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# Durability of wood and wood-based products — Quantitative determination of pentachlorophenol in wood — Gas chromatographic method

ICS 71.100.50; 79.040

British Standards

# National foreword

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The UK participation in its preparation was entrusted by Technical Committee B/515, Wood preservation, to Subcommittee B/515/2, Specifications and chemical testing of wood preservatives, which has the responsibility to:

- aid enquirers to understand the text;
- present to the responsible European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;
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**Cross-references**

The British Standards which implement international or European publications referred to in this document may be found in the *BSI Catalogue* under the section entitled "International Standards Correspondence Index", or by using the "Search" facility of the *BSI Electronic Catalogue* or of British Standards Online.

**Summary of pages**

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TECHNICAL REPORT
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**CEN/TR 14823**

September 2003

ICS 71.100.50; 79.040

English version

# Durability of wood and wood-based products – Quantitative determination of pentachlorophenol in wood – Gas chromatographic method

Durabilité du bois et des matériaux dérivés du bois – Analyse quantitative du pentachlorophénol dans le bois – Méthode par chromatographie en phase gazeuse

Dauerhaftigkeit von Holz und Holzprodukten – Quantitative Bestimmung von Pentachlorphenol in Holz – Gaschromatographische Verfahren

This Technical Report was approved by CEN on 27 July 2003. It has been drawn up by the Technical Committee CEN/TC 38.

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# Contents

Page

# Foreword

This document (CEN/TR 14823:2003) 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 status of this document as Technical Report has been chosen because this method described is an example of a laboratory method validated by a ring test. It is recognized that, for example, different techniques of extraction might be employed by the analyst without loss of analytical performance and quality. However, when using different techniques the comparability to the method described in this Technical Report should be demonstrated, e.g. by using a certified reference material (CRM).

# Introduction

At present, no standardised method for the analysis of pentachlorophenol (PCP) in wood is recognized in Europe. Only a few national standards are available world wide, e g BS 5666, Part 6 and AWPA Standard A-5 and these methods are designed for the analysis of timber treated with levels of PCP that are necessary to prevent degradation of the treated timber by fungi.

This Technical Report has been issued in order to facilitate the analysis of PCP-treated wood and panels and in particular low levels of PCP that can be present in packaging timbers and pallets. Low levels of PCP and other chlorophenols, and the anisoles derived from them, can cause taints in foodstuffs. PCP can be present as residues from old anti-sapstain treatment or some timber treated against decay could inadvertently have been incorporated into the panel product.

# 1 Scope

This Technical Report specifies a laboratory method of determining the pentachlorophenol content of wood. The method is applicable to all types of PCP- treated wood and wood-based materials as well as for the analysis of waste timber with respect to its PCP content.

The method has a quantification limit corresponding to 100 µg PCP per kilogram of wood material expressed as dry matter. The method described has a measurement range up to PCP contents of 25 mg/kg of dry matter. These figures refer to the given example (where an aliquot of 1 ml of the extract is used for acetylation, see 8.4).

NOTE 1 If lower quantification limits are required, a higher volume of extract aliquot can be used for derivatisation.

NOTE 2 This method could have some modifications with some wood species as hardwoods.

# 2 References

EN 212, *Wood preservatives - General guidance on sampling and preparation for analysis of wood preservatives and treated timber.*

EN 322, *Wood-based panels – Determination of moisture content.*

EN ISO 3696, *Water for analytical laboratory use - Specification and test methods (ISO 3696:1987).*

ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.*

ISO 11465, *Soil quality - Determination of dry matter and water content on a mass basis - Gravimetric method.*

# 3 Safety precautions

Persons using this method should be familiar with normal analytical laboratory procedures and practice.

This method does not purport to address all the safety problems, if any, associated with its use.

It is the responsibility of the user to establish safety and health practices and to ensure compliance with any European or national regulatory conditions.

# 4 Principle

Pentachlorophenol is extracted from the wood material using methanol. The extracted pentachlorophenol is transformed to pentachlorophenol acetate by derivatisation of an aliquot of the extract in aqueous potassium carbonate solution with acetic anhydride. The acetate derivative formed is extracted from this aqueous solution with n-hexane and analysed by gas chromatography with electron capture detection.

# 5 Reagents

During the analysis, unless otherwise specified, use only reagents of recognized analytical grade which have been checked in advance as to not interfere with the analytical results, and water complying with grade 3 as defined in EN ISO 3696.

