FOOD STANDARDS Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

20 September 2023
262-23

Supporting document

Risk and Technical Assessment – Application A1271

Cellulase from GM *Aspergillus niger* as a processing aid

Executive summary

Novozymes Australia Pty Ltd has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of the enzyme cellulase (EC 3.2.1.4) as a processing aid in brewing and the production of distilled alcohol (potable alcohol)[1]. The enzyme is a protein engineered variant of cellulase sourced from genetically modified (GM) *Aspergillus niger* containing the cellulase gene from *Trichoderma reesei*.

The proposed use of the cellulase enzyme as a processing aid in brewing and the production of potable alcohol is consistent with its typical function of catalysing the endohydrolysis of (1→4)-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans. Cellulase performs its technological purpose during the production of food and is not performing the technological purpose in the food for sale. It is therefore functioning as a processing aid for the purposes of the Code.

There are relevant identity and purity specifications for the enzyme in the Code and the applicant provided evidence that their enzyme meets these specifications.

A. *niger* has a long history of safe use as a production microorganism of enzyme processing aids, including several that are already permitted in the Code. The production organism is neither pathogenic nor toxigenic. Analysis of the GM production strain confirmed the presence and stability of the inserted DNA.

Bioinformatics analysis found no significant homology of the cellulase enzyme itself with any known toxins or food allergens. Toxicity testing of the cellulase enzyme showed no evidence of genotoxicity *in* vitro and the no observed adverse effect level (NOAEL) in a 90-day oral toxicity study in rats was 1233 mg total organic solids (TOS)/kg body weight (bw)/day, the highest dose tested.

The theoretical maximum daily intake (TMDI) of the TOS from the cellulase preparation was calculated to be 0.05 mg TOS/kg bw. A comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of approximately 25,000.

[1] The application refers to the production of ‘distilled alcohol’, however the term ‘potable alcohol’ can be used as an alternative term. The Code permits the use of certain enzymes in ‘potable alcohol’ rather than ‘distilled alcohol’ and the applicant confirmed that ‘potable alcohol’ is appropriate in this case.

Based on the reviewed data it is concluded that in the absence of any identifiable hazard, an acceptable daily intake (ADI) ‘not specified’ is appropriate.

Contents

1 Introduction

Novozymes Australia Pty Ltd (Novozymes) has applied to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of a protein engineered variant of the enzyme cellulase (EC 3.2.1.4) as a processing aid. This cellulase enzyme is produced from a genetically modified (GM) BO-1 strain of *Aspergillus niger* containing the cellulase gene from *Trichoderma reesei*.

The enzyme is intended to be used as a processing aid in brewing and the production of distilled alcohol at the minimum level required to achieve the desired effect, in accordance with the principles of Good Manufacturing Practice (GMP). Although the application refers to the production of 'distilled alcohol', the term 'potable alcohol' can be used as an alternative term. The Code permits the use of certain enzymes in 'potable alcohol' rather than 'distilled alcohol' and the applicant confirmed that 'potable alcohol' is appropriate in this case. Hereinafter the term 'potable alcohol' will be used.

The applicant markets a liquid preparation containing this enzyme as the active constituent under the commercial name Ultraflo Key in other countries where use of the enzyme is permitted.

The objectives of this technical and risk assessment were to:

- determine whether the proposed purpose is a solely technological purpose and that the enzyme achieves its technological purpose as a processing aid in the quantity and form proposed to be used
- evaluate potential public health and safety issues that may arise from the use of this enzyme as a processing aid in brewing and the production of potable alcohol, specifically by considering the:
  - safety and history of use of the host and gene donor organisms
  - characterisation of the genetic modification(s)
  - safety of the enzyme.

2 Food technology assessment

2.1 Identity of the enzyme

Novozymes provided relevant information regarding the identity of the enzyme, and this has been verified using the IUBMB[2] enzyme nomenclature reference database (McDonald et al 2009). Details of the identity of the enzyme are provided below.

