

# Certificate of Analysis

**NRC-CNRC**

Certified Reference Material

## MYCO-1

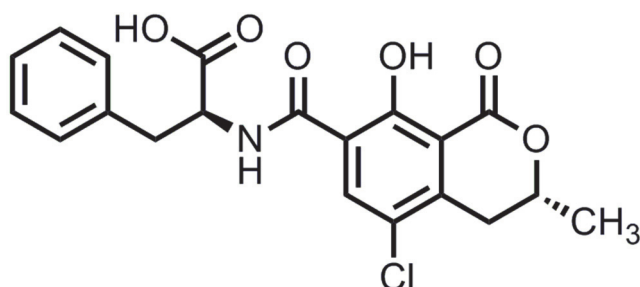
Certified Reference Material of mycotoxin contaminated rye flour

The following tables show those constituents for which certified and information values have been established for this rye flour certified reference material (CRM).

The certified value represents the mass fraction of ochratoxin A in MYCO-1 (as supplied) based on results generated at the National Research Council Canada (NRC) using the NRC certified reference materials OTAN-1 (native ochratoxin A) and OTAL-1 ( $[^{13}\text{C}_6]$ -ochratoxin A) as primary calibrator and internal standard, respectively. The expanded uncertainty ( $U_{\text{CRM}}$ ) in the certified value is equal to  $U = ku_c$  where  $u_c$  is the combined standard uncertainty calculated according to the JCGM Guide [1] and  $k$  is the coverage factor. A coverage factor of two (2) was applied for ochratoxin A. It is intended that  $U_{\text{CRM}}$  accounts for every aspect that reasonably contributes to the uncertainty of the certified value.

**Table 1: Certified quantity value for MYCO-1**

Substance	Molecular formula	Mass fraction $\mu\text{g/kg}$
ochratoxin A (a,b)	$\text{C}_{20}\text{H}_{18}\text{ClNO}_6$	$4.05 \pm 0.82$



### ochratoxin A

CAS registry number: 303-47-9

InChI Key: RWQKHEORZBHNRI-BMIGLBTASA-N

Molecular formula:  $\text{C}_{20}\text{H}_{18}\text{ClNO}_6$

Molar mass:  $403.814 \pm 0.012$  g/mol



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**Table 2: Information value for MYCO-1**

Substance	Molecular formula	Mass fraction g/kg
water (c)	H <sub>2</sub> O	127

**Coding**

The coding refers to the instrumental method used for value assignment:

- a** Double isotope dilution liquid chromatography tandem mass spectrometry (ID<sup>2</sup>-LC-MS/MS)
- b** Double isotope dilution liquid chromatography high resolution mass spectrometry (ID<sup>2</sup>-LC-HRMS)
- c** Karl Fischer titration

**Certified values**

Certified values are considered to be those for which NRC has the highest confidence in accuracy and that all known and suspected sources of bias have been taken into account and are reflected in the stated expanded uncertainties. Certified values are the best estimate of the mean and uncertainty (Table 1).

**Information values**

Information values are those for which insufficient data are available to provide a comprehensive estimate of uncertainty (Table 2).

**Intended use**

This reference material is primarily intended for use in method development, validation, and quality control for the analysis of mycotoxins in flour.

**Storage**

It is recommended that the material be stored in a controlled cold temperature environment such as a freezer at approximately -20 °C.

**Instructions for use**

Each bottle should be allowed to warm to room temperature and the contents thoroughly mixed prior to opening. After accessing contents, bottles should be tightly sealed and immediately returned to the freezer. Repeated sampling is permitted, although care must be taken not to introduce contamination. A minimum sample mass of 5 g is recommended.

**Preparation of material**

The material was prepared at the NRC using commercially sourced rye flour from local vendors blended with highly contaminated flour generated at NRC containing ochratoxin A. Mycotoxin producing fungal cultures were identified and incubated on sterile wheat kernels via solid state fermentation. The kernels were then autoclaved, freeze-dried, ground, blended, and sieved to generate highly contaminated sources of flour. Small amounts of contaminated flour were blended with the commercial rye flour in a multi-step process to attain the target level of ochratoxin A. The material was bottled into 100 g units in a controlled environment at 40 % relative humidity. MYCO-1 contains several other mycotoxins such as T-2 toxin, neosolaniol, zearalenone, deoxynivalenol, deoxynivalenol-3-glucoside, and the ergot alkaloids.



