29 April 2021
154-21

Supporting document 1

Risk assessment – Application A1218

β-Galactosidase from *Bacillus subtilis* (Enzyme)

Executive summary

Danisco New Zealand Ltd applied to Food Standards Australia New Zealand (FSANZ) to amend Schedule 18 – Processing Aids of the Australia New Zealand Food Standards Code (the Code) to include a new source of β-galactosidase (EC 3.2.1.23) from a genetically modified (GM) strain of *Bacillus subtilis.* The source organism for the enzyme gene is *Lactobacillus delbrueckii* subsp. *bulgaricus*. The proposed use of β-galactosidase is as a processing aid in the production of dairy foods, to reduce lactose content.

FSANZ has undertaken an assessment to determine whether the enzyme achieves its technological purpose in the quantity and form proposed to be used and to evaluate public health and safety concerns that may arise from the use of this enzyme.

FSANZ concludes that the proposed use of this β-galactosidase, as an enzyme in the production of lactose reduced dairy foods, is consistent with its typical function of catalysing the hydrolysis of lactose. Analysis of the evidence provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used, which must be consistent with Good Manufacturing Practice (GMP) controls and processes, is technologically justified.

β-Galactosidase performs its technological purpose during the production of dairy foods and is not performing a technological purpose in the final food, therefore functioning as a processing aid as defined in the Code. There are relevant identity and purity specifications for the enzyme in the Code.

There are no public health and safety concerns associated with the use of β-galactosidase produced by the genetically modified *B. subtilis*, as a food processing aid at GMP levels in dairy foods.

The safety assessment did not identify any concerns associated with the host organism, *B. subtilis,* or the gene donor organism, *L. delbrueckii subsp. bulgaricus*. The host is neither pathogenic nor toxigenic and has a long history of safe use in food. Characterisation of the GM production strain confirmed both presence and stable inheritance of the inserted β-galactosidase gene. Bioinformatic analyses found no similarity of the enzyme protein to known toxins or allergens.

The no observed adverse effect level (NOAEL) in a 13-week repeated dose oral toxicity study in rats was the highest dose tested and corresponds to 1000 mg/kg bw/day total organic solids (TOS). This is more than 100-fold higher than the theoretical maximum daily intake (TMDI) estimated by FSANZ when using worst case assumptions (9.7 mg TOS/kg body weight/day), and more than 200-fold higher than FSANZ's estimate of exposure over a long period of time or a lifetime (4.8 mg TOS/kg body weight/day), based on the proposed use, as stated in the Application.

Based on the reviewed toxicological and dietary exposure data, it was concluded that an acceptable daily intake (ADI) 'not specified' is appropriate.

Contents

1 Introduction

Danisco New Zealand Ltd (Danisco) applied to FSANZ for permission to use the enzyme β-galactosidase (EC 3.2.1.23) as a processing aid in dairy foods, to reduce lactose content. This β-galactosidase is derived from a strain of *Bacillus subtilis* which is genetically modified to overexpress the β-galactosidase gene from *Lactobacillus delbrueckii bulgaricus.* The applicant states that the enzyme would be used at the minimum level required to achieve the desired effect and in accordance with the principles of Good Manufacturing Practice (GMP).

There are permissions for β-galactosidase from other microbial sources in the Australia New Zealand Food Standards Code (the Code), including one for the production of lactose reduced dairy foods. If permitted following a pre-market assessment, the β-galactosidase that is the subject of this application would provide an additional option for manufacturers seeking to reduce the lactose content of dairy foods.

The application was made by Danisco on behalf of DuPont Industrial Biosciences (IB), the manufacturer and marketer of the enzyme. On 4 February 2021, FSANZ was informed that ownership of Danisco had changed from DuPont to International Flavours & Fragrances Inc. (IFF). Danisco remains the applicant for this application.

1.1 Objectives of the assessment

The objectives of this technical and safety assessment were to:

- determine whether the proposed purpose is a solely technological purpose (function) 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, produced by a genetically modified organism, as a processing aid, specifically by considering the:
  - history of use of the host and gene donor organisms
  - characterisation of the genetic modification(s)
  - safety of the enzyme.

