Paper and board - Determination of the cytotoxicity of aqueous extracts using a metabolically competent hepatoma cell line (HepG2)

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ICS 85.060

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16418

April 2014

ICS 85.060

English Version

Paper and board - Determination of the cytotoxicity of aqueous extracts using a metabolically competent hepatoma cell line (HepG2)

Papier et carton - Détermination de l'effet cytotoxique d'extraits aqueux en utilisant une lignée cellulaire d'hépatome possédant des enzymes du métabolisme (cellules HepG2)

Papier und Pappe - Bestimmung der Zytotoxizität von wässrigen Extrakten unter Verwendung einer metabolisch kompetenten Hepatom-Zelllinie (HepG2)

This European Standard was approved by CEN on 8 February 2014.

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Foreword

This document (EN 16418:2014) has been prepared by Technical Committee CEN/TC 172 “Pulp, paper and board”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by October 2014 and conflicting national standards shall be withdrawn at the latest by October 2014.

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1 Scope

This European Standard specifies a test method for the laboratory assessment of the potential cytotoxic effect of paper and board intended to come into contact with foodstuffs using specifically the HepG2 cell line.

Compared to the EN 15845[1], HepG2 cells are more representative of a human oral exposure to xenobiotics, due to the presence in the cells of phase I, II and III enzymes of the metabolism.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 645, *Paper and board intended to come into contact with foodstuffs - Preparation of a cold water extract*

EN 647, *Paper and board intended to come into contact with foodstuffs - Preparation of a hot water extract*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
reference water
tap water which undergoes the following treatment sequence: pre-filtration, reverse osmosis, filtering through activated carbon powder (adsorption) then through cartridges of mixed-bed ion exchange microresins (demineralisation), ultrafiltration (molecular weight cut-off at 10 kDa), and UV photo-oxidation

Note 1 to entry: Alternatively, any other purification regime, which produces HPLC-quality water (resistance > 18,0 MΩ/cm, total organic carbon < 3 ppb, no microorganism) or waters of grade 1 or 2 according to EN ISO 3696, can be used.

3.2
water extract
reference water that has been exposed to contact with paper or board

Note 1 to entry: See EN 645 or EN 647.

3.3
negative control water
reference water that has been treated according to the same conditions as the water extract but without being in contact to paper or board

3.4
positive control waters
1) fresh 2,5 mg/l solution of potassium dichromate (CAS 7778-50-9) prepared in reference water and sterilized by filtration through 0,22 µm filter and kept at room temperature in the dark

and

2) a stock 2 mg/ml solution of benzo[|a]pyrene (CAS 50-32-8) prepared in dimethylsulfoxide (DMSO) and kept at 4 °C in the dark. Dilute this stock solution 1 000 times in reference water before use

3.5
reference sample
culture medium prepared with reference water as specified in 3.1

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