Adopted: 6 December 2023

DOI: 10.2903/j.efsa.2024.8513

SCIENTIFIC OPINION

efsa JOURNAL

Safety evaluation of the food enzyme phosphoinositide phospholipase C from the genetically modified *Pseudomonas fluorescens* strain PIC

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 | Yrjö Roos | Cristina Fernàndez-Fraguas | Ana Gomes | Yi Liu | Andrew Chesson

Correspondence: fip@efsa.europa.eu

Abstract

The food enzyme phosphoinositide phospholipase C (1-phosphatidyl-1D-myo-inositol-4,5-bisphosphate inositoltrisphosphohydrolase EC 3.1.4.11.) is produced with the genetically modified *Pseudomonas fluorescens* strain PIC by DSM Food specialties B.V. 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 the processing of fats and oils for the production of refined edible fats and oils by degumming. Since residual amounts of the total organic solids are removed by the washing and purification steps applied during degumming, dietary exposure estimation and toxicity testing were considered unnecessary. A search for the similarity of the amino acid sequence of the food enzyme to known allergens was made and no matches were found. The Panel considered that the risk of allergic reactions by dietary exposure cannot be excluded, but the likelihood for this to occur is low. Based on the data provided, the Panel concluded that this food enzyme does not give rise to safety concerns under the intended conditions of use.

KEYWORDS

1-phosphatidyl-1D-myo-inositol-4,5-bisphosphate inositoltrisphosphohydrolase, EC 3.1.4.11, food enzyme, genetically modified microorganism, phosphoinositide phospholipase C, *Pseudomonas fluorescens*

CONTENTS

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 microorganisms or products thereof including a product obtained by a fermentation process using microorganisms: (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 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.

On 8 September 2021, a new application has been introduced by the applicant 'DSM Food Specialities B.V.' for the authorisation of the food enzyme Phosphoinositide phospholipase C from a genetically modified strain of *Pseudomonas fluorescens* (strain PIC).

1.1.2 | Terms of reference

The European Commission requests the European Food Safety Authority to carry out the safety assessment and the assessment of possible confidentiality requests of the following food enzyme: Phosphoinositide phospholipase C from a genetically modified strain of *Pseudomonas fluorescens* (strain PIC) in accordance with Regulation (EC) No 1331/2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

1.1.3 | Interpretation of the terms of reference

The present scientific opinion addresses the request to carry out the safety assessment of the food enzyme phosphoinositide phospholipase C from the genetically modified *Pseudomonas fluorescens* strain PIC.

[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.

2 | DATA AND METHODOLOGIES

2.1 | Data

The applicant has submitted a dossier in support of the application for authorisation of the food enzyme phosphoinositide phospholipase C from a genetically modified *Pseudomonas fluorescens* strain PIC.

Additional information was requested from the applicant during the assessment process on 12 April 2022 and 14 December 2022 and received on 29 September 2022 and 28 September 2023 (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 guidance on the 'Food manufacturing processes and technical data used in the exposure assessment of food enzymes' (EFSA CEP Panel, 2023).

2.3 | Public consultation

According to Article 32c(2) of Regulation (EC) No 178/2002[3] and to the Decision of EFSA's Executive Director laying down the practical arrangements for the pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 5 to 26 May 2023, for which no comments were received.

3 | ASSESSMENT

IUBMB nomenclature	Phosphoinositide phospholipase C
Systematic name	1-phosphatidyl-1D-myo-inositol-4,5-bisphosphate inositoltrisphosphohydrolase
Synonyms	triphosphoinositide phosphodiesterase; phosphoinositidase C; 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase; monophosphatidylinositol phosphodiesterase; phosphatidylinositol phospholipase C
IUBMB no	EC 3.1.4.11
CAS no	63551-76-8
EINECS no	849-293-4

Phosphoinositide phospholipases C catalyse the hydrolysis of phosphatidyl inositol at the sn-3 position of phospholipids, resulting in the generation of 1,2-diacylglycerol and inositol phosphate. The enzyme under application is intended to be used in the processing of fats and oils for the production of refined edible fats and oils by degumming.