2 Food technology assessment

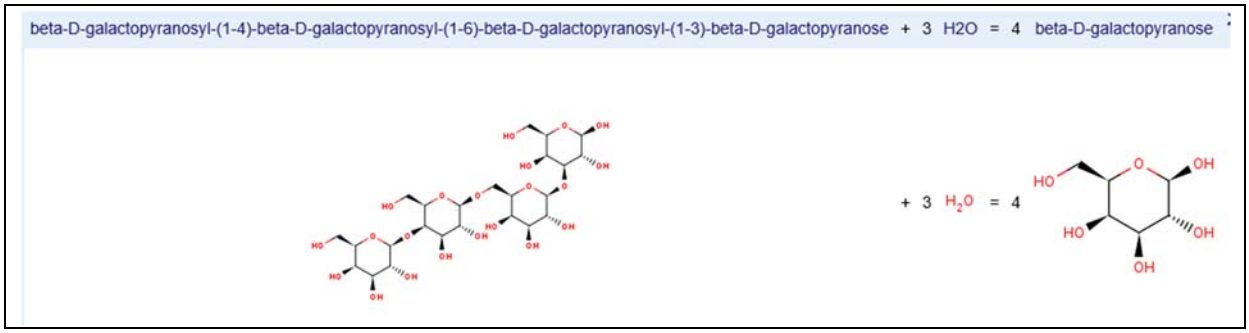
2.1 Characterisation of the enzyme

2.1.1 Identity of the enzyme

The applicant provided relevant information regarding the identity of the β-galactosidase enzyme. FSANZ verified this using an appropriate enzyme nomenclature reference (IUBMB 2020).

Accepted IUBMB[1] name:	β-galactosidase
Systematic name:	β-D-galactoside galactohydrolase
Other names:	lactase; β-lactosidase; maxilact; hydrolact; β-D-lactosidase; S 2107; lactozym; trilactase; β-D-galactanase; oryzatym; sumiklat
IUBMB enzyme nomenclature:	EC 3.2.1.23
CAS[2] number:	9031-11-2
Reaction:	Hydrolysis of terminal non-reducing β-D-galactose residues in β-D-galactosides (Figure 1).

Figure 1 *Representation of a hydrolysis reaction of a β-D-galactoside catalysed by β-galactosidase*



Source: BRENDA:EC 3.2.1.23 (https://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.23)

2.2 Manufacturing process

2.2.1 Production of the enzyme

The enzyme is produced using a standard manufacturing process comprised of a three-part process: fermentation (growth of organism and production of enzyme), recovery (separation of cell mass from enzyme and concentration/purification of enzyme) and formulation/drying (preparation of a stable enzyme formulation). Detail is provided in Appendix A of the application.

[1] International Union of Biochemistry and Molecular Biology.
[2] Chemical Abstracts Service.

The applicant states that all raw materials used in the fermentation and recovery process for the β-galactosidase enzyme concentrate are standard ingredients used in the enzyme industry. All the raw materials are reported to conform to the specifications of the Food Chemical Codex, 6th edition (FCC 2008), except for those raw materials which do not appear in the FCC. For those not appearing in the FCC, according to the applicant, the enzyme manufacturer has internal requirements in line with FCC requirements and has in place a supplier quality program and manufactures their β-galactosidase in accordance with GMP.

Enzymes are generally sold as enzyme preparations, which consist of the enzyme(s) and other ingredients, to facilitate their storage, sale, standardisation, dilution or dissolution. Full details on the manufacturing process, raw materials and ingredients used in the production of the β-galactosidase enzyme preparation were provided as Confidential Commercial Information by the applicant. The fermentation medium used for culturing the *B. subtilis* production strain in relation to the potential for allergenicity is discussed in Section 3.4 below.

2.2.2 Specifications

There are international 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 and in the Food Chemicals Codex (FCC). These specifications are included in the primary sources listed in section S3—2 of Schedule 3 of the Code and enzymes used as a processing aid must meet either of these specifications. Schedule 3 of the Code also includes specifications for 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 the β-galactosidase preparation. Table 1 provides a comparison of the analyses with international specifications established by JECFA and Food Chemicals Codex, as well as those in the Code (as applicable). Based on these results, the enzyme preparation met all relevant specifications for metals and the microbiological criteria.