Accepted IUBMB name:	cellulase
Systematic name:	4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase
Other names/common names:	endo-1,4-β-D-glucanase; β-1,4-glucanase; β-1,4-endoglucan hydrolase; celluase A; cellulosin AP; endoglucanase D; alkali cellulase; cellulase A 3; celludextrinase; 9.5 cellulase; avicelase; pancellase SS; 1,4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase
IUBMB enzyme nomenclature:	EC 3.2.1.4
CAS number:	9012-54-8
Reaction:	Endohydrolysis of (1→4)-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans

2.2 Manufacturing process

2.2.1 Production of the enzyme

Enzymes produced from microorganisms are typically produced by controlled fermentation followed by removal of the production microorganism, purification and concentration of the enzyme. Final standardisation with stabilisers, preservatives, carriers, diluents, and other approved food-grade additives and ingredients is carried out after the purification and concentration steps. The formulated enzymes are referred to as enzyme preparations, which, depending upon the application in food, may be a liquid, semi-liquid or dried product. Enzyme preparations may contain either one major active enzyme that catalyses a specific reaction during food processing or two or more active enzymes that catalyse different reactions (FAO/WHO 2020a).

2.2.2 Specifications for identity and purity

There are international general specifications for enzyme preparations used in the production of food. These have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in its Compendium of Food Additive Specifications (FAO JECFA Monographs 26 (2021)), and in the United States Pharmacopeial Convention (2022) Food chemicals codex 13th edition). These specifications are included in earlier publications of the primary sources listed in section S3—2 of Schedule 3 of the Code, and enzymes used as processing aid need to meet either of these specifications. In addition, under JECFA, enzyme preparations must meet the specifications criteria contained in the individual monographs. In the case of cellulase from GM *A. niger*, there is no individual monograph.[3]

[2] International Union of Biochemistry and Molecular Biology.

[3] For the functional use 'enzyme preparation', the JECFA database can be searched for individual monographs: http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/

Schedule 3 of the Code also includes specifications for arsenic and heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

The applicant provided the results of analysis of three different batches of their cellulase enzyme concentrate. Table 1 provides a comparison of the results of those analyses with international specifications established by JECFA and Food Chemicals Codex, as well as those in the Code (as applicable). Based on those results, the enzyme met all relevant specifications.

Table 1 *Analysis of Novozymes' cellulase enzyme concentrate compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes*

Test parameters	*Novozymes' test results*	*Specifications*		
		JECFA	*Food Chemicals Codex*	*The Code section S3—4*
Lead (mg/kg)	ND	≤5	≤5	≤2
Arsenic (mg/kg)	ND	-	-	≤1
Cadmium (mg/kg)	ND	-	-	≤1
Mercury (mg/kg)	ND	-	-	≤1
Coliforms (cfu/g)	<4	≤30	≤30	-
***Salmonella* (in 25 g)**	ND	Absent	Negative	-
***Escherichia coli* (in 25 g)**	ND	Absent	-	-
Antimicrobial activity	ND	Absent	-	-

ND= not detected. Limits of detection 0.5 mg/kg for lead, 0.3 mg/kg for arsenic, 0.05 mg/kg for cadmium, 0.05 mg/kg for mercury
cfu = colony forming units

The specification for the enzyme preparation used by Novozymes (as provided in section A.5 of the application) includes a test for the absence of the production strain.

2.3 Technological purpose

Novozymes requested permission to use the enzyme cellulase from GM *A. niger* as a processing aid in brewing and the production of potable alcohol. The enzyme would be used at levels in accordance with the principles of GMP.

As identified by the IUBMB (section 2.1 above), cellulase catalyses the endohydrolysis of (1→4)-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans. For a schematic of the reaction catalysed by cellulase, refer to its record in the enzyme database BRENDA[4] (Chang et al 2021).

Novozymes stated that in brewing, cellulase degrades the polymeric beta-glucans present in the endosperm cell wall of grain into smaller less viscous molecules, thereby lessening the filtration time and reducing problems of haze.

In the production of potable alcohol, the technological purpose of cellulase as stated by the applicant, is to degrade gelatinised starch and dextrins into glucose and other fermentable sugars.

[4] EC explorer - BRENDA Enzyme Database (brenda-enzymes.org)

The technological purpose, as stated by the applicant, of cellulase in brewing and the production of potable alcohol is consistent with the typical function of cellulase.

Novozymes provided information on the physical and chemical properties of their enzyme preparation. Table 2 summarises this information. The enzyme is either heat-denatured or removed during the brewing process and during the distillation of potable alcohol. Therefore, the enzyme is inactivated in brewed products and potable alcohol and would have no technological effect in these foods after they are produced.