3.1 | Source of the food enzyme

The phosphoinositide phospholipase C is produced with the genetically modified bacterium *Pseudomonas fluorescens* strain PIC (DS 71006), which is deposited at the Westerdijk Fungal Biodiversity Institute Culture Collection (the Netherlands) with the deposit number CBS 146324.[4]

The production strain was identified as *P. fluorescens* by whole genome sequence (WGS) analysis, which showed an average nucleotide identity of 99.02% with the reference strain *P. fluorescens* ASM523369v1 (NCBI Assembly resource GCF_900215245.1). WGS was also used to search for genes involved in toxin production and virulence factors, and none were found.[5]

[3]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, p. 1–24.

[4]Technical dossier/Node C.4.1/Annex 7.

[5]Technical dossier/Node C.4.1/Annex 3.

3.1.1 | Characteristics of the parental and recipient microorganism

The parental strain is the natural isolate *P. fluorescens* Biovar I strain MB101. This strain was modified by deleting the *pyrF* gene (encoding orotidine-5′-phosphate decarboxylase) and inserting the *Escherichia coli lacI* gene near the levansucrase (*lsc*) locus under the control of the *lacIQ1* promoter, giving rise to recipient strain DC454.[6] For these modifications, plasmids containing antimicrobial resistance marker genes for kanamycin (*kanR*), ampicillin (*bla*) and tetracycline (*tetA* and *tetR*) were used.[7]

3.1.2 | Characteristics of introduced sequences

The sequence encoding the phosphoinositide phospholipase C ([redacted]) was isolated from a soil sample. It was subsequently modified to improve the thermal stability of the enzyme and codon-optimised to match the codon usage of *P. fluorescens*. The amino acid sequence of the enzyme shows the highest homology with the *Bacillus cereus* phosphoinositide phospholipase C and other phosphoinositide phospholipases C from related *Bacillus* species. The [redacted] coding sequence was placed under the control of a chimeric promoter ([redacted]) based on the [redacted] promoters, and the [redacted] terminator from the [redacted].

The [redacted] gene (encoding [redacted]) from [redacted], which enables growth in the absence of uridine, was used as a [redacted].

Plasmid pDOW1169_BD27719 was derived from pMYC5088, a self-replicating, non-conjugative and non-mobilisable derivative of the broad host-range RSF1010 plasmid. It also contains fragments from the broad host-range plasmid [redacted] and the [redacted] plasmid [redacted]. The plasmid pDOW1169_BD27719 lacks the tetracycline resistance genes (*tetA* and *tetR*) originally present in pMYC5088; it harbours the [redacted] expression cassette and the [redacted] gene described above.[8]

3.1.3 | Description of the genetic modification process

The purpose of the genetic modification was to enable the production strain to synthesise the phosphoinositide phospholipase C. For this, plasmid pDOW1169_BD27719 was introduced in the recipient strain by electroporation, resulting in the production strain PIC.

WGS data showed that the production strain PIC contains multiple copies of plasmid pDOW1169_BD27719, lacks the native *pyrF* gene and has the *lacI* gene integrated into its chromosome.[9] WGS analysis also showed the absence of antimicrobial resistance genes and backbone sequences of the plasmids involved in the construction of the recipient strain.[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 *P. fluorescens* PIC differs from the recipient strain in its capacity to produce the phosphoinositide phospholipase C and in its ability to grow in the absence of uridine. It also differs in the presence of the *lac* operator, which enables the induced expression of the phosphoinositide phospholipase C gene.

The production strain does not contain antimicrobial resistance genes, including those used during the genetic modifications, as shown by WGS analysis.[11]

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,[12] with food safety procedures based on Hazard Analysis and Critical Control Points and in accordance with current good manufacturing practice.[13]

[6]Technical dossier/Node C.4.1/06.Source of the food enzyme – confidential.
[7]Technical dossier/Node C.4.1/Annex 3.
[8]Technical dossier/Node C.4.1/06.Source of the food enzyme - confidential.
[9]Technical dossier/Node C.4.1/Annex 3.
[10]Technical dossier/Node C.4.1/Annex 3.
[11]Technical dossier/Node C.4.1/Annex 3.
[12]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.
[13]Technical dossier/Node C.4.2/Annex 11.