**5.1 Acetic anhydride** ($C_4H_6O_3$).

**5.2 Methanol** ($CH_3OH$).

NOTE Other solvents can be used instead of methanol, some extraction difficulties can occur with some wood species (e.g. hardwoods). It is recommended to cross check the extraction efficiency of any other solvent or solvent mixture with that of methanol.

**5.3 n-Hexane** ($C_6H_{14}$).

**5.4 Potassium carbonate**, solution, c(K $CO_3$) = 0,1 mol/l.

**5.5 Sodium sulfate**, anhydrous ($Na_2SO_4$).

**5.6 Pentachlorophenol** (PCP, ($C_6Cl_5OH$)) of certified purity (> 99%), e.g. IPO 560[1].

**5.7 2,4,6-tribromophenol** (TBP, ($C_6H_2Br_3OH$)) of certified purity (> 98%) as an internal standard.

**5.8 Purified sand**, (ignited at 900 °C after acid treatment)

NOTE An untreated wood sample (of the same wood species as the sample for analysis) known to be free of pentachlorophenol can also be used instead of purified sand but before use it should be checked for interference with the analytical method.

## 6 Apparatus

Ordinary laboratory apparatus and the following.

NOTE Glassware should be thoroughly cleaned prior to use.

**6.1 Analytical balance**, accurate to 0,01 mg.

**6.2 Ultra-sonic bath** equipped with a thermostat capable of controlling and maintaining a temperature of 40 °C.

**6.3 Disposable Pasteur pipettes** made of glass, 2,0 ml capacity.

**6.4 Separating funnel**, 150 ml capacity.

**6.5 Volumetric pipettes**, 1,0 ml and 10 ml or 20 ml capacity.

**6.6 Microlitre syringes**, 25 µl, 100 µl and 500 µl capacity.

**6.7 Volumetric flasks**, 10 ml, 25 ml and 50 ml capacity.

**6.8 Conical flasks** 100 ml capacity, with screw caps provided with an insert of polytetrafluoroethylene (PTFE) or alternatively with standard ground stoppers provided with PTFE standard ground sockets and conical joint clips.

**6.9 Gas chromatograph** equipped with a splitless/split or a non-discriminating injection system and an electron capture detector (ECD)

**6.10 Separation column**. One capillary column, or preferably two, with stationary phase of different polarity e.g. DB 1, DB 5, DB 17[2]; length: 25 m to 30 m; internal diameter: 0,2 mm to 0,32 mm; film thickness: 0,25 µm to 0,33 µm.

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[1] IPO 560 is an example of a suitable product available commercially, supplied by Promochem. This information is given for the convenience of users of this CEN Technical Report and does not constitute and endorsement by CEN of this product.

**6.11 Pre-column** coated with deactivated silica.

## 7 Preparation of the test sample

Collect at least 10 g of the sample material taken according to e.g. EN 212. This sample material is preferably ground under mild conditions (e.g. cryogenic grinding) to chips with an approximate particle size of 0,5 mm to 1,0 mm diameter.

NOTE 1 Wood shavings can also be made for analysis (especially if other compounds e.g. trichlorophenols or trichloroanisols have to be analysed at the same time).

Homogenise the ground sample material to obtain a representative sample, and store it in a brown glass vessel with screw caps with a PTFE insert. This is the test sample.

NOTE 2 If possible, the sample for analysis should be prepared from a minimum of five independent pieces or sections representatively taken from the quantity of wood material to be analysed.

## 8 Procedure

### 8.1 General

It is recommended to carry out at least two parallel analyses. If results differ by more than 10 % an additional analysis shall be carried out.

### 8.2 Standard solutions

#### 8.2.1 Preparation of stock solutions (calibrant and internal standard)

Prepare the stock solutions by weighing about 12,5 mg of PCP (5.6) and TBP (5.7) separately to the nearest of 0,01 mg and dissolve each in 25 ml methanol (5.2).

NOTE These stock solutions with nominal concentrations of 0,5 mg/ml can be stored in the dark at –18 °C for three months.

#### 8.2.2 Preparation of working solutions

Transfer by pipette (6.5) 1,00 ml of stock PCP solution (8.2.1) to a 10 ml one-mark volumetric flask and make up to the mark with methanol (5.2) to give a solution having a nominal concentration of 50 µg/ml PCP. Repeat with the TBP stock solution to give a solution having a nominal concentration of 50 µg/ml TBP.

