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Safety evaluation of the food enzyme non-reducing end α-L-arabinofuranosidase from the genetically modified *Trichoderma reesei* strain NZYM-GV

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Abstract

The food enzyme non-reducing end α-L-arabinofuranosidase (α-L-arabinofuranoside non-reducing end α-L-arabinofuranosidase; EC 3.2.1.55) is produced with the genetically modified *Trichoderma reesei* strain NZYM-GV by Novozymes A/S. The genetic modifications do not give rise to safety concerns. The food enzyme is free from viable cells of the production organism and its DNA. It is intended to be used in grain treatment for the production of starch and gluten fractions. Since residual amounts of total organic solids (TOS) are removed by washing and purification steps applied during grain treatment, the estimation of a dietary exposure is considered not necessary. Genotoxicity tests did not indicate a safety concern. The systemic toxicity was assessed by means of a repeated dose 90-day oral toxicity study in rats. The Panel identified a no observed adverse effect level of 1,116 mg TOS/kg body weight per day, the highest dose tested. Similarity of the amino acid sequence to those of known allergens was searched and no match was found. The Panel considered that, under the intended conditions of use, the risk of allergic sensitisation and elicitation reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is considered to be low. Based on the data provided and the removal of TOS during the grain treatment, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

Keywords: Food enzyme, non-reducing end α-L-arabinofuranosidase, α-L-arabinofuranoside non-reducing end α-L-arabinofuranosidase, EC 3.2.1.55, *Trichoderma reesei*, genetically modified microorganism

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008[1] provides definition for 'food enzyme' and 'food enzyme preparation'.

'Food enzyme' means a product obtained from plants, animals or micro-organisms or products thereof including a product obtained by a fermentation process using micro-organisms: (i) containing one or more enzymes capable of catalysing a specific biochemical reaction; and (ii) added to food for a technological purpose at any stage of the manufacturing, processing, preparation, treatment, packaging, transport or storage of foods.

'Food enzyme preparation' means a formulation consisting of one or more food enzymes in which substances such as food additives and/or other food ingredients are incorporated to facilitate their storage, sale, standardisation, dilution or dissolution.

Before January 2009, food enzymes other than those used as food additives were not regulated or were regulated as processing aids under the legislation of the Member States. On 20 January 2009, Regulation (EC) No 1332/2008 on food enzymes came into force. This Regulation applies to enzymes that are added to food to perform a technological function in the manufacture, processing, preparation, treatment, packaging, transport or storage of such food, including enzymes used as processing aids. Regulation (EC) No 1331/2008[2] established the European Union (EU) procedures for the safety assessment and the authorisation procedure of food additives, food enzymes and food flavourings. The use of a food enzyme shall be authorised only if it is demonstrated that:

- it does not pose a safety concern to the health of the consumer at the level of use proposed;
- there is a reasonable technological need;
- its use does not mislead the consumer.

All food enzymes currently on the European Union market and intended to remain on that market, as well as all new food enzymes, shall be subjected to a safety evaluation by the European Food Safety Authority (EFSA) and approval via an EU Community list.

The 'Guidance on submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) lays down the administrative, technical and toxicological data required.

1.1. Background and Terms of Reference as provided by the requestor

1.1.1. Background as provided by the European Commission

Only food enzymes included in the European Union (EU) Community list may be placed on the market as such and used in foods, in accordance with the specifications and conditions of use provided for in Article 7 (2) of Regulation (EC) No 1332/2008 on food enzymes.

An application has been introduced by the applicant "Novozymes A/S" for the authorization of food enzyme Arabinofuranosidase from a genetically modified *Trichoderma reesei* (strain NZYM-GV).

Following the requirements of Article 12.1 of Regulation (EC) No 234/2011[3] implementing Regulation (EC) No 1331/2008, the Commission has verified that the three applications fall within the scope of the food enzyme Regulation and contain all the elements required under Chapter II of that Regulation.

1.1.2. Terms of Reference

In accordance with Article 29 of Regulation (EC) No 178/2002[4], the European Commission asks the European Food Safety Authority to carry out the safety assessment on the following food enzyme: Arabinofuranosidase from a genetically modified *Trichoderma reesei* (strain NZYM-GV), in accordance

[1] Regulation (EC) No 1332/2008 of the European Parliament and of the Council of 16 December 2008 on Food Enzymes and Amending Council Directive 83/417/EEC, Council Regulation (EC) No 1493/1999, Directive 2000/13/EC, Council Directive 2001/112/EC and Regulation (EC) No 258/97. OJ L 354, 31.12.2008, pp. 7–15.