Table 2 *Cellulase enzyme preparation physical/chemical properties*

Physical/chemical properties of commercial enzyme preparation	
Appearance	Brown liquid
Temperature range	Activity up to 90°C, optimum 73-77°C
Temperature stability	Retains almost full activity up to 67°C, residual activity drastically dropped at 80°C and negligible activity remained at 95°C
pH range and optimum	Activity within range pH 3.0-8, optimal activity at pH 3-5 at 37°C

2.4 Allergen considerations

Novozymes provided information as Confidential Commercial Information (CCI) about the raw materials used during the fermentation process to produce their cellulase enzyme. Novozymes also confirmed that analytical testing shows no detectable amounts of the relevant allergens (to the raw materials used) that are required to be declared by the Code (Standard 1.2.3 and Schedule 9) in the enzyme preparation. Based on the information provided, FSANZ considers that the allergens of concern required to be declared by the Code are highly unlikely to be present in Novozymes final commercial enzyme preparation (a mixture of the ultra-filtered enzyme concentrate and sodium benzoate, potassium sorbate, sorbitol and water).

2.5 Conclusion

FSANZ concludes that the use of this cellulase enzyme as a processing aid in brewing and the production of potable alcohol is consistent with its typical function of catalysing the endohydrolysis of (1→4)-β-D-glucosidic linkages in cellulose, lichenin and cereal β-D-glucans.

Cellulase performs its technological purpose during brewing and the production of potable alcohol, after which it is inactivated or removed, and is not performing a technological purpose in the final food. It is therefore functioning as a processing aid for the purposes of the Code.

There are relevant identity and purity specifications for the enzyme in the Code, and the applicant provided evidence that the enzyme meets these specifications.

3 Safety assessment

The objective of this safety assessment was to evaluate any potential public health and safety concerns that may arise from the use of this enzyme as a processing aid.

Some information relevant to this section is CCI, so full details cannot be provided in this public report.

3.1 History of use

3.1.1 Host organism

A. *niger* is widely used as a production organism and host for the manufacture of food ingredients and enzymes. *A*. *niger* is recognised as neither pathogenic nor toxigenic. FSANZ has previously assessed the safety of host organisms from the *A*. *niger* BO-1 strain lineage in applications A1221, A1248 and A1252. The identity of the host organism was determined using standard molecular techniques.

3.1.2 Gene donor organism

The donor for the cellulase gene is identified as *Trichoderma reesei*.

3.2 Characterisation of the genetic modification(s)

3.2.1 Description of the DNA to be introduced and method of transformation

An expression cassette containing a gene encoding a protein engineered variant of the cellulase enzyme was introduced into the genome of the host *A. niger* strain using standard methodologies. The cellulase gene is from *Trichoderma reesei* and encodes an enzyme with nine amino acid changes compared to the wild type cellulase enzyme. The cellulase gene was placed under the control of an *A. niger*-derived promoter and terminator. The expression cassette also contained a signal peptide sequence derived from a xylanase gene from *T. reesei*, which was integrated to improve production yield, and the *amdS* selectable marker gene from *Aspergillus nidulans*, allowing transformants to be selected based on their ability to grow on media supplemented with acetamide. Data provided by Novozymes and analysed by FSANZ confirmed the identity of the protein engineered cellulase enzyme.

3.2.2 Characterisation of inserted DNA

Data provided by Novozymes and analysed by FSANZ confirmed the presence of the inserted DNA in the production strain. The applicant also provided genome sequencing results which confirmed the absence of antibiotic resistance genes in the production strain.

3.2.3 Genetic stability of the inserted gene

The assessment confirmed that the inserted gene is integrated into the genome of the production strain and does not have the ability to replicate autonomously. The inserted gene is therefore considered to be genetically stable.

To provide further evidence of the stability of the introduced gene, the applicant provided phenotypic data from large-scale fermentation of the production strain. These data confirmed that the cellulase gene is expressed over multiple generations and is stable.

3.3 Safety of the cellulase enzyme

3.3.1 History of safe use

Cellulase from *Aspergillus niger*, as well as from other microbial sources, is currently permitted as a processing aid in Schedule 18 of the Code. However, cellulase from *A. niger* containing the gene for cellulase from *T. reesei* is not permitted and does not have a history of safe use in Australia or New Zealand.

