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Safety evaluation of the food enzyme D-psicose 3-epimerase from the genetically modified *Corynebacterium glutamicum* strain FIS002

EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP),
Claude Lambré, José Manuel Barat Baviera, Claudia Bolognesi, Pier Sandro Cocconcelli,
Riccardo Crebelli, David Michael Gott, Konrad Grob, Evgenia Lampi, Marcel Mengelers,
Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren,
Laurence Vernis, Holger Zorn, Boet Glandorf, Lieve Herman, Yi Liu, Joaquim Maia,
Elsa Nielsen and Andrew Chesson

Abstract

This assessment addresses a food enzyme preparation consisting of the immobilised intact but non-viable cells of the genetically modified *Corynebacterium glutamicum* strain FIS002 by CJ-Tereos Sweeteners Europe SAS. The production strain produces the food enzyme D-fructose 3-epimerase (D-psicose 3-epimerase; EC 5.1.3.30). The food enzyme preparation is used in processing fructose to produce a speciality carbohydrate D-allulose (synonym D-psicose). Since residual amounts of total organic solids (TOS) are removed by the purification steps applied during the production of D-allulose, dietary exposure was not calculated. Genotoxicity tests did not raise 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 (NOAEL) of 1,796 mg TOS/kg body weight (bw) per day, the highest dose tested. A search for similarity of the amino acid sequence of the enzyme to known allergens was made 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 of such reactions to occur is low. The food enzyme preparation contains multiple copies of an antimicrobial resistance gene, which is considered a hazard. However, under the specific intended conditions of use described by the applicant, and based on the evidence showing the removal of TOS during the production of D-allulose and the absence of recombinant DNA in the D-allulose, the Panel concluded that the identified hazard associated with the food enzyme D-psicose 3-epimerase produced with the genetically modified *C. glutamicum* strain FIS002 will not result in a risk.

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Requestor: European Commission

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Correspondence: fip@efsa.europa.eu

Panel members: José Manuel Barat Baviera, Claudia Bolognesi, Andrew Chesson, Pier Sandro Cocconcelli, Riccardo Crebelli, David Michael Gott, Konrad Grob, Claude Lambré, Evgenia Lampi, Marcel Mengelers, Alicja Mortensen, Gilles Rivière, Inger-Lise Steffensen, Christina Tlustos, Henk Van Loveren, Laurence Vernis and Holger Zorn.

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1. Introduction

Article 3 of the Regulation (EC) No 1332/2008¹ 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 1331/2008² 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:

- i) it does not pose a safety concern to the health of the consumer at the level of use proposed;
- ii) there is a reasonable technological need;
- iii) 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, 2009) 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 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 “CJ-Tereos Sweeteners Europe SAS” for the authorisation of the food enzyme D-psicose 3-epimerase from *Corynebacterium glutamicum* strain FIS002.

Following the requirements of Article 12.1 of Commission Regulation (EC) No 234/2011³ implementing Regulation (EC) No 1331/2008², the Commission has verified that the application 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

The European Commission requests the European Food Safety Authority to carry out the safety assessment on the food enzyme D-psicose 3-epimerase from *Corynebacterium glutamicum* strain FIS002 in accordance with the article 17.3 of Regulation (EC) No 1332/2008 on food enzymes.

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, 11.3.2011, pp. 15–24.

1.2. Interpretation of the Terms of Reference

The technical dossier contains information about the creation of two strains (FIS002 and FIS002-1).⁴ In accordance to the Terms of Reference, only the production strain FIS002 has been evaluated in this scientific opinion.

2. Data and methodologies

2.1. Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme D-psicose 3-epimerase from a genetically modified *Corynebacterium glutamicum* strain FIS002 on 13 February 2018. The technical dossier was spontaneously updated on 23 September 2019 by the applicant.

Additional information was requested from the applicant during the assessment process on 23 December 2019 and on April 2021 was consequently provided (see 'Documentation provided to EFSA').

