

# CERTIFICATE OF ANALYSIS

## ERM<sup>®</sup>-BC720

T-2 and HT-2 toxin in oat flakes		
Compound <sup>a</sup>	Certified value <sup>b</sup>	Uncertainty <sup>c</sup>
	Mass fraction in $\mu\text{g kg}^{-1}$	
T-2 toxin <sup>d</sup>	82	4
HT-2 toxin <sup>e</sup>	81	4

<sup>a</sup> T-2 and HT-2 toxin measured using sample preparation, instrumental separation (HPLC) and mass spectrometric detection as specified on page 3 of this certificate.

<sup>b</sup> The value given represents the unweighted mean value of 80 results (obtained by BAM). Certified values are traceable to the SI

<sup>c</sup> Estimated expanded uncertainty  $U$  with a coverage factor of  $k = 2$ , corresponding to a confidence level of about 95 %, as defined in the Guide to the expression of uncertainty in measurement (GUM), ISO/IEC Guide 98-3 (2008). Uncertainty contributions arising from characterisation as well as from homogeneity and stability testing were taken into account.

<sup>d</sup> CAS number: 21259-20-1

<sup>e</sup> CAS number: 26934-87-2

The certified property values will be valid for 12 months beginning with the dispatch of the material from BAM; this validity may be extended as further evidence of stability becomes available.

The minimum sample intake is 1g.

### NOTE

European Reference Material ERM<sup>®</sup>-BC720 was produced and certified under the responsibility of BAM Federal Institute for Materials Research and Testing according to the principles laid down in the technical guidelines of the European Reference Materials<sup>®</sup> co-operation agreement between BAM-LGC-IRMM. Information on these guidelines is available on the Internet (<http://www.erm-crm.org>).

Accepted as an ERM<sup>®</sup>, Berlin, 15 July 2014 Sample

No.:

Date of dispatch:

BAM  
Department 1  
Analytical Chemistry; Reference Materials

BAM  
Division 1.7  
Food Analysis

Prof. Dr. U. Panne  
(Head of Department)

Dr. M. Koch  
(Head of Division)

## DESCRIPTION OF THE SAMPLE

The intended purpose of reference material ERM<sup>®</sup>-BC720 is i) validation of analytical procedures for the determination of T-2 and HT-2 toxin in oat, and ii) quality assurance in the analytical laboratory. The ERM<sup>®</sup>-BC720 material is an oat flakes sample from a commercial source intended for human consumption.

3.0 kg of oat flakes for human consumption (six aliquots, each of 500 g) out of a batch of 25 kg procured from German retail markets in 2010 were inoculated with spores of *Fusarium sporotrichioides* (DSM No.: 62425) and incubated over 3 weeks at 28 °C. After the incubation period, the material was frozen and freeze-dried. Both materials, inoculated/incubated and non-contaminated, were milled with a centrifugal mill to obtain a particle size smaller than 1.0 mm and subsequently analysed for their T-2/HT-2 toxin contents. Based on the results, 2,220 g of the inoculated/incubated material were mixed with about 21.98 kg of non-contaminated oat flakes for 20 hours using a drum hoop mixer to obtain final contents of 82 µg kg<sup>-1</sup> (T-2 toxin) and 81 µg kg<sup>-1</sup> (HT-2 toxin), respectively. The total quantity was then milled to a final particle size smaller than 0.5 mm and homogenised by means of a drum hoop mixer. Further homogenisation and bottling were carried out by means of an 8 port rotary sample divider using the “cross riffling” procedure (for details see report). A total number of 256 units were bottled in 250 mL amber glass bottles containing (94.2 ± 0.9) g sealed with screw caps containing PTFE-inlays, and numbered in the order of leaving the bottling process. The material is stored at BAM at -21 °C until dispatch.

The between-bottle homogeneity was evaluated by analysis of variance (ANOVA) on 16 out of 256 bottles (4 replicate analyses per bottle). Extensive stability tests provided sound evidence for a minimum validity of the certified value as indicated on page 1 of this certificate provided the material is stored according to the instructions given on page 3.

## PARTICIPANTS

An interlaboratory comparison study (ILC) involving the following 24 laboratories was conducted in order to support the in-house certification study at BAM.