[2] Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, pp. 1–6.

[3] Commission Regulation (EU) No 234/2011 of 10 March 2011 implementing Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 64, 11.03.2011, pp. 15–24.

[4] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, pp. 1–24.

with the Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme arabinofuranosidase from the genetically modified *T. reesei* strain NZYM-GV. The dossier was updated on 1 February 2021.

Additional information was requested from the applicant during the assessment process on 16 September 2021 and was consequently provided (see 'Documentation provided to EFSA').

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA 'Guidance on transparency in the scientific aspects of risk assessment' (EFSA, 2009b) and following the relevant guidance documents of the EFSA Scientific Committee.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009a) as well as the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) have been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance with the updated 'Scientific Guidance for the submission of dossiers on food enzymes' (EFSA CEP Panel, 2021).

3. Assessment

IUBMB nomenclature	Non-reducing end α-L-arabinofuranosidase
Systematic name	α-L-arabinofuranoside non-reducing end α-L-arabinofuranosidase
Synonyms	α-Arabinofuranosidase, α-arabinosidase
IUBMB No	EC 3.2.1.55
CAS No	9067-74-7
EINECS No	232-957-7

Non-reducing end α-L-arabinofuranosidases catalyse the hydrolysis of non-reducing α-L-arabinofuranoside residues of α-L-arabinans and arabinoxylans, resulting in the release of arabinose. The food enzyme is intended to be used in grain treatment for the production of starch and gluten fractions.

3.1. Source of the food enzyme

The non-reducing end α-L-arabinofuranosidase is produced with the genetically modified filamentous fungus *T. reesei* strain NZYM-GV, which is deposited in the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany), with deposit number [redacted][5]

The production strain was identified as *T. reesei* [redacted][6]

[redacted][7]

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain [redacted]

The recipient strain [redacted][8]

[5] Technical dossier/2nd submission/Annex A2.
[6] Technical dossier/2nd submission/Annex D1.
[7] Technical dossier/ Additional data December 2021/Annex A1 (2).
[8] Technical dossier/2nd submission/Annex 4/p. 7.

3.1.2. Characteristics of the introduced sequences

The sequence encoding the non-reducing end α-L-arabinofuranosidase [redacted][9]

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise non-reducing end α-L-arabinofuranosidase [redacted][10][11]

The production strain contains [redacted]

3.1.4. Safety aspects of the genetic modification

The technical dossier contains all necessary information on the recipient microorganism, the donor organism and the genetic modification process.

The production strain *T. reesei* NZYM-GV differs from the recipient strain in its capacity to produce the non-reducing end α-L-arabinofuranosidase [redacted][12]

No issues of concern arising from the genetic modifications were identified by the Panel.

3.2. Production of the food enzyme

The food enzyme is manufactured according to the Food Hygiene Regulation (EC) No 852/2004[13], with food safety procedures based on hazard analysis and critical control points and in accordance with current Good Manufacturing Practice.[14]

The production strain is grown as a pure culture using a typical industrial medium in a submerged, [redacted] fermentation system with conventional process controls in place. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is then further purified and concentrated, including an ultrafiltration step in which the enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[15] The applicant provided information on the identity of the substances used to control the fermentation and in the subsequent downstream processing of the food enzyme.[16]

[9] Technical dossier/2nd submission/Annex 4/p. 13.
[10] Technical dossier/2nd submission/Annex C1.
[11] Technical dossier/2nd submission/Annex C2.
[12] Technical dossier/2nd submission/Annex D2.
[13] Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of food additives. OJ L 226, 25.6.2004, pp. 3-21.
[14] Technical dossier/2nd submission/p. 44-45/Annex 5.
[15] Technical dossier/2nd submission/p. 44-51.
[16] Technical dossier/2nd submission/p. 33/Annexes: 2_05 and 6.