## Stability

The short-term, freeze-thaw, and long-term stability of ochratoxin A in MYCO-1 was assessed at NRC via ID<sup>2</sup>–LC–MS/MS. The short-term stability was carried out using an isochronous approach. Bottles of MYCO-1 were stored at +37, +20, +4, –20, and –80 °C and sub-samples were taken at one week, one month, and six months. Results were evaluated using the –80 °C samples as a reference and no significant degradation was observed. The freeze-thaw stability was assessed over ten freeze-thaw cycles from –20 to +20 °C. Sub-samples were analysed simultaneously and no significant degradation was observed. The long-term stability at –20 °C was assessed after one year using sub-samples taken from three randomly selected bottles. No significant differences in the measured mass fraction were observed over this period. Uncertainty components for short-term, freeze-thaw, and long-term stability of ochratoxin A in MYCO-1 were thus considered negligible and assigned to zero.

## Homogeneity

The material was tested for homogeneity at NRC via ID<sup>2</sup>–LC–HRMS. Results from sub-samples (5 g) were evaluated using two-way analysis of variance (ANOVA) statistical model [2, 3] to determine both within-bottle and between-bottle inhomogeneity components. A combined uncertainty was included in the calculation of the certified value.

## Uncertainty

Included in the combined uncertainty estimate ( $u_c$ ) are uncertainties in the batch characterization ( $u_{\text{char}}$ ), uncertainties related to within-bottle and between-bottle variation ( $u_{\text{hom}}$ ), uncertainties related to stability ( $u_{\text{stability}}$ ), and uncertainties related to different analytical methods ( $u_{\text{method}}$ ). Expressed as standard uncertainties, these components are listed in Table 3.

**Table 3: Uncertainty components for MYCO-1**

Substance	$U_{k=2}$ µg/kg	$u_c$ µg/kg	$u_{\text{char}}$ µg/kg	$u_{\text{hom}}$ µg/kg	$u_{\text{stability}}$ µg/kg	$u_{\text{method}}$ µg/kg
ochratoxin A	0.82	0.41	0.10	0.40	0.00	0.00

## Metrological traceability

Results presented in this certificate are traceable to the SI through gravimetrically prepared standards of established purity (NRC OTAN-1) and international measurement intercomparisons. As such, MYCO-1 serves as a suitable reference material for laboratory quality assurance programs, as outlined in ISO/IEC 17025.

## Quality System (ISO/IEC 17025, ISO Guide 34)

This material was produced in compliance with the documented NRC Measurement Science and Standards (MSS) Quality System, which conforms to the requirements of ISO/IEC 17025 and ISO Guide 34. The MSS Quality System supporting NRC calibration and measurement capabilities, as listed in the Bureau international des poids et mesures (BIPM) key comparison database (<http://kcdb.bipm.org/>), has been reviewed and approved under the authority of the Inter-American Metrology System (SIM), and found to be in compliance with the expectations of the Comité international des poids et mesures (CIPM) Mutual Recognition Arrangement. The SIM certificate of approval is available upon request.



## Updates

Users should ensure that the certificate they have is current. Our website at [www.nrc.gc.ca/crm](http://www.nrc.gc.ca/crm) will contain any new information.

## References

- [1] JCGM (2008), Evaluation of measurement data: Guide to the expression of uncertainty in measurement. JCGM 100:2008.
- [2] A.M.H. van der Veen, J. Pauwels, Uncertainty calculations in the certification of reference materials. 1. Principles of analysis of variance, Accred Qual Assur (2000), 5:464-469.
- [3] ISO (2017), Reference materials – Guidance for characterization and assessment of homogeneity and stability. ISO Guide 35:2017.

## Authorship

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## Acknowledgements

We thank Dr. Sheryl Tittlemier of the Canadian Grain Commission (Winnipeg, MB, Canada) for helpful discussions.

The contributions of NRC staff members Adilah Bahadoor, Phuong Mai Le, Donald M. Leek, Garnet McRae, Kenny Nadeau, Bradley Stocks, and Marie-Pier Thibeault are also acknowledged.



**MYCO-1***Date of issue: March 2018**Date of expiry: March 2020***Approved by:**

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**This Certificate is only valid if the corresponding product was obtained directly from NRC or one of our authorized vendors.**

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