Following the reception of additional data on 19 November 2020, EFSA requested a clarification teleconference on 25 January 2021, after which the applicant provided additional data on 7 April 2021.

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, 2009) as well as in the 'Statement on characterisation of microorganisms used for the production of food enzymes' (EFSA CEP Panel, 2019) and following the relevant existing guidance's of EFSA Scientific Committees.

The current 'Guidance on the submission of a dossier on food enzymes for safety evaluation' (EFSA, 2009) has been followed for the evaluation of the application with the exception of the exposure assessment, which was carried out in accordance to the methodology described in the 'CEF Panel statement on the exposure assessment of food enzymes' (EFSA CEF Panel, 2016).

3. Assessment

IUBMB nomenclature:	D-psicose 3-epimerase
Systematic name:	D-psicose 3-epimerase
Synonyms:	D-allulose 3-epimerase, fructose epimerase
IUBMB No.:	EC 5.1.3.30
CAS No.:	1219591-85-1

D-psicose 3-epimerase catalyses the epimerisation of D-fructose at the C3 position to produce D-allulose (also known as D-psicose). The enzyme is retained in the non-viable *Corynebacterium glutamicum* (FIS002) cells, which are used to produce D-allulose.

3.1. Source of the food enzyme

The D-psicose 3-epimerase is produced with a genetically modified bacterium *Corynebacterium glutamicum* strain FIS002, which is deposited at the Korean Culture Centre of Microorganisms (KCCM, Republic of Korea) with deposit number [REDACTED]⁵

The production strain was identified as *C. glutamicum* by whole genome sequence analysis, showing an average nucleotide identity of 99.9% with the type strain *C. glutamicum* [REDACTED]⁶

The whole genome sequence analysis identified a [REDACTED] resistance gene within the genome of the production strain, which is the result of the genetic modification. No other antimicrobial resistance genes were found using 80% identity and 70% minimum length as threshold values.⁶

3.1.1. Characteristics of the parental and recipient microorganisms

The parental strain is *C. glutamicum* [REDACTED]

[REDACTED] It is also the recipient strain.

⁴ Additional data November 2020.

⁵ Updated Technical dossier September 2019/Annex D.

⁶ Additional data August 2021/Appendix 1.

3.1.2. Characteristics of introduced sequences

The sequence encoding the D-psicose 3-epimerase (*dpe-2*) is a variant of the wild-type [REDACTED] gene from [REDACTED]. The introduced sequence [REDACTED]

The expression plasmid pFIS-1-DPE-2 is derived from the replicative plasmid [REDACTED] and contains the following elements [REDACTED]

[REDACTED] a [REDACTED] resistance gene [REDACTED]. No other antimicrobial resistance or virulence genes were identified on the expression plasmid pFIS-1-DPE-2.⁸

In plasmid pFIS-1-DPE-2, the *dpe-2* gene [REDACTED] [REDACTED].⁴ [REDACTED].⁹

3.1.3. Description of the genetic modification process

The purpose of genetic modification was to enable the production strain to synthesise a variant of the D-psicose 3-epimerase from [REDACTED]. For this purpose, the variant *dpe-2* gene was obtained by [REDACTED]

[REDACTED] Finally, the recipient strain *C. glutamicum* [REDACTED] was transformed with plasmid pFIS-1-DPE-2 and selected for resistance to [REDACTED], resulting in the production strain FIS002.