Table 1 *Comparison of manufacturer's β-galactosidase preparation compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes*

Analysis	Analysis provided by manufacturer*	Specifications		
		JECFA (2006)	*Food Chemicals Codex (FCC, 2018)*	*Australia New Zealand Food Standards Code (section S3—4)*
Lead (mg/kg)	<0.1	≤ 5	≤ 5	≤2
Arsenic (mg/kg)	<0.1	-	-	≤1
Cadmium (mg/kg)	<0.01	-	-	≤1
Mercury (mg/kg)	<0.01	-	-	≤1
Coliforms (cfu/g)	<1	≤30	≤30	-
***Salmonella* (in 25 g)**	Absent	Absent	Negative	-
***E. coli* (in 25 g)**	Absent	Absent	-	-

* across three samples

2.3 Technological purpose of the processing aid

β-Galactosidase is intended for use in dairy products such as milk, UHT milk, yoghurt, ice cream and cheese. Dairy products naturally contain lactose, a galactoside to which some consumers are intolerant (Stipanuk 2000). As described in the reaction in section 2.1.1 above, β-galactosidase catalyses the hydrolysis of lactose, producing the monosaccharides glucose and galactose. Thus, β-galactosidase used in dairy processing reduces the lactose content of the dairy product.

The proposed purpose of the β-galactosidase enzyme, to reduce the lactose content of dairy products, is supported by scientific literature (e.g. Nagodawithana and Reed,1993).

The applicant provided information on the physical and chemical properties of the enzyme preparation. Table 2 summarises this information.

Table 2 *Physical and chemical properties of β-galactosidase enzyme preparation*

Physical and chemical properties of commercial enzyme preparation	
Enzyme activity	Minimum lactase activity 15250 SDLU/g* (from three batches)
Appearance	Clear to dark brown liquid
Temperature range	Optimum range 50 - 60°C.
Temperature stability	> 80% activity retained after 15 seconds of incubation over the temperature range 60 to 75°C. Enzyme activity significantly reduced at temperature ≥ 70°C
pH range	Maximum activity at pH 7.0.
Storage stability	Approximately 95% activity remaining after two years at 4°C

*The principle of the assay method used to measure the activity of β-galactosidase is that lactase hydrolyses o-nitrophenyl-β-D-galactopyranoside into o-nitophenol (ONP) and galactose. The reaction is stopped after ten minutes at 30°C with sodium carbonate and the liberated ONP is measured in a spectrophotometer. A detailed assay method was provided with the application.

Use of commercial enzyme preparations should follow GMP, where use is at a level that is not higher than that necessary to achieve the desired enzymatic reaction. The conditions of use of the enzyme to reduce lactose content will depend on a number of factors including the nature of the application and the individual food manufacturers' production processes. The optimum use level should be assessed and adjusted using trials that reflect their particular processes.

2.3.1 Technological justification for the enzyme

As outlined above, the enzyme can be used in the processing of dairy products to reduce the lactose content, benefiting consumers who are intolerant to lactose.

The enzyme performs its function of catalysing the hydrolysis of lactose during the production of dairy foods and is inactivated by the pasteurisation process. It is therefore performing as a processing aid as defined by the Code.

The applicant has provided evidence of a dosage advantage using the enzyme that is the subject of this application, in lactose reduction of 20-50% compared to other commercially available lactase, subject to the hydrolysis conditions used and the lactose concentration in the product (Appendix A of the application).

2.4 Food technology conclusion

FSANZ concludes that the proposed use of this β-galactosidase as an enzyme in the production of lactose reduced dairy foods, is consistent with its typical function of catalysing the hydrolysis of lactose.

Analysis of the evidence provides adequate assurance that the use of the enzyme, in the quantity and form proposed to be used, which must be consistent with GMP controls and processes, is technologically justified.

β-Galactosidase performs its technological purpose during the production of dairy products and is not performing a technological purpose in the final food, therefore functioning as a processing aid as defined in 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 of the enzyme

3.1 History of use of host and gene donor microorganisms

3.1.1 Host organism

Bacillus subtilis occurs ubiquitously in the environment and can be found in soil, water and plants. *B. subtilis* has been used for many years as a source of food-processing enzymes, and is non-pathogenic to humans (Olempska-Beer et al., 2006; US EPA 1997). FSANZ has previously assessed the safety of *B. subtilis* as the source organism for a number of food processing aids (derived from both GM and non-GM organisms). Schedule 18 of the Code currently permits the use of the following enzymes derived from *B. subtilis*: α-acetolactate decarboxylase, α-amylase, β-amylase, asparaginase, endo-1,4-beta-xylanase, β-glucanase, hemicellulase multicomponent enzyme, maltogenic α-amylase, metalloproteinase, pullulanase and serine proteinase.