The applicant stated that the production organism, *A. niger*, has a long history of safe use as a production strain for food-grade enzyme preparations and has given rise to a number of food enzyme production strains. The applicant provided a confidential statement confirming that substantial quantities of cellulase from GM *A. niger* has been sold in a range of countries since 2022.

There are no known reports of adverse effects arising from the consumption of *A. niger* cellulase, nor *T. reesei* cellulase, when used as a processing aid.

3.3.2 Bioinformatic assessment of toxicity

A Clustal alignment (ClustalW 2.0.10[5]) was performed by the applicant comparing the cellulase produced by the production organism *A. niger* against all sequences in the Uniprot database [6] (release: 2021-02-15) that contained the search word 'toxin' and not 'fragment'. There were no toxin sequences identified with a sequence similarity to the enzyme above 20% identity, indicating that the cellulase enzyme has no substantial homology to any known toxin sequence.

3.3.3 Toxicity studies

Toxicity studies conducted with the cellulase that is the subject of this application include a 13-week repeat-dose oral gavage study in rats, and two genotoxicity studies; a bacterial reverse mutation assay (Ames test) and an *in vitro* micronucleus assay.

Animal studies

Cellulase, Batch PPC70565: Toxicity Study by Oral Gavage Administration to Han Wistar Rats for 13 Weeks (Labcorp, 2021). Regulatory Status: GLP; conducted according to OECD TG 408.

Cellulase was administered to Han Wistar (RccHan WIST strain) rats (10/sex/group) at doses of 123.3, 406.9, or 1233.1 mg TOS/kg bw/day by oral gavage for 13 weeks. The vehicle/negative control was water and the dose volume was 10 mL/kg bw/day. The rats were group housed under standard laboratory conditions of environment and husbandry.

Animals were observed daily for clinical condition. Body weight, food consumption and detailed physical examinations for signs of toxicity were recorded weekly. Sensory reactivity, grip strength, and motor activity assessments were performed on all animals in week 12. Ophthalmic examination was conducted on all animals prior to treatment and on control and high-dose animals in week 12. At the end of the treatment period, blood samples were collected for haematology and clinical chemistry analysis. All females were assessed for

[5] Clustal W2: http://www.clustal.org/clustal2

[6] UniProt database: https://www.uniprot.org

stage of oestrous cycle at study termination. All animals underwent a detailed necropsy at study termination, including full macroscopic examination of an extensive range of organs and tissues. Histopathological examination was conducted on the control and high-dose test groups, as well as one mid-dose test male that died prematurely.

One male from the mid-dose test group (406.9 mg TOS/kg bw/day) was found dead on day 71. The cause of death was undetermined: no clinical signs were observed *ante mortem* and no significant histopathological findings were observed. The study report concluded that the death of this animal was incidental and not related to treatment, given that it was a mid-dose animal. No treatment-related effects were observed on feed consumption, body weights, clinical chemistry, ophthalmology, or functional observations (functional performance or sensory reactivity). No treatment-related macroscopic or histopathological findings were observed at necropsy.

It was concluded that oral administration of cellulase was well tolerated with no evidence of any adverse findings at any of the test doses. The no observed adverse effect level (NOAEL) was therefore 1233.1 mg TOS/kg bw/day, the highest dose tested.

Genotoxicity studies

Cellulase, Batch PPC70565: Bacterial Reverse Mutation Test (Treat-and-wash method) (Covance Laboratories Ltd, 2021a). Regulatory Status: GLP; conducted according to OECD TG 471.

The potential mutagenicity of cellulase (batch PPC70565) was evaluated in *Salmonella enterica* ser. Typhimurium strains TA98, TA100, TA1535 and TA1537, and in the *Escherichia coli* strain WP2 *uvrA*, with and without metabolic activation using rat liver homogenate (S9 mix). Appropriate positive control articles, as recommended by the OECD guideline, were used. Sterile water was used as the vehicle/negative control.

Two independent mutation tests were performed according to the treat-and-wash method: the plate incorporation method (experiment I) and the pre-incubation method (experiment II). All tests were conducted in triplicate. Concentrations of cellulase up to 5000 µg TOS/mL were tested. Other concentrations used were a series of *ca* half-$\log_{10}$ dilutions (diluted with sterile water). No precipitation or toxicity was observed at any concentration of the test article.