Strain FIS002 contains multiple copies of the pFIS-1-DPE-2 replicative plasmid.¹⁰

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 FIS002 differs from the recipient *C. glutamicum* [REDACTED] by the presence of the multicopy replicative plasmid pFIS-1-DPE-2, which enables the strain to produce a variant of the D-psicose 3-epimerase from [REDACTED] and to grow in the presence of [REDACTED]. The plasmid pFIS-1-DPE-2 was isolated from the production strain and sequenced entirely. The result showed that the transformation process did not introduce changes to the nucleotide sequence of the expression plasmid.¹¹ Using genomic DNA isolated from the bacterial culture taken before and after fermentation, Southern analysis showed that no integration of the replicative plasmid into the genome occurred during the fermentation process.¹²

C. glutamicum is included in the list of organisms considered suitable for Qualified Presumption of Safety (QPS) approach (EFSA BIOHAZ Panel, 2019). However, due to the presence of multiple copies of the [REDACTED] gene that confers resistance to [REDACTED], the QPS approach cannot be applied to the production strain FIS002. [REDACTED] is classified as a critically important antimicrobial for human (WHO, 2018). The presence of multiple copies of the [REDACTED] gene is considered a hazard.

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

3.2. Production of the food enzyme preparation

The food enzyme preparation is manufactured according to the Food Hygiene Regulation (EC) No 852/2004¹³, with food safety procedures based on hazard analysis and critical control points,¹⁴ audited by the Food Safety System Certification 22000.¹⁵

⁷ Updated Technical dossier September 2019/Table 3.2.1.2.4.1-1 and Annex J & Additional data November 2020.

⁸ Additional data April 2021.

⁹ Updated Technical dossier September 2019/p. 19-21 & Additional data November 2020.

¹⁰ Updated Technical dossier September 2019/Figure 3.2.1.2.4.1-4.

¹¹ Updated Technical dossier September 2019/Annex C.

¹² Updated Technical dossier September 2019/Annex E.

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 Additional data/Appendix 3.

¹⁵ Updated Technical dossier September 2019/p. 35.

The production strain is grown as a pure culture using a typical industrial medium in a submerged, fed-batch fermentation system with conventional process controls in place. [REDACTED] is added to the fermentation medium to maintain selection pressure to prevent plasmid loss from the production strain.¹⁶ After completion of the fermentation, the cells are heat-killed at [REDACTED].¹⁷ The heat-killed cells are then harvested by centrifugation and washed with water to remove the fermentation medium. In order to generate the immobilised cell system, the harvested *C. glutamicum* cells are mixed with [REDACTED] solution [REDACTED], to fix the cells in a matrix.¹⁸ 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 preparation.¹⁹

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

The use of [REDACTED] during the fermentation process is considered a hazard. However, the panel considers that the washing of cells before immobilisation is likely to ensure its removal.

3.3. Characteristics of the food enzyme

3.3.1. Properties of the food enzyme

The D-psicose 3-epimerase is a single polypeptide chain of [REDACTED] amino acids.²⁰ The molecular mass of the mature protein, derived from the amino acid sequence, was calculated to be [REDACTED] kDa.²¹ The food enzyme was analysed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis. A consistent protein pattern was observed across all samples of the purified enzyme concentrate. The gel showed a single major protein band migrating slightly above the marker protein of [REDACTED] kDa consistent with the expected mass of the enzyme.²² No other enzymatic activities were reported.²³

The in-house determination of the immobilised cell beads activity is based on the conversion of D-fructose to D-allulose (reaction conditions: [REDACTED]). The enzymatic activity is determined by measuring the release of D-allulose by high-performance liquid chromatography. The activity of the enzyme is expressed as the percentage D-allulose converted from the initial D-fructose.²⁴ The 'relative activity' of the enzyme is expressed as the activity of the enzyme, divided by the maximum observed activity.

The food enzyme preparation has a temperature optimum [REDACTED] and a pH optimum [REDACTED].²⁵ The thermostability of the immobilised food enzyme preparation was tested by incubating the enzyme solution [REDACTED], the enzyme activity was generally greater than 70%.

3.3.2. Chemical parameters

Data on the chemical parameters of the immobilised food enzyme preparation were provided for 10 batches of cell beads (Table 1). The mean total organic solids (TOS) of the food enzyme preparation was 11.2%. The mean enzyme activity/TOS ratio is 2.5% conversion/% TOS.