The Panel considered that sufficient information has been provided on the manufacturing process and the quality assurance system implemented by the applicant to exclude issues of concern.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The non-reducing end α-L-arabinofuranosidase is a single polypeptide chain of [redacted] amino acids. The molecular mass of the mature protein, calculated from the amino acid sequence, is [redacted] kDa.[17] The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A consistent protein pattern was observed across all batches. The gel showed a major protein band corresponding to an apparent molecular mass of about [redacted] kDa, consistent with the expected mass of the enzyme, and a second major band around 66 kDa.[18] The food enzyme was tested for α-amylase, glucoamylase, lipase and protease activities, and none were detected. Minor cellulase and β-glucanase activities were reported by the applicant.[19]

The in-house determination of non-reducing end α-L-arabinofuranosidase activity is based on hydrolysis of wheat arabinoxylan (reaction conditions: pH 4.4, 37°C, 10 min). The released L-arabinose is oxidised to L-arabinonic acid, and the activity is determined by a coupled enzymatic assay. The enzyme activity is expressed in ARabinofuranosidase eXo monosubstituted Units/g (ARXU(M)/g) relative to an internal arabinofuranosidase enzyme standard.[20]

The food enzyme has a temperature optimum around 40°C (pH 4.0, 15 min) and a pH optimum between pH 3.0 and pH 5.0 (37°C, 15 min). Thermostability was tested after a pre-incubation of the food enzyme for 30 min at different temperatures (pH 4.0). The enzyme activity decreased by around 60% above 40°C and showed no activity at 50°C.[21]

3.3.2. Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches used for commercialisation and one batch for toxicological tests (Table 1).[22] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 11.1% and the mean enzyme activity/TOS ratio was 2.8 ARXU(M)/mg TOS.

Table 1: Composition of the food enzyme

Parameters	Unit	Batches			
		1	2	3	4[(a)]
Non-reducing end α-L-arabinofuranosidase activity	ARXU(M)/g batch[(b)]	312.7	296.6	321.9	300.3
Protein	%	9.3	9.0	9.4	9.2
Ash	%	0.3	0.3	0.3	0.6
Water	%	88.7	88.4	88.8	88.7
Total organic solids (TOS)[(c)]	%	11.0	11.3	10.9	10.7
Activity/mg TOS	ARXU(M)/mg TOS	2.8	2.6	3.0	2.8

(a): Batch used for the toxicological studies.
(b): ARXU(M): ARabinofuranosidase eXo monosubstituted (see Section 3.3.1).
(c): TOS calculated as 100% – % water – % ash.

3.3.3. Purity

The lead content in the four batches was below 0.5 mg/kg[23], which complies with the specification for lead as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006). In

[17] Technical dossier/2nd submission/p. 30/Annex 1.
[18] Technical dossier/2nd submission/p. 31-32.
[19] Technical dossier/2nd submission/p. 37-38/Annexes: 3_02, 3_03, 3_04, 3_05, 3_06, 3_07.
[20] Technical dossier/2nd submission/p. 35/Annex 3_01.
[21] Technical dossier/2nd submission/p. 36-37/Annex 9.
[22] Technical dossier/2nd submission/p. 31, 55-56/Annexes: 2, 7_01, 7_02, 7_03 and 10.
[23] Technical dossier/2nd submission/p. 33-34, 56/Annexes: 2_04 and 10.

addition, the levels of arsenic, cadmium and mercury were below the limits of detection of the employed methodologies.[23,24]

The food enzyme complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[25] No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).[26]

Strains of *Trichoderma*, in common with most filamentous fungi, have the capacity to produce a range of secondary metabolites (Frisvad et al., 2018). The applicant did not provide information on specific potential secondary metabolites produced under the conditions of fermentation which might be present in the food enzyme TOS. This issue is addressed by the toxicological examination of the food enzyme TOS.[27]

The Panel considered that the information provided on the purity of the food enzyme is sufficient.

3.3.4. Viable cells and DNA of the production strain

The absence of viable cells of the production strain in the food enzyme was demonstrated [redacted][28] No colonies were produced.

The absence of recombinant DNA in the food enzyme was demonstrated [redacted] No DNA was detected [redacted][29,30]

3.4. Toxicological data

A battery of toxicological tests has been provided, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian cell micronucleus assay and a repeated dose 90-day oral toxicity study in rats. The batch 4 (Table 1) used in these studies has similar protein pattern and purity as the batches used for commercialisation, and thus is considered suitable as a test item.