#### 8.2.3 Preparation of calibration standards

To a series of five conical flasks (6.8) containing (5,0 ± 0,1) g of purified sand (5.8), transfer 25 µl, 50 µl, 100 µl, 250 µl and 500 µl of PCP working solution (8.2.2). To each of the five flasks and another flask containing sand but no added PCP, add 250 µl of TBP working solution (8.2.2) to give a series of standards containing 0,0 µg, 1,25 µg, 2,5 µg, 5 µg, 12,5 µg and 25 µg of PCP with 12,5 µg TBP as internal standard in each.

NOTE This procedure assumes that 1 ml of the methanol extract will be taken for preparation of the calibration solutions. If a greater volume of extract is to be taken, for example 5 ml, the above standards should be prepared from working solutions each with a nominal concentration of 10 µg/ml .

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2) DB1, DB5, DB17 are examples of a suitable product available commercially, supplied by J & W. This information is given for the convenience of users of this CEN Technical Report and does not constitute an endorsement by CEN of these products.

## 8.3 Preparation of the calibration solutions

### 8.3.1 Extraction

To each flask add 50 ml of methanol and swirl to ensure that all the wood is wetted. To quantify possible evaporation losses of the solvent during the extraction procedure determine the gross masses of all flasks (dry and clean outside) at ambient temperature before and after sonication. Place the flasks in an ultra-sonic bath for 1 h at 40 °C. Perform all the following steps under gravimetric control using an analytical balance. After weighing, allow the wood to settle and, by means of a series (one per flask) of 10 ml or 20 ml volumetric pipettes (6.5) and/or Pasteur pipettes (6.3), transfer as much as possible of the clear supernatant solution to a series of 50 ml one-mark volumetric flasks (6.7). Make the contents of each flask up to the mark with methanol.

**Table 1 — Example of calibration solutions**

| Calibration solution | PCP concentration (ng/ml) | TBP concentration (ng/ml) |
|---|---|---|
| 1 | 1,0 | 10,0 |
| 2 | 2,0 | 10,0 |
| 3 | 4,0 | 10,0 |
| 4 | 10,0 | 10,0 |
| 5 | 20,0 | 10,0 |

The maximum storage time of these solutions is one month at approximately 5 °C, e.g. in a refrigerator.

NOTE The concentration of the calibration solutions as well as the volume of the aliquot of the methanolic extract taken for acetylation can be varied to cover the expected level of PCP contamination of the wood sample up to at least 100 mg/kg.

### 8.3.2 Acetylation

Add 30 ± 1 ml of the potassium carbonate solution (5.4) to a series of six separating funnels (6.4). Using a pipette (6.5), transfer 1 ml of the extract solution (8.3.1) to its allotted separating funnel, shake to mix the contents of the separating funnel thoroughly. To each separating funnel, add (2 ± 0,2) ml of the acetic anhydride (5.1) and shake the mixture for 2 min. Extract the acetylated pentachlorophenol and tribromophenol with 10 ml of the n-hexane (5.3) by shaking the mixture for 10 min. By means of a clean Pasteur pipette, transfer the upper (organic) layer into a 25 ml one-mark volumetric flask (6.7) via filter funnel fitted with a filter paper containing anhydrous sodium sulfate (5.5). Repeat the extraction with another 10 ml quantity of n-hexane and wash the filter paper and sodium sulfate with small quantities of n-hexane. Make up to the mark with n-hexane.

## 8.4 Determination

### 8.4.1 Extraction

In accordance with the expected PCP content, weigh, to the nearest 0,1 mg, 1 g to 5 g of the test sample (clause 7) and transfer to a conical flask (6.8). Add (under gravimetric control) 250 µl (see note 1) of the TBP working solution (8.2.2) to the test sample, close the flask with the screw cap and leave for 30 min. Perform all the following steps under gravimetric control using an analytical balance.

Add 50 ml of methanol to the flask and swirl to ensure that all the wood is wetted. Place the flask in an ultra-sonic bath (6.2) for 1 h at 40°C. After removing the flask from the bath dry its outer surface by a clean tissue and determine its gross mass at ambient temperature to quantify possible evaporation losses during the extraction procedure. Allow the wood to settle and, by means of a 20 ml volumetric pipette (6.5) and/or a Pasteur pipette (6.3), transfer as much as possible of the clear supernatant solution to a 50 ml one-mark volumetric flask (6.7).