¹⁶ Updated Technical dossier September 2019/p. 28.

¹⁷ Additional data November 2020/Appendix 1.

¹⁸ Updated Technical dossier September 2019/p. 31-33.

¹⁹ Updated Technical dossier September 2019/Table 3.2.1.2.5.1-1 & Additional data November 2020/Appendix 4.

²⁰ Updated Technical dossier September 2019/p. 9.

²¹ Additional data/November 2020.

²² Updated Technical dossier September 2019/Figure 3.2.1.1.2.1-1.

²³ Updated Technical dossier September 2019/Annex B.

²⁴ Updated Technical dossier September 2019/Annex A.

²⁵ Updated Technical dossier September 2019//Figure 3.2.1.1.2.3-3b and Figure 3.2.1.1.2.3-6b & Additional data November 2020.

Table 1: Compositional data of the food enzyme preparation^(c)

Parameter	Unit	Food enzyme preparation (n = 10)	
		Mean	Minimum–Maximum
D-psicose 3-epimerase activity ^(a)	% conversion	26.5	24.8–27.2
Protein	%	–	–
Water	%	84.9	82.5–89.0
Ash	%	1.9	1.4–2.2
Immobilisation agent	%	2.0	2.0–2.0
Total organic solids (TOS) ^(b)	%	11.2	7.2–13.5
Activity/TOS ratio	% conversion/% TOS	2.5	2.0–3.7

(a): Relative activity (see Section 3.3.1).

(b): TOS calculated as 100% – % water – % ash – % carriers or diluent.

(c): Updated Technical dossier September 2019/Annex K.

3.3.3. Purity

The lead content in 10 commercial batches of the immobilised cells was below the level of detection (LoD),²⁶ which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006). In addition, the levels of mercury were below the LoD of the employed method. The concentrations of arsenic and cadmium in the commercial batches were up to 0.09 and 0.06 mg/kg, respectively. The Panel considered these concentrations as not of concern.²⁷

The food enzyme preparation (immobilised cells) complies with the microbiological criteria (for total coliforms, *Escherichia coli* and *Salmonella*) as laid down in the general specifications and considerations for enzymes used in food processing (FAO/WHO, 2006).²⁸ No antimicrobial activity was detected in any of the tested batches (FAO/WHO, 2006).²⁹

Aflatoxin and ochratoxin measured in 11 batches of immobilised cells were below the LoDs of the applied analytical methods.³⁰

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 preparation (i.e. [redacted] beads) was demonstrated in four independent batches of the [redacted] beads. [redacted] beads were mixed with [redacted] spread onto agar plates [redacted], which were incubated [redacted]. No colonies were found.⁸

DNA from the production strain remains in the [redacted] beads.³¹

3.4. Toxicological data

A battery of toxicological tests, including a bacterial gene mutation assay (Ames test), an *in vitro* mammalian chromosomal aberration test and a repeated dose 90-day oral toxicity study in rats, has been provided.³² The test material used in these studies is the heat-treated *C. glutamicum* FIS002 cells in the form of a powder. The cell batch used for genotoxicity tests contained 10.7% water, 4% ash and 84.9% TOS, and the batch used for the 90-day toxicity study contained 4.9% water, 5.3% ash and 89.8% TOS.²⁶ The Panel accepted the use of heat-killed cells as a suitable substitute for testing the potential toxicity of the immobilised cells.

²⁶ Updated Technical dossier September 2019/Table 3.2.1.2.5.3-1 and Annex K. LoD = 0.01 mg/kg.

²⁷ Updated Technical dossier September 2019/Annex K: LoDs: Pb = 0.1 mg/kg; As = 0.2 mg/kg; Cd = 0.1 mg/kg; Hg = 0.1 mg/kg.

²⁸ Updated technical dossier September 2019/Annex K: CoAs for Immobilized FIS002.

²⁹ Updated technical dossier September 2019/Annex K – CoAs for Immobilized FIS002.