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. Isopropylthio-β-galactoside (IPTG), a lactose analogue, is added to the medium to induce the enzyme synthesis. After completion of the fermentation, the solid biomass is removed from the fermentation broth by filtration. The filtrate containing the enzyme is further purified and concentrated, including an ultrafiltration step in which enzyme protein is retained, while most of the low molecular mass material passes the filtration membrane and is discarded.[14] 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.[15]

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

3.3 | Characteristics of the food enzyme

3.3.1 | Properties of the food enzyme

The phosphoinositide phospholipase C is a single polypeptide chain of 298 amino acids.[16] The molecular mass of the mature protein, calculated from the amino acid sequence, is around 34 kDa. The food enzyme was analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A consistent protein pattern was observed across all batches. The gels showed the target protein migrating between the marker proteins of 31 and 36.5 kDa in all batches, consistent with the expected molecular mass of the enzyme.[17] No other enzymatic activities were reported.

The in-house determination of phosphoinositide phospholipase C activity is based on the hydrolysis of 4-methylumbelliferyl myo-inositol-1-phosphate (reaction conditions: pH 7.5, 37°C, 1 min). The enzymatic activity is determined by measuring the release of 4-methylumbelliferone spectrophotometrically at 380 nm and is expressed in Inositol Phosphate Releasing Unit/g (IPRU/g). One IPRU is defined as the amount of enzyme required to liberate 1 µmol of 4-methylumbelliferone in 1 min under the assay conditions.[18]

The food enzyme has a temperature optimum around 40°C (pH 7.5) and a pH optimum around pH 6 (37°C). Thermostability was tested after incubation of the food enzyme for 5 min at different temperatures. The enzyme activity decreased above 67°C, showing no residual activity after pre-incubation at 75°C.[19]

3.3.2 | Chemical parameters

Data on the chemical parameters of the food enzyme were provided for three batches (Table 1).[20] The mean total organic solids (TOS) of the three food enzyme batches for commercialisation was 11.8% and the mean enzyme activity/TOS ratio was 101.9 IPRU/mg TOS.

TABLE 1 Composition of the food enzyme.

Parameters	Unit	1	2	3
Phosphoinositide phospholipase C activity	IPRU/g batch[a]	12,430	10,150	12,910
Protein	%	8.1	7.2	10.5
Ash	%	0.5	0.5	0.8
Water	%	89.4	88.8	84.5
Total organic solids (TOS)[b]	%	10.1	10.7	14.7
Activity/TOS ratio	IPRU/mg TOS	123.1	94.9	87.8

[a]IPRU: Inositol Phosphate Releasing Unit (see Section 3.3.1).
[b]TOS calculated as 100% – % water – % ash.

[14]Technical dossier/ Node C.4.2/*07. Manufacturing process* and Annex 12.
[15]Technical dossier/ Node C.4.2/Annex 13 and Additional information September 2022/Annex 22.
[16]Technical dossier/ Node C.4.1/Annex 3 Confidential. Appendix 6.
[17]Technical dossier/ Node C.4.3/Annex 16.
[18]Technical dossier/ Node C.4.3/Annex 15.
[19]Technical dossier/ Node C.4.3/Annex 17.
[20]Technical dossier/ Node C.3.1/Annex 1 and Additional information September 2022/Annex 1.

3.3.3 | Purity

The lead content in the three commercial batches was below 2 mg/kg, which complies with the specification for lead (≤ 5 mg/kg) as laid down in the general specifications for enzymes used in food processing (FAO/WHO, 2006).[21,22]

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).[23] No antimicrobial activity was detected in any of the tested batches.[24]

IPTG was used as an inducer in the fermentation process. IPTG was not detected in the food enzyme, but the Panel noted that the analytical method had a high limit of detection (LoD) (23 mg/kg) and poor recovery (10%). However, any residual IPTG is expected to be removed by repeated washing applied during the degumming process.