B. subtilis has been granted Qualified Presumption of Safety[3] (QPS) status by the European Food Safety Authority, with the qualification that there is absence of toxigenic activity. Using the safe strain lineage concept[4], the information provided by the applicant showed that the the risk of toxin production by the production strain was very low. As shown in Section 3.3.3 below, no toxicological hazard was identified in bioassays with a representative enzyme preparation.

The production strain *B. subtilis* DH 617 was derived from the host strain *B. subtilis* BG125. The taxonomy of the production strain was confirmed as *B. subtilis,* based on 100% identity of the 16s RNA sequence.

Modifications were made to the parental *B. subtilis* strain to prepare an appropriate recipient strain for introducing the novel β-galactosidase gene and to optimise the organism for industrial enzyme production. The modifications include a series of genetic modification steps. A description of these changes was provided and has been assessed. No risks were identified.

3.1.2 Gene donor organism(s)

The gene for β-galactosidase was sourced from *Lactobacillus delbrueckii* subsp. *bulgaricus* using PCR. The information provided by the applicant stated that the donor strain for this application, *L. delbrueckii* subsp. *bulgaricus* DGCC1261, was isolated from yoghurt in 1996. Other strains have been approved for use in commercial yoghurt in Japan for over 20 years (Shortt 1999), and classed as Biosafety Level 1 organisms, based on the United States Public Health Service Guidelines[5]. This implies there are no safety concerns with the use of *L. delbrueckii* subsp. *bulgaricus* as the donor organism.

[3] For more information please see following EFSA webpage: https://www.efsa.europa.eu/en/topics/topic/qualified-presumption-safety-qps

[4] The term *safe strain lineage* refers to related strains that have all been derived by genetic modification from a single precursor that has been thoroughly characterized and shown to be non-toxigenic and non-pathogenic (Pariza and Cook 2010; EFSA 2018).

[5] For more information please see the following CDC webpage: https://www.cdc.gov/biosafety/publications/bmbl5/index.htm

3.2 Characterisation of the genetic modification[6]

3.2.1 Description of DNA to be introduced

A typical expression cassette was generated, containing the β-galactosidase gene flanked by a specific promoter and terminator. The sequence of the gene was identical to the sequence found in the donor organism.

3.2.2 Characterisation of inserted DNA

DNA sequencing was used to characterise the insertion of the expression cassette in DH617. Evidence provided by the applicant indicated the enzyme gene had been integrated into the genome of the host, had the expected sequence and had not undergone rearrangement.

3.2.3 Genetic stability of the inserted gene

A genotypic analysis was performed on DH617, comparing sequences before and after a model fermentation run. Evidence provided confirmed the expression of the gene was consistent across several generations, indicating the production strain is genetically stable.

3.3 Potential toxicity of the processing aid

3.3.1 History of safe use of the enzyme

L. delbrueckii bulgaricus β-galactosidase (EC 3.2.1.23) that has been expressed in *B. subtilis* is approved for use as a food processing aid in Denmark and France, and has been used for processing dairy products in Europe since 2019. FSANZ is not aware of any reports of safety concerns associated with the use of the enzyme.

3.3.2 Bioinformatics concerning potential for toxicity

A BLAST search was performed using the mature amino acid sequence of β-galactosidase in the UniProt[7] database. With a conservative E-value[8] threshold of 0.1, the top 1000 matches were to β-galactosidase and related enzymes and isoforms found across various species. No matches were found to any toxins or venoms.

3.3.3 Evaluation of enzyme toxicity studies

The *L. delbrueckii bulgaricus* β-galactosidase test item used in the toxicity studies was expressed in an alternative *B. subtilis* research and development strain. Both *L. delbrueckii bulgaricus* β-galactosidase enzymes were identical and production strains were derived from a common lineage. The only difference between the *B. subtilis* strain used to express the toxicology test item and the *B. subtilis* strain under review was a single genomic deletion of a metabolic enzyme distinct from the genetic modifications driving β-galactosidase expression. This difference is not considered to be of toxicological significance such that FSANZ considers the test article to be representative to the material that is the subject of this application.

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[6] Due to the requirements of Confidential Commercial Information, specific information cannot be provided in this section

[7] https://www.uniprot.org/ .