3.4.1. Genotoxicity

3.4.1.1. Bacterial reverse mutation test

A bacterial reverse mutation assay (Ames test) was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 471 (OECD, 1997a,b) and following Good Laboratory Practice (GLP).[31] Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* strain WP2 *uvrA* pKM101 were used in the presence or absence of metabolic activation (S9-mix), applying the treat and plate assay. Two experiments were carried out with triplicate plating using six different concentrations of the food enzyme (16, 50, 160, 500, 1,600 and 5,000 μg TOS/plate) in a first experiment. Since no cytotoxicity was seen (as a reduction in the background lawns) at any test concentration in the first experiment, the maximum concentration of 5,000 μg TOS/plate was retained for all strains in the second experiment, but with a narrower concentration interval (160, 300, 625, 1,250, 2,500 and 5,000 μg TOS/plate). Upon treatment with the food enzyme, there was no relevant increase in revertant colony numbers above the control values in any strain tested, with or without S9-mix.

The Panel concluded that the food enzyme did not induce gene mutations under the test conditions employed in this study.

[24] LoDs: Pb = 0.5 mg/kg; As = 0.3 mg/kg; Cd and Hg = 0.05 mg/kg each.
[25] Technical dossier/2nd submission/p. 34, 56/Annexes: 2_07, 2_08, 2_09, 2_10.
[26] Technical dossier/2nd submission/p. 34, 56/Annexes: 2_06 and 10.
[27] Additional data December 2021.
[28] Technical dossier/2nd submission/Annex E1.
[29] Technical dossier/2nd submission/Annex E2.
[30] Additional data December 2021/Annex E2.
[31] Technical dossier/2nd submission/p. 57-58/Annex 7_01.

3.4.1.2. *In vitro* mammalian cell micronucleus assay

The *in vitro* micronucleus test was carried out according to OECD Draft Guideline 487 (OECD, 2014) and following GLP.[32] A single experiment was performed with duplicate cultures of human peripheral blood lymphocytes with and without metabolic activation (S9-mix). Based on the results of a dose finding test, the cells were exposed to the food enzyme at 500, 1,000, 2,000, 3,000, 4,000 and 5,000 μg TOS/mL in a short-term treatment (3 h treatment followed by 21 h recovery period) with or without S9-mix and in a long-term treatment (24 h treatment followed by 24 h recovery) without S9-mix. No pronounced cytotoxicity was seen: a maximum reduction of replication index (20%) was observed at 5,000 μg TOS/mL in the long-term treatment in the absence of S9-mix. The three highest concentrations tested were scored for micronuclei in all three tests. After the short-term treatment without S9-mix, the frequencies of binucleated cells with micronuclei (MNBN) were similar to and not significantly higher than those observed in concurrent controls. A statistically significant increase of MNBN frequency was seen in a single culture at the lowest concentration scored (3,000 μg TOS/mL) in the short-term treatment with S9-mix and at all the concentrations tested in the long-term treatment, against a low concurrent control response. All the values were within the historical vehicle control range and therefore not considered to be of biological relevance.

The Panel concluded that, under the test conditions employed in this study, the food enzyme did not induce an increase in the frequency of MNBNs in cultured human peripheral blood lymphocytes.

3.4.2. Repeated dose 90-day oral toxicity study in rodents

The repeated dose 90-day oral toxicity study was performed in accordance with OECD Test Guideline 408 (OECD, 1998) and following GLP.[33] Groups of 10 male and 10 female Han Wistar rats received by gavage the food enzyme in doses of 112, 368 or 1,116 mg TOS/kg body weight (bw) per day. Controls received the vehicle (reverse osmosis water).

No mortality was observed.

A statistically significant decrease in forelimb grip strength in low- and mid-dose males and increase in hindlimb grip strength in mid- and high-dose females was observed. The Panel considered these changes as not toxicologically relevant since there was no dose-response relationship (in males), no consistency between the changes in males and females and the changes were within the historical control values.

The haematological investigation revealed a statistically significant increase in the mean corpuscular haemoglobin concentration (MCHC) in treated females (+2.4%, +1.8%, +2.1%), a decrease in the reticulocyte count (Retic) in high-dose females (−18%), a decrease in the white blood cell (WBC) count in treated females (−22%, −27%, −19%), a decrease in the lymphocyte count in high-dose males (−15%) and in treated females (−23%, −30%, −21%), a decrease in large unstained cells (LUC) in mid- and high-dose females (−50%, −50%), an increase in the prothrombin time (PT) in treated males (+6%, +8%, +4%) and in high-dose females (+6%), and a decrease in the activated partial thromboplastin times (APTT) in high-dose males (−9%) and in treated females (−13%, −8%, −3%). The Panel considered these changes as not toxicologically relevant owing to the absence of a dose–response relationship (MCHC, WBC count, lymphocyte count in females, LUC, PT in males, APTT in females) and a low magnitude of the changes (all parameters).