No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed at any concentration level (up to and including 5000 µg TOS/plate), with or without S9 mix, in both experiments I and II. The expected increases in revertant colony numbers were observed with all the positive control articles used, therefore confirming the validity of the assay.

It was concluded that cellulase (batch PPC70565) showed no evidence of mutagenic activity under the conditions of the assay.

Cellulase, Batch PPC70565: In Vitro Micronucleus Test in Human Lymphocytes (Covance Laboratories Ltd, 2021b). Regulatory status: GLP; conducted according to OECD TG 487

The potential of cellulase (batch PPC70565) to induce micronuclei formation in mammalian cells was tested using human lymphocytes isolated from peripheral blood, collected and pooled from two healthy, non-smoking adult volunteers (aged 25-35 years; of unspecified sex).

The study consisted of a preliminary toxicity study (to determine concentrations for the main

test) and a main test, with test procedures being the same. In the main test, lymphocyte cultures were treated with cellulase either in the presence or absence of rat liver homogenate (S9 mix) for 3 hours; and then treated with cellulase for 20 hours in the absence of S9 mix. Before treatment with cellulase, lymphocyte cultures were stimulated with phytohaemagglutinin. Following treatment (3-hour exposure) or during treatment (20-hour exposure), cytokinesis was blocked with cytochalasin B.

Cellulase concentrations up to 5000 µg TOS/mL were tested, dosed at 10% v/v. The vehicle/negative control was water; the positive controls were mitomycin C and colchicine in the absence of S9 mix; and cyclophosphamide in the presence of S9 mix. Cultures were performed in duplicate.

Cellulase did not cause any statistically significant increases in the frequency of micronucleated binucleate cells compared with vehicle controls, either in the presence and absence of S9 mix (following 3-hour treatment); or in the absence of S9 mix (following 20-hour treatment). All positive controls induced significant increases in the proportion of cells with micronuclei, confirming the validity of the test system.

It was concluded that cellulase (batch PPC70565) did not induce micronuclei under the conditions of the study.

3.3.4 Potential for allergenicity

The applicant provided details of recent searches (2021) for amino acid sequence homology of the cellulase enzyme to known allergens, using the FARRP allergen protein database[7] and the Allergen Nomenclature database[8], using four sequence alignments: the full length protein (more than 35% identity), an 80 mer sliding window (more than 35% identity), a scaled 80 mer sliding window (more than 35% identity), and an 8 mer sliding window (100% identity).

No homology to sequences of known allergens was identified using these search parameters.

3.3.5 Assessments by other regulatory agencies

There are no known safety assessments by other regulatory agencies.

The applicant provided documentation to show that the enzyme is approved by Mexico's Regulatory Health Authority (Comision Federal para la Proteccion contra Riesgos Sanitarios; COFEPRIS). This does not constitute a safety assessment report.

3.4 Dietary exposure assessment

The objective of the dietary exposure assessment was to review the budget method calculation presented by the applicant as a 'worst-case scenario' approach to estimating likely levels of dietary exposure, assuming that all of the TOS from the cellulase enzyme preparation remained in the food.

The budget method is a valid screening tool for estimating the theoretical maximum daily intake (TMDI) of a food additive (Douglass et al 1997). The calculation is based on physiological food and liquid requirements, the food additive concentration in foods and beverages, and the proportion of foods and beverages that may contain the food additive.

[7] AllergenOnline: http://www.allergenonline.org/

[8] Allergen Nomenclature: http://allergen.org/

The TMDI can then be compared to an acceptable daily intake or a NOAEL to estimate a margin of exposure (MOE) for risk characterisation purposes. Whilst the budget method was originally developed for use in assessing food additives, it is also appropriate to use for estimating the TMDI for processing aids (FAO/WHO 2020). The method is used by international regulatory bodies and the FAO/WHO Joint Expert Committee on Food Additives (JECFA) (FAO/WHO 2021) for dietary exposure assessments for processing aids.

In their budget method calculation, the applicant made the following assumptions:

- the maximum physiological requirement for liquid is 100 mL/kg bw/day (the standard level used in a budget method calculation for non-milk beverages)
- 25% of non-milk beverages are processed
- all processed beverages contain 12% cellulose (or cellulose-derived) dry matter
- the densities of non-milk beverages are ~ 1
- all non-milk beverages contain the highest use level of 17 mg TOS/kg in the raw material (cellulose (or cellulose-derived) dry matter)
- all of the TOS from the enzyme preparation remains in the final food.