Make the content of the flask up to the mark with methanol. Under gravimetric control a well defined and large part of the methanolic extract can be taken for analysis.

NOTE 1 This assumes that 1 ml of the extract will be taken for analysis. If a greater volume, for example, 5 ml is to be taken, 50 µl of the 50 µg/ml working solution should be taken (compare to 8.2.3).

NOTE 2 Other extraction techniques, like Soxhlet extraction, microwave assisted extraction and pressurised fluid extraction can be suitable. However, when using other extraction techniques the comparability to the method described in this Technical report should be demonstrated, e.g. by using a certified reference material (CRM).

### 8.4.2 Acetylation

Follow the procedure given in 8.3.2

NOTE A higher volume of the aliquot of the extract can be used for derivatisation if a quantification limit below a content of 100 µg PCP per kilogram of wood dry matter is required (see clause 1).

### 8.4.3 Gas chromatographic conditions

Guidance on the gas chromatographic conditions is given in the following:

| | |
|---|---|
| Carrier gas: | helium (minimum purity (see 5.6) or 99,9996 %); |
| Gas flow: | 20 cm/s to 30 cm/s; |
| Injection mode: | preferably splitless/split, 1 µl (any other non-discriminating injection technique is applicable); |
| Injector temperature: | 250 °C; |
| Detector temperature: | 350 °C; |
| Oven temperature programme: | 50 °C (1 min); 50 °C to 160 °C at 20 °C/min; 160 °C to 310 °C at 8 °C/min and 310 °C for 5 min. |

Please consult the user instructions of the manufacturer for optimal GC conditions. Please check if the chromatograph is equipped with a gas filter as it is needed for the trace analysis of volatile environmental pollutants.

NOTE 1 The oven temperature programme can be different if polychlorophenols and polychloroanisols have to be analysed at the same time.

NOTE 2 By use of on column injection techniques interference can occur caused by substances extracted from the wood matrix. In such cases it is recommended to perform an additional clean-up procedure (e.g. by silica gel) to improve the chromatographic performance.

### 8.4.4 Calibration

The calibration is carried out using the internal standard method.

The linear calibration function is based on five calibration points as described in (8.3) and the determination of PCP based on this function is described in the following. This calibration function includes the whole analytical procedure. It is established by plotting the ratio of the peak area of the acetylated PCP standard to that of the acetylated TBP standard versus the relating ratio of concentrations according to the following equation:

$$\frac{A_{\mathrm{CPC}}}{A_{\mathrm{TBP}}} = s \times \frac{C_{\mathrm{PCP}}}{C_{\mathrm{TBP}}} + \mathrm{b}$$

where:

| | |
|---|---|
| $A_{\mathrm{PCP}}$ | is the measurement parameter of acetylated PCP standard (e.g. peak area); |
| $A_{\mathrm{TBP}}$ | is the measurement parameter of acetylated TBP standard (e.g. peak area); |
| $s$ | is the slope of the linear calibration function; |
| $c_{\mathrm{PCP}}$ | is the mass concentration of acetylated PCP in the related calibration solution in nanograms per millilitre; |
| $c_{\mathrm{TBP}}$ | is the mass concentration of acetylated TBP in the related calibration solution in nanograms per millilitre; |
| $\mathrm{b}$ | is the intercept of the linear calibration function on the axis of ordinates ; |

### 8.4.5 Validity check of the calibration function

The validity of the calibration function shall be checked within each batch of samples by analysis of calibration solutions as unknown analytical samples (e.g. two for every ten samples). If the linear function calculated from these control measurements falls within the 95% confidence interval of the actual calibration function this function is assumed to be valid. If not, a new calibration function shall be established.

NOTE A Quality Control (QC) should be added to validate calibration. The QC procedure can be a set of calibration solutions from another supplier.

## 9 Calculation and expression of results

The PCP content *w*, in milligrams per kilogram of the test sample is calculated from this multiple point linear calibration function (8.3) using the following equation:

$$w = \frac{\left(A'_{\mathrm{PCP}} / A_{\mathrm{TBP}}\right) - b}{s \times m \times 1000} \times C'_{\mathrm{TBP}} \times f \times V$$

where:

| | |
|---|---|
| $C'_{\mathrm{TBP}}$ | is the mass concentration of acetylated TBP in the final n-hexane solution in nanograms per millilitre; |
| $m$ | is the dry mass of the test sample, in grams, determined from the mass weighed for extraction according to relevant standards (e.g. ISO 11465, EN 322); |
| $A'_{\mathrm{TBP}}$ | is the measurement parameter of acetylated TBP in the final n-hexane solution (e.g. peak area); |
| $A'_{\mathrm{PCP}}$ | is the measurement parameter of acetylated PCP in the final n-hexane solution (e.g. peak area); |
| $f$ | is the dilution factor, ratio of extract volume to volume of aliquot for derivatisation, e.g. 50 ml / 1 ml = 50; |
| $V$ | is the volume, in millilitres, of final n-hexane solution for GC analysis, e.g. 25 ml. |