The Panel considered that the information provided on the purity of the food enzyme was 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 in three independent batches analysed in triplicate. One millilitre of product was added to 100 mL of a medium selective for pseudomonads, plated and incubated at 37°C for 4 days. No colonies were produced. A positive control was included.[25]

The absence of recombinant DNA in the food enzyme was demonstrated by polymerase chain reaction (PCR) analysis of three batches in triplicate. No DNA was detected with primers that would amplify a 179-bp fragment specific to the production strain, with an LoD of 10 ng spiked DNA/g food enzyme.[26]

3.4 | Toxicological data

The need for toxicity testing is waived when it can be demonstrated that there is no (or negligible) carry-over of the food enzyme TOS into the final food products (EFSA CEP Panel, 2021).

In degumming of fats and oils, the food enzyme-TOS is water-soluble and is expected to follow the aqueous fraction and not the lipid fraction. Experimental evidence has been supplied by industry confirming this expectation (> 99% removal; EFSA CEP Panel, 2023). Consequently, the Panel considered that toxicity testing, other than allergenicity, was not necessary for this food manufacturing process.

3.4.1 | 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 phosphoinositide phospholipase C produced with *P. fluorescens* strain PIC 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.[27]

No information was available on oral and respiratory sensitisation or elicitation reactions of this phosphoinositide phospholipase C.

Allergic reactions to phospholipases from insect bites have been reported, as well as food allergy to a phospholipase from a red food colouring pigment (Ohgiya et al., 2009). However, the phosphoinositide phospholipase C does not possess sequence homologies with these phospholipases and no allergic reactions upon dietary exposure to phosphoinositide phospholipase have been reported in the literature.

Yeast extract, a known source of allergens, is present in the media fed to the microorganisms. However, during the fermentation process, this product will be degraded and utilised by the microorganisms for cell growth, cell maintenance and production of enzyme protein. In addition, the microbial biomass and fermentation solids are removed. Taking into account the fermentation process and downstream processing, the Panel considered that potentially allergenic residues from this source are not expected to be present in the food enzyme.

[21]Technical dossier/ Node C.3.1/*05*. Identity of the enzyme and specifications p. 1 and Annexes 1 and 2.

[22]LoD: Pb = 0.01 μg/L.

[23]Technical dossier/ Node C.3.1/05. Identity of the enzyme and specifications p. 1 and Annexes 1 and 2.

[24]Technical dossier/ Node C.3.1/05. Identity of the enzyme and specifications p. 1 and Annexes 1 and 2.

[25]Technical dossier/Node C.4.1/Annex 8.

[26]Technical dossier/Additional information September 2023/Annex 6.

[27]Technical dossier/Annex 10 and 18 and Additional information September 2022/Annex 23.

The Panel considered that the risk of allergic reactions upon dietary exposure to this food enzyme cannot be excluded, but the likelihood is low.

3.5 | Dietary exposure

3.5.1 | Intended use of the food enzyme

The food enzyme is intended to be used in the processing of fats and oils for the production of refined edible fats and oils by degumming at a recommended use level of 1.5–15.3 mg TOS/kg vegetable oils.[28]

When added to crude oil, phosphoinositide phospholipase C hydrolyses phosphatidyl inositol into inositol phosphate and diacylglycerol. The hydrophilic inositol phosphate together with the phosphoinositide phospholipase C migrate into the aqueous phase and are subsequently removed as a water-based sludge.[29] The enzymatic treatment helps to reduce the amount of gum phospholipids, resulting in higher oil yields and cleaner final products. The food enzyme–TOS is removed by repeated washing applied during the degumming process (EFSA CEP Panel, 2023).

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 refined vegetable oils is negligible. Consequently, a dietary exposure was not calculated.

3.6 | Margin of exposure

Since the estimation of dietary exposure and toxicity testing were considered unnecessary by the Panel, the margin of exposure was not calculated.

4 | CONCLUSIONS

Based on the data provided and the removal of TOS during the intended food production process, the Panel concluded that the food enzyme phosphoinositide phospholipase C produced with the genetically modified *Pseudomonas fluorescens* strain PIC 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

Dossier 'Phosphoinositide phospholipase C from a GM strain of *Pseudomonas fluorescens* (PIC)'. February 2022. Submitted by DSM Food Specialties B.V.

Additional information. September 2022. Submitted by DSM Food Specialties B.V.

Additional information. September 2023. Submitted by DSM Food Specialties B.V.