Based on these assumptions, the applicant calculated the TMDI of the TOS from the enzyme preparation to be 0.05 mg TOS/kg bw/day.

The inputs and assumptions used by FSANZ were the same as those used by the applicant. The TMDI of the TOS from the enzyme preparation based on FSANZ's calculations for non-milk beverages is 0.05 mg TOS/kg bw/day.

The estimate of the TMDI undertaken by the applicant and FSANZ will be overestimates of the dietary exposure given the conservatisms in the budget method. This includes the assumption that all of the TOS from the enzyme preparation remains in beverages whereas the applicant has stated that the enzyme is likely to either be inactivated or removed during processing. If any inactivated enzyme remained after processing, it would be present in insignificant quantities and perform no function in the final food.

4 Discussion

No public health or safety concerns were identified in the assessment of cellulase produced by GM *A. niger*. *A. niger* has a long history of safe use as a source of enzyme processing aids, including several that are already permitted in the Code. The *A. niger* host is neither pathogenic or toxigenic. Analysis of the GM production strain confirmed the presence and stability of the inserted DNA.

Bioinformatics analysis found no significant homology of the cellulase enzyme itself with any known toxins or food allergens. Toxicity testing of the cellulase enzyme showed no evidence of genotoxicity *in* vitro and the NOAEL in a 90-day oral toxicity study in rats was 1233 mg TOS/kg bw/day, the highest dose tested. The TMDI of the TOS from the cellulase preparation was calculated to be 0.05 mg TOS/kg bw. A comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of approximately 25,000.

5 Conclusion

Based on the reviewed data it is concluded that in the absence of any identifiable hazard an Acceptable Daily Intake (ADI) 'not specified' is appropriate.

6 References

Chang A, Jeske L, Ulbrich S, Hofmann J, Koblitz J, Schomburg I, Neumann-Schaal M, Jahn D, Schomburg D (2021) BRENDA, the ELIXIR core data resource in 2021: new developments and updates. Nucleic Acids Res 49:D498–D508. DOI:10.1093/nar/gkaa1025.

Covance Laboratories Ltd. (2021a) Cellulase, Batch PPC70565: Bacterial Reverse Mutation Test (Treat-and-wash method). Study Number: 8448881. Covance Laboratories Ltd, Huntingdon, UK.

Covance Laboratories Ltd. (2021b) Cellulase, Batch PPC70565: *In Vitro* Micronucleus Test in Human Lymphocytes. Study Number: 8448880. Covance Laboratories Ltd, Huntingdon, UK.

Douglass JS, Barraj LM, Tennant DR, Long WR and Chaisson CF (1997) 'Evaluation of the Budget Method for screening food additive intakes, Food Additives and Contaminants' 14:791-802.

FAO and WHO (2021) *Compendium of Food Additive Specifications. Joint FAO/WHO Expert Committee on Food Additives (JECFA), 91st Meeting – Virtual meeting, 1–12 February 2021.* FAO JECFA Monographs No. 26. Rome. https://doi.org/10.4060/cb4737en

FAO/WHO (2020) Environmental Health Criteria 240. Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 6: Dietary exposure assessment of chemicals in food. Second Edition 2020. WHO, Geneva. https://www.who.int/docs/default-source/food-safety/publications/chapter6-dietary-exposure.pdf?sfvrsn=26d37b15_6

FAO/WHO (2020a) Environmental Health Criteria 240. Principles and Methods for the Risk Assessment of Chemicals in Food. Chapter 9.1.4: Processing aids. Second Edition 2020. WHO, Geneva. Principles and methods for the risk assessment of chemicals in food (who.int)

FAO/WHO (2021) Evaluation of certain food additives: eighty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 1027.

Labcorp (2021) Cellulase, Batch PPC70565: Toxicity Study by Oral Gavage Administration to Han Wistar Rats for 13 Weeks. Study Number: 8446408. Labcorp Early Development Laboratories Ltd., Suffolk, UK.

McDonald AG, Boyce S, Tipton KF (2009) ExplorEnz: the primary source of the IUBMB enzyme list. Nucleic Acids Res 37:D593–D597. DOI:10.1093/nar/gkn582.