³⁰ Updated technical dossier September 2019/Annex K. LoD: Aflatoxin = 1 µg/kg; Ochratoxin = 3 µg/kg.

³¹ Technical dossier/additional data November 2020/Appendix 2 & Additional data April 2021.

³² Updated Technical dossier September/Annex O.

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, 1997) and following Good Laboratory Practice. Four strains of *Salmonella* Typhimurium (TA98, TA100, TA1535 and TA1537) and *Escherichia coli* WP2 *uvrA* were used in the presence or absence of metabolic activation (S9-mix), applying the direct plate incorporation assay.

A range-finding test was carried out with eight concentrations of the FIS002 cells ranging from 5 to 5,000 µg/plate (corresponding to 4.25–4,247 µg TOS/plate). Precipitation in the plates was observed at 3,000 µg/plate and above. No significant increases of revertants per plate or cytotoxicity were observed at any dose level in any test strains.

The main experiment was carried out in triplicate using five different concentrations of the FIS002 cells (50, 150, 500, 1,500 and 5,000 µg/plate, corresponding to 42.5, 127, 425, 1,274 and 4,247 µg TOS/plate). Precipitation was observed at 5,000 µg/plate. No cytotoxicity was observed at any concentration level of the test substance. Upon treatment with the food enzyme, there was no significant increase in revertant colony numbers above the control values in any strain 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.

3.4.1.2. *In vitro* mammalian chromosomal aberration test

The *in vitro* mammalian chromosomal aberration test was carried out in Chinese hamster lung (CHL) cells according to OECD Test Guideline 473 (OECD, 2014) and following good laboratory practice (GLP). Concentration ranges for the main study were selected based on a range-finding test with eight concentrations of the FIS002 cells ranging from 5 to 5,000 µg/mL (corresponding to 4.25–4,247 µg TOS/mL) with and without metabolic activation system (S9-mix). The main experiment was carried out in duplicate cultures by short-term treatment (6 h followed by 18 h recovery period) and by continuous treatment (24 h). The relative cell growth rate with respect to negative controls was the indicator of cytotoxicity.³³

In the short-term treatments, the CHL cells were exposed to the FIS002 cells at concentrations of 0, 1,250, 2,500 and 5,000 µg/mL (corresponding to 0, 1,062, 2,123 and 4,247 µg TOS/mL) with S9-mix and at concentrations of 0, 600, 1,200 and 2,400 µg/mL (corresponding to 0, 510, 1,018 and 2,037 µg TOS/mL) without S9-mix. In the presence of the S9-mix, the relative cell growth rate was 71%, 72% and 58% at 1,062, 2,123 and 4,247 µg TOS/mL, respectively. Without S9-mix, the relative cell growth rate was 83%, 65% and 41% at 510, 1,018 and 2,037 µg TOS/mL, respectively. In the continuous treatment, the CHL cells were exposed to the FIS002 cells at concentrations of 0, 200, 400 and 800 µg/mL (corresponding to 0, 170, 340 and 679 µg TOS/mL) without S9-mix. The relative cell growth rate was 85%, 67% and 40% at 170, 340 and 679 µg TOS/mL, respectively.

In the short-term treatment with S9-mix, a statistically significant increase in the mean frequency of metaphases with structural aberrations was observed at the highest concentration tested (2.67% vs. 0.33% in controls), exceeding the range of historical control data (0–2%). In a confirmatory analysis, a second set of slides from the same experiment were scored. No statistically significant increase in the frequency of structural chromosomal aberrations was detected (0.67% at the 4,247 µg TOS/mL vs. 0.33% in controls). Thus, the positive result detected in the first evaluation was not reproducible in a second separate analysis. When the data from the two evaluations were pooled, the frequency of structural chromosomal aberrations was statistically significantly increased (1.67% vs. 0.33% in controls) but was within the range of historical control data. No statistically significant increases in the mean frequencies of numerical chromosomal aberrations were detected. In the short-term treatment without metabolic activation and in the continuous treatment, no statistically significant increases in the mean frequencies of structural and numerical aberrations were observed.