ABBREVIATIONS

CAS	Chemical Abstracts Service
CEP	EFSA Panel on Food Contact Materials, Enzymes and Processing Aids
EINECS	European Inventory of Existing Commercial Chemical Substances
FAO	Food and Agricultural Organization of the United Nations
GMO	genetically modified organism
IUBMB	International Union of Biochemistry and Molecular Biology
kDa	kiloDalton
LoD	limit of detection
PCR	polymerase chain reaction
TOS	total organic solids
WGS	whole genome sequence
WHO	World Health Organization

[28]Technical dossier/Node C.4.9.

[29]Technical dossier/Node C.4.8.

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

REQUESTOR

European Commission

QUESTION NUMBER

EFSA-Q-2021-00654

COPYRIGHT FOR NON-EFSA CONTENT

EFSA may include images or other content for which it does not hold copyright. In such cases, EFSA indicates the copyright holder and users should seek permission to reproduce the content from the original source.

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.

LEGAL NOTICE

The scientific output published implements EFSA's decision on the confidentiality requests submitted on specific items. As certain items have been awarded confidential status by EFSA, they are consequently withheld from public disclosure by redaction.

REFERENCES

EFSA (European Food Safety Authority). (2009a). Guidance of EFSA prepared by the scientific panel of food contact material, enzymes, Flavourings and processing aids on the submission of a dossier on food enzymes. *EFSA Journal*, *7*(8), 1305. https://doi.org/10.2903/j.efsa.2009.1305

EFSA (European Food Safety Authority). (2009b). Guidance of the scientific committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: General principles. *EFSA Journal*, *7*(5), 1051. https://doi.org/10.2903/j.efsa.2009.1051

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids). (2019). Statement on the characterisation of microorganisms used for the production of food enzymes. *EFSA Journal*, *17*(6), 5741. https://doi.org/10.2903/j.efsa.2019.5741

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes, Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Liu, Y., & Chesson, A. (2021). Statement on the process-specific technical data used in exposure assessment of food enzymes. *EFSA Journal*, *19*(12), 7010. https://doi.org/10.2903/j.efsa.2021.7010

EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., van Loveren, H., Vernis, L., Zorn, H., Roos, Y., Apergi, K., … Chesson, A. (2023). Food manufacturing processes and technical data used in the exposure assessment of food enzymes. *EFSA Journal*, *21*(7), 8094. https://doi.org/10.2903/j.efsa.2023.8094

EFSA GMO Panel (EFSA Panel on Genetically Modified Organisms). (2010). Scientific opinion on the assessment of allergenicity of GM plants and micro-organisms and derived food and feed. *EFSA Journal*, *8*(7), 1700. https://doi.org/10.2903/j.efsa.2010.1700

FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). (2006). General specifications and considerations for enzyme preparations used in food processing in compendium of food additive specifications. 67th meeting. *FAO JECFA Monographs*, *3*, 63–67. https://www.fao.org/3/a-a0675e.pdf

Ohgiya, Y., Arakawa, F., Akiyama, H., Yoshioka, Y., Hayashi, Y., Sakai, S., Ito, S., Yamakawa, Y., Ohgiya, S., Ikezawa, Z., & Teshima, R. (2009). Molecular cloning, expression, and characterization of a major 38-kd cochineal allergen. *Journal of Allergy and Clinical Immunology*, *123*, 1157–1162. https://doi.org/10.1016/j.jaci.2008.12.1111

How to cite this article: EFSA CEP Panel (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids), Lambré, C., Barat Baviera, J. M., Bolognesi, C., Cocconcelli, P. S., Crebelli, R., Gott, D. M., Grob, K., Lampi, E., Mengelers, M., Mortensen, A., Rivière, G., Steffensen, I.-L., Tlustos, C., Van Loveren, H., Vernis, L., Zorn, H., Roos, Y., Fernàndez-Fraguas, C., Gomes, A. … Chesson, A. (2024). Safety evaluation of the food enzyme phosphoinositide phospholipase C from the genetically modified *Pseudomonas fluorescens* strain PIC. *EFSA Journal*, *22*(1), e8513. https://doi.org/10.2903/j.efsa.2024.8513