The PCP content of the test sample is given in milligrams per kilogram of dry matter as the mean of independent determinations together with its measurement uncertainty.

## 10 Quality assurance

Within each series of test samples to be analysed, a blank measurement of the whole analytical procedure and the recovery measurement of the acetylated TBP internal standard shall be carried out with purified sand (5.8) or test samples prepared from the same wood species as to be analysed known to be free of pentachlorophenol.

NOTE 1 For laboratories with less experience in this method it is advisable to check the recovery of the acetylated TBP internal standard while analysing the wood material by using an additional internal standard such as PCB 52 (2, 2’, 5, 5’-tetrachlorobiphenyl). It can be added to the final solution which is filled to the mark with n-hexane. In this way the absence of any interference in the position of the internal standard in the chromatogram can also be checked.

NOTE 2 Results achieved with single column GC-ECD are strongly recommended to be confirmed by additional GC-ECD analysis using another column of different polarity or alternatively by GC-MS analysis.

When establishing this method in the laboratory it is recommended that a certified reference material (CRM), e.g. BCR 683, a ground wood sample with a certified content of pentachlorophenol is used for verification.

NOTE 3 The CRM BCR 683 will be available from Institute for Reference Materials and Measurements(IRMM).Retieseweg, B-2440 Geel, Belgium (www.irmm.jrc.be/mrm.html).

## 11 Precision

**11.1** Calibration curve (curve linear fit): $r^2 > 0{,}985$.

**11.2** This method was validated in a laboratory intercomparison with 21 participating laboratories for three test samples at different levels of PCP-contamination. The performance characteristics of the intercomparison were evaluated according to ISO 5725-2 and the repeatability and the reproducibility were determined. A summary of the intercomparison data is given in annex A.

## 12 Test report

The test report shall contain the following information:

a) a reference to this Technical Report;

b) the date of sampling;

c) the sampling procedure followed e.g. EN 212;

d) the date of testing.

# Annex A

# Ring test results

The summary of the results of an intercomparison study evaluated according to ISO 5725-2 with 21 participating laboratories on three test samples with different levels of PCP contamination is given in Table A.1[3] :

**Table A.1 — Ring test results**

| | PCP content mg/kg | $CV_r$ % | $CV_R$ % | $n$ |
|---|---|---|---|---|
| Sample 1 | 18,37 | 10,29 | 24,39 | 21 |
| Sample 2 | 4,46 | 11,23 | 18,63 | 21 |
| Sample 3 | 0,60 | 17,79 | 40,61 | 21 |

The given PCP content is a consensus value (mean of 21 laboratory means)

$CV_r$ is the repeatability coefficient of variation (relative standard deviation related to the mean of means) according to the definition of ISO 5725-1 ;

$CV_R$ is the reproducibility coefficient of variation (relative standard deviation related to the mean of means) according to the definition of ISO 5725-1 ;

$n$ is the number of participating laboratories.

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[3] This details of these ring test results are given in the publication : R. Becker: Methodenvergleich zur Bestimmung von Pentachlorphenol in Holz (Method intercomparison for the determination of pentachlorophenol (PCP) in wood), Final report in German (June 2000), BAM – Federal Institute for Materials Research and Testing, D – 12200 Berlin, Germany German Federal Environmental Agency research grant No. 29931303 (13 January 2000)

# Bibliography

[1] BS 5666 Part 6 (1983) *Methods of analysis of wood preservatives and treated timber, Part 6, Quantitative analysis of preservative solutions and treated timber containing pentachlorophenol, pentachlorophenyl laurate, $\gamma$-hexachlorocyclohexane and dieldrin*, British Standards Institute, London.

[2] AWPA Standard A-5 *Standard methods for analysis of oil-borne preservatives* (1999) American Wood Preservers' Association, Book of Standards 1999.

[3] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results - Part 1: General principles and definitions.*

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