The Panel concluded that the food enzyme did not induce chromosome aberrations under the test conditions employed for this study.

³³ Updated technical dossier September 2019/Annex O.

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. Groups of 10 male and 10 female Sprague–Dawley [CrI: CD(SD)] rats received by gavage the FIS002 cells in doses of 500, 1,000 and 2,000 mg/kg body weight (bw) per day (corresponding to 449, 898 and 1,796 mg TOS/kg bw per day). Controls received the vehicle (sterile water). Furthermore, the recovery control and high-dose groups each comprising five males and five females, terminated 4 weeks after the end of treatment, were included in the study.³³

One mid-dose female animal was found dead on day 54. The cause of the death was not clarified at necropsy because of cannibalism. The Panel considered this death as accidental and not test compound-related.

A statistically significant increase in water intake was observed in high-dose males on day 21 and on day 105 during the recovery period. As these were sporadic events without dose-response relationship, they were considered by the Panel not to be treatment-related.

Statistically significant differences to controls in haematological parameters were recorded only in the recovery period. High-dose males had lower red blood cell distribution width (RDW), relative reticulocyte count and platelet count. High-dose females had higher RDW and eosinophil count. As these findings were not present in the main groups, they lacked consistency between sexes, the differences to the recovery control group in the RDW and in the eosinophil count were small, and the values for reticulocytes and platelets were comparable to those in the high-dose group at the end of the treatment, these findings were not considered toxicologically relevant.

Clinical chemistry investigation revealed a statistically significantly lower albumin to globulin (A/G) ratio in mid- and high-dose males compared to controls. In the recovery period, high-dose males had higher levels of blood urea nitrogen and creatinine. These changes were not considered toxicologically relevant by the Panel due to their low magnitude, lack of dose-response or lack of these changes during the treatment period.

In urinalysis, statistically significant findings were limited to increase of ketone bodies in low-dose males at the end of the dosing period and of specific gravity in high-dose females at the end of the recovery period. These sporadic findings of low magnitude and lacking a dose-response relationship (at the end of the dosing period) were considered by the Panel as not toxicologically relevant.

No other statistically significant differences to controls were observed.

Overall, the Panel identified a no observed adverse effect level (NOAEL) of 1,796 mg TOS/kg bw per day, the highest dose tested.

3.4.3. Allergenicity

The allergenicity assessment considers only the food enzyme and not any carrier or other excipient which may be used in the final formulation.

The potential allergenicity of the D-psicose 3-epimerase produced with the genetically modified *C. glutamicum* strain FIS002 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, 2017). Using higher than 35% identity in a sliding window of 80 amino acids as the criterion, no match was found.³⁴

No information is available on oral and respiratory sensitisation or elicitation reactions of this D-psicose 3-epimerase. In addition, no allergic reactions to ingestion or respiratory exposure to epimerases have been reported in the literature.

According to the information provided, substances or products that may cause allergies or intolerances (corn steep liquor) are used as a raw material in the media fed to the microorganisms.³⁵ However, during the fermentation process, these products will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues of these foods employed as protein sources are not expected to be present.

³⁴ Updated technical dossier September 2019/Annex P.

³⁵ Updated technical dossier September 2019/Table 3.2.1.2.5.1-1.

Quantifying the risk for allergenicity is not possible in view of the individual susceptibility to food allergens. Allergenicity can be ruled out only if the proteins are fully removed. However, traces of protein could be present in the D-allulose product.

The Panel considered 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 occurring is considered to be low.