[8] The E value (or Expect value) indicates the significance of a match found when searching a sequence database. The closer an E value gets to zero, the less likely an alignment could have been produced by chance.

Genotoxicity

Bacterial reverse mutation test (DuPont, 2019). Regulatory Status: GLP; conducted according to OECD test guideline (TG) 471.

The potential mutagenicity of β-galactosidase was evaluated in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* strain WP2 *uvrA*, with and without metabolic activation using rat-liver homogenate (S9). Mutation tests, pre-incubated with the test item, were conducted just once, using three experimental replicates per treatment. The maximal concentration of the test item was 5000 µg/plate of protein (equal to 6429 ug TOS/plate), based on the findings of a preliminary dose range finding study.

Positive controls in the absence of metabolic activation were 4-nitroquinoline-*N*-oxide (TA100, WP2 *uvrA*), acridine mutagen ICR-191 (TA1537), 2-nitrofluorene (TA98) and *N*-methyl-*N'*nitro-*N*-nitrosoguanidine (TA1535), while the positive control in the presence of metabolic activation was 2-aminoanthracene (all strains). Sterile deionized water was used as the vehicle control.

No concentration-related increases in revertant colonies were observed in cultures treated with the test item, with or without metabolic activation. All positive control treatments showed the anticipated increases in mutagenic activity demonstrating the validity of the assay. It was concluded that β-galactosidase was not mutagenic in any of the bacterial test strains tested under the conditions of this study.

In vitro mammalian chromosomal aberration test in human peripheral blood lymphocytes (DuPont, 2019). Regulatory status: GLP; conducted according to OECD test guideline TG 473

The potential of β-galactosidase to cause chromosomal aberrations in dividing mammalian cells was tested in human lymphocyte cells, isolated from peripheral blood drawn from a healthy 30 year old female volunteer. Cells were exposed to the test item dissolved in sterile water with or without S9 for 4 hours (short-term) before a 22 hour rest period, or exposed to the test item continuously for 22 hours (continuous) without S9. Positive control assays were conducted in parallel using mitomycin C in the absence of S9 and cyclophosphamide in the short-term treatment with S9.

Dose selection experiments observed cytotoxic activity at a total protein concentration of 500 µg/mL. As a result, the test item total protein concentration was adjusted to: 50-300 µg/mL in the short-term assay with metabolic activation; 75-250 µg/mL in the short-term in the absence of metabolic activation; and 25-100 µg/mL for the continuous treatment study. The adjusted concentrations were based on the preliminary dose selection experiments and chosen to achieve a mitotic reduction of approximately 55% at the maximal concentration. The experiment was conducted twice independently. Scoring was undertaken on 300 metaphase cells for each test item concentration, across both independent experiments.

There were no increases in the incidence of chromosomal aberration in human peripheral blood lymphocytes following exposure to the test item, relative to the vehicle controls, in any of the conditions tested. The positive controls demonstrated a statistically significant increase in chromosomal aberration, validating the sensitivity of the experimental methodology.
It was concluded that the test β-galactosidase did not cause chromosomal aberrations in human peripheral blood lymphoma cells, under the conditions of this study.

Animal studies

90-day repeated dose oral toxicity study in rats (DuPont, 2019). Regulatory Status: GLP; conducted according to OECD TG 408

The β-galactosidase test article was administered to Sprague-Dawley SPF Crl:CD(SD) rats (10 rats / sex / test group) at doses of 0, 250, 500 and 1000 mg/kg bw/day TOS by oral gavage for 13 weeks. The vehicle control was distilled water. Animals were housed in pairs with *ad libitum* access to food and water. Qualitative clinical observations were performed daily. Body weight, food consumption and detailed clinical observations were recorded weekly.

Ophthalmological examination was conducted on all test animals prior to treatment and at study termination in Week 13. Manipulative tests, grip strength and motor activity were assessed in week 13. Urinalysis was performed in week 13 and blood for haematological, coagulation and clinical chemistry was collected at necropsy. Gross pathology, measurement of organ weights and a histopathological examination was conducted on all animals at study termination.

No mortality occurred during the study. No treatment related effects were observed in body weight, food consumption, manipulative testing, grip strength, motor activity, urinalysis, haematology, coagulation. clinical chemistry or ophthalmology examinations in any of the test animals. No remarkable macroscopic or histopathological changes were observed at necropsy. The no observed adverse effect level (NOAEL) was 1000 mg/kg bw/day TOS, which was the highest dose tested.

