 areas each less than 3 mm in diameter.

2 – slight attack: superficial attack (less than 1 mm) limited in extent to not more than $^1/_{10}$ of the surface area of the test specimen *or* a single tunnelling of a depth less than 3 mm.

3 – average attack: superficial attack (< 1 mm) over more than $^1/_{10}$ of the surface area of the test speci-