3.5. Dietary exposure

3.5.1. Intended use of the food enzyme

The non-viable cells expressing the food enzyme is intended to be used in immobilised form to produce D-allulose at a use level of up to 480 mg TOS/kg D-fructose.⁴

As the food enzyme is intended to be used only in its immobilised form, the transfer of food enzyme TOS into the final product, i.e. highly purified D-allulose, is expected to be negligible. Following the epimerisation step, the reaction product (non-purified D-allulose) is subjected to a series of purification steps (ion exchange and chromatography) to obtain the D-allulose syrup and an additional crystallisation step to obtain the crystalline D-allulose,⁴ which is expected to eliminate residual food enzyme TOS from the final products (D-allulose syrup and crystalline D-allulose).

When analysing five batches of D-allulose syrups and five batches of crystalline D-allulose, █████ used in the immobilisation process, was not detected in by inductively coupled plasma mass spectrometry.³⁶ █████, used for plasmid selection during the fermentation process, was not detected.³⁷ The enzyme protein was also not detected by enzyme-linked immunosorbent assay³⁸ or by analysis of total protein content using three different techniques (silver staining,³⁹ Bioanalyser,⁴⁰ NanoOrange staining⁴¹).

The *dpe-2* gene and the █████ gene from the expression plasmid were not detected in five batches of D-allulose syrups and five batches of D-allulose crystalline by polymerase chain reaction (PCR).⁴²

3.5.2. Dietary exposure estimation

The technical information and experimental data provided on the removal of food enzyme TOS during the production of speciality carbohydrate (D-allulose) were considered by the Panel as sufficient (see Section 3.5.1) to exclude this process from the exposure assessment (Annex B in EFSA CEF Panel, 2016). Consequently, a dietary exposure was not calculated.

4. Conclusions

The food enzyme preparation consists of a non-viable genetically modified organism containing an antimicrobial resistance gene on a multicopy replicative plasmid, which is considered a hazard.

Under the specific intended conditions of use described by the applicant, and based on the evidence showing the removal of TOS during the production of D-allulose and the absence of recombinant DNA in the D-allulose, the Panel concluded that the identified hazard associated with the food enzyme preparation containing D-psicose 3-epimerase produced with the genetically modified *C. glutamicum* strain FIS002 will not result in a risk.

5. Recommendation

The applicant should ensure that during the manufacture of the food enzyme preparation (the █████ encapsulated cells of the production strain), no recombinant DNA is released into the environment. The D-allulose manufacturer(s) should similarly ensure that during the production of D-allulose using this food enzyme preparation, no recombinant DNA is released into the environment.

³⁶ Additional data November 2020, LoD=0.002 mg/kg.

³⁷ Updated Technical dossier September 2019/Annex G, LoD = 0.076 mg/kg.

³⁸ Updated Technical dossier September 2019/Annex F, LoD = 15.63 mg/g.

³⁹ Updated Technical dossier September 2019/Annex F, LoD = 0.32 mg/g.

⁴⁰ Updated Technical dossier September 2019/Annex F, LoD = 7 mg/g.

⁴¹ Updated Technical dossier September 2019/Annex F, LoD = 10 mg/g.

⁴² Updated Technical dossier September 2019/Annex F, LoD = 0.01 ng/g.

Documentation provided to EFSA

- 1) Technical dossier 'Application for the authorisation of D-psicose 3-epimerase from a genetically modified strain of *Corynebacterium glutamicum* (strain FIS002) as a food enzyme in the European Union'. February 2018. Submitted by CJ-Tereos Sweeteners Europe SAS.
- 2) Technical dossier. September 2019. Updated by CJ-Tereos Sweeteners Europe SAS.
- 3) Additional information. November 2020. Submitted by CJ-Tereos Sweeteners Europe SAS.
- 4) Additional information. April 2021. Submitted by CJ-Tereos Sweeteners Europe SAS.
- 5) Additional information. August 2021. Submitted by CJ-Tereos Sweeteners Europe SAS.

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Abbreviations

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
CHL	Chinese hamster lung
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
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Cooperation and Development
PCR	polymerase chain reaction
RDW	red blood cell distribution width
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TOS	Total Organic Solids
WHO	World Health Organization