Laboratory	City, Country	Compound	
		T-2 toxin	HT-2 toxin
AGES Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH	Linz, Austria	x	x
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit	Oberschleißheim, Germany	x	x
Chemisches und Veterinäruntersuchungsamt Rheinland	Leverkusen, Germany	x	x
chemlab GmbH	Bensheim, Germany	x	x
Coop	Pratteln, Switzerland	x	x
Eurofins Analytik GmbH Wiertz-Eggert-Jörissen	Hamburg, Germany	x	x
Food GmbH	Jena, Germany	x	x
Institut Kirchoff Berlin GmbH	Berlin, Germany	x	x
Kantonales Laboratorium Thurgau	Frauenfeld, Switzerland	x	x
Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern	Rostock, Germany	x	x
Landesamt für Verbraucherschutz Sachsen-Anhalt	Halle (Saale), Germany	x	x
Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer	Speyer, Germany	x	x
Landwirtschaftliches Technologiezentrum Augustenberg	Karlsruhe, Germany	x	x
Lebensmittelversuchsanstalt	Wien, Austria	x	x
LUFA NORD-WEST	Hameln, Germany	x	x

Laboratory	City, Country	Compound	
		T-2 toxin	HT-2 toxin
LUFA-ITL GmbH	Kiel, Germany	X	X
Max Rubner Institut	Detmold, Germany	X	X
Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit	Stade, Germany	X	X
R-Biopharm AG	Darmstadt, Germany	X	
SGS Germany GmbH, Laboratory Service Hamburg	Hamburg, Germany	X	X
Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft	Leipzig, Germany	X	X
Staatliches Veterinäruntersuchungsamt Arnsberg	Arnsberg, Germany	X	X
Stadt Bochum, Chemisches Untersuchungsamt	Bochum, Germany	X	X
Thüringer Landesanstalt für Landwirtschaft	Jena, Germany	X	X

## ANALYTICAL METHODS AND RESULTS OF THE CERTIFICATION STUDY

In-house certification study at BAM was conducted by using high performance liquid chromatography hyphenated to positive electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) based on a stable isotope dilution assay using [<sup>13</sup>C<sub>24</sub>]-T-2 and [<sup>13</sup>C<sub>22</sub>]-HT-2 toxin as internal standards.

The ILC participant laboratories applied methods of their own choice. The methods used involved instrumental determination by high performance liquid chromatography and gas chromatography coupled to mass spectrometry. The methods mainly varied in their initial extraction and clean-up procedures. Details of the methods used are given in the certification report.

## SAFETY INFORMATION

The usual laboratory safety precautions have to be applied. No hazardous effects are to be expected when the material is used under conditions usually adopted for the analysis of foodstuff matrices low or moderately contaminated with T-2 and HT-2 toxin. Although the mycotoxin content in the sample is at trace levels, any use other than the intended one should be avoided. Personnel handling of the material must adequately be trained and follow regular laboratory safety precautions. It is strongly recommended to handle and dispose of the reference material in accordance with the guidelines for hazardous materials legally in force at the site of end use and disposal.

## INSTRUCTIONS FOR USE

Before taking a sub-sample the bottle should be allowed to reach room temperature and be mixed thoroughly. Thereafter, the bottle is to be closed tightly. To the best of our knowledge, the stability of the reference material is not affected by short periods of handling at ambient temperature during transport and use. However, BAM cannot be held responsible for any alteration of the material occurring during handling and storage at the customer's premises, especially of opened samples.

## STORAGE

The material has to be stored at a temperature equal to or lower than -18 °C in its original bottle.

## LEGAL NOTICE

Neither BAM, its contractors nor any person acting on their behalf:

- (a) make any warranty or representation, express or implied, that the use of any information, material, apparatus, method or process disclosed in this document does not infringe any privately owned intellectual property rights; or
- (b) assume any liability with respect to, or for damages resulting from, the use of any information, material, apparatus, method or process disclosed in this document save for loss or damage arising solely and directly from the negligence of BAM.

## TECHNICAL REPORT

A detailed technical report (pdf file or paper copy) describing the production, general characterisation as well as the analytical procedures applied and the treatment of the analytical data during certification of ERM<sup>®</sup>-BC720 is available on request from BAM.

Supply of Reference Materials by BAM Federal Institute for Materials Research and Testing  
Richard-Willstätter-Str. 11, D-12489 Berlin, Germany

Phone: +49 30 8104 1179

e-mail: [sales.crm@bam.de](mailto:sales.crm@bam.de)

Fax: +49 30 8104 1177

internet: [www.bam.de](http://www.bam.de)