The clinical chemistry investigation revealed a statistically significant decrease in alkaline phosphatase (ALP) in high-dose males (−14%). The Panel considered this change as not toxicologically relevant because of the absence of a dose-response relationship and the low magnitude of the change.

There was a statistically significant increase in the relative kidney weight in high-dose males (+5%). The Panel considered this change as not toxicologically relevant because of the low magnitude of the change.

No other statistically significant or biologically relevant differences to controls were reported.

The Panel identified the no observed adverse effect level (NOAEL) of 1,116 mg TOS/kg bw per day, the highest dose tested.

[32] Technical dossier/2nd submission/p. 58-59/Annex 7_02.
[33] Technical dossier/2nd submission/p. 59-60/Annex 7_03.

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not carriers or other excipients that may be used in the final formulation.

The potential allergenicity of the non-reducing end α-L-arabinofuranosidase produced with the genetically modified *T. reesei* strain NZYM-GV was assessed by comparing its amino acid sequence with those of known allergens according to the 'Scientific opinion on the assessment of allergenicity of GM plants and microorganisms and derived food and feed of the Scientific Panel on Genetically Modified Organisms' (EFSA GMO Panel, 2010). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.[34,35]

No information is available on oral and respiratory sensitisation or elicitation reactions of this non-reducing end α-L-arabinofuranosidase.

Respiratory allergy following occupational inhalation of an α-L-arabinofuranosidase has been reported (Huerta-Ocampo et al., 2020). However, several studies have shown that adults with occupational asthma to a food enzyme may be able to ingest the corresponding allergen without acquiring clinical symptoms of food allergy (Cullinan et al., 1997; Poulsen, 2004; Armentia et al., 2009). Information on adverse reactions upon ingestion of α-L-arabinofuranosidases in individuals sensitised through the respiratory route have not been reported.[36]

The Panel considers that under the intended conditions of use the risk of allergic sensitisation and elicitation reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood of such reactions to occur is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The food enzyme is intended to be used in grain treatment for the production of starch and gluten fractions at the recommended use levels of 10-90 ARXU(M)/kg cereal grains or grist, corresponding to 3.6–32.1 mg TOS/kg cereal grains or grist.[37] It is added to the milled grain to remove arabinose side chains in the cell wall arabinoxylan fraction, allowing a higher yield and better purity of the gluten and starch fractions. The food enzyme–TOS is removed from the final processed foods by washing and purification steps applied during grain treatment (EFSA CEP Panel, 2021).

3.5.2. Dietary exposure estimation

The Panel accepted the evidence provided as sufficient to conclude that the residual amounts of food enzyme-TOS in the final gluten and starch is negligible. Consequently, a dietary exposure was not calculated.

3.6. Margin of exposure

In the absence of an estimate for the dietary exposure, the margin of exposure is not calculated.

4. Conclusions

Based on the data provided and the removal of TOS during the grain treatment for the production of starch and gluten fractions, the Panel concluded that the food enzyme non-reducing end α-L-arabinofuranosidase produced with the genetically modified *T. reesei* strain NZYM-GV does not give rise to safety concerns under the intended conditions of use.

The CEP Panel considered the food enzyme free from viable cells of the production organism and recombinant DNA.

5. Documentation as provided to EFSA

Application for authorisation of Arabinofuranosidase from a genetically modified *Trichoderma reesei* (strain NZYM-GV). February 2021. Submitted by Novozymes A/S.

Additional information. December 2021. Submitted by Novozymes A/S.

[34] Technical dossier/2nd submission/p. 61-63/Annex 8.
[35] Additional data December 2021/Annex 8.1.
[36] Additional data December 2021/Annex 8.2.
[37] Technical dossier/p. 53-55.

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Abbreviations

ANI	average nucleotide identity
ARXU(M)	ARabinofuranosidase eXo, monosubstituted
bw	body weight

CAS	Chemical Abstracts Service
CEF	EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
CFU	colony forming units
DRF	dose-range finding
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GLP	Good Laboratory Practice
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LoD	limit of detection
MNBN	binucleated cells with micronuclei
MoE	margin of exposure
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
SDS–PAGE	sodium dodecyl sulfate–polyacrylamide gel electrophoresis
TOS	total organic solids
WGS	whole genome sequence
WHO	World Health Organization