3.4 Potential for allergenicity

A BLAST search was performed using the mature amino acid sequence of β-galactosidase in the AllergenOnline[9] database (queried in February 2020). Three sequence alignments were performed: full length protein (E-value 0.1), an 80 mer sliding window (>35% homology) and an 8 mer sliding window search (100% homology). Results did not identify any similarity of the β-galactosidase enzyme to known allergens.

Two case reports were available in the scientific literature describing allergic sensitisation to ingested β-galactosidase enzyme preparations (Binkley, 1996; Voisin & Borici-Mazi, 2016). Both events occurred in reaction to orally ingested β-galactosidase from *Aspergillus oryzae*, which was confirmed by the prick test method. However, considering the number of β-galactosidase enzymes approved worldwide, these two allergenic sensitivity events appear to be extremely rare events, occurring 20 years apart and arising as a reaction to an alternative β-galactosidase enzyme than the β-galactosidase enzyme under review.

The fermentation medium used for culturing the *B. subtilis* production strain contains soy meal and glucose derived from wheat sources. An ELISA could not detect any soy protein in the final product (limit of detection of 2.5 ppm), while a Bradford protein assay could not detect any protein in the commercially sourced glucose (limit of detection of 3 ppm) before being added to the fermentation medium.

[9] http://www.allergenonline.org/

3.5 Dietary Exposure Assessment

The objective of the dietary exposure assessment was to review the budget method calculation presented by the applicant as a 'worse-case scenario' approach to estimating likely levels of dietary exposure assuming all added β-galactosidase 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. The TMDI can then be compared to an ADI or a NOAEL to estimate a margin of exposure for risk characterisation purposes.

In their budget method calculation, provided in Appendix C of the application, the applicant made the following assumptions:

- The maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day.
- The maximum physiological requirement for non-milk beverages is 100 mL/kg body weight/day (the standard level used in a budget method calculation).
- 15% of solid foods and 5% of non-milk beverages contain β-galactosidase, at the maximum level of 1290 mg TOS/kg.
- All of the enzyme remains in the final food.

The applicant calculated that the TMDI of β-galactosidase, based on these assumptions, is 11.29 mg total organic solids (TOS)/kg body weight/day.

As some assumptions made by the applicant differ to those that FSANZ would have made in applying the budget method, FSANZ recalculated the TDMI in two ways:

- Firstly using the following assumptions which are highly conservative and are reflective of a first tier in estimating dietary exposure:
  - The maximum physiological requirement for solid food (including milk) is 50 g/kg body weight/day (the standard level used in a budget method calculation where there is potential for the enzyme to be in baby foods or general purpose foods that would be consumed by infants).
  - Consumption of dairy-based drinks, including flavoured milk, yoghurt drinks and whey drinks, is captured within the maximum physiological requirement for solid food (including milk). Therefore, given the application of the enzyme is only applicable to dairy products, the non-milk beverages component of the budget method was not included in the calculation.
  - 15% of solid foods contain β-galactosidase, all at the maximum level of 1290 mg TOS/kg.
  - All of the enzyme remains in the final food.
  - The concentration of β-galactosidase in any final foods will not exceed the maximum level of 1290 mg TOS/kg.

- Secondly using the following assumptions which are also conservative, but more representative of lifetime exposure and are a second tier or refinement of the estimate of dietary exposure:
  - The maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day (the standard level used in a budget method calculation where the additive is used in foods other than baby foods).

- Consumption of dairy-based drinks, including flavoured milk, yoghurt drinks and whey drinks, is captured within the maximum physiological requirement for solid food (including milk). Therefore, given the application of the enzyme is only applicable to dairy products, the non-milk beverages component of the budget method was not included in the calculation.
- 15% of solid foods contain β-galactosidase, all at the maximum level of 1290 mg TOS/kg.
- All of the enzyme remains in the final food.
- The concentration of β-galactosidase in any final foods will not exceed the maximum level of 1290 mg TOS/kg.

The TDMIs based on FSANZ's calculations are 9.7 mg TOS/kg body weight/day and 4.8 mg TOS/kg body weight/day respectively. The second TDMI is more representative of exposure over a long period of time, or over a lifetime. These are both likely to be overestimates of the dietary exposure given the conservatisms in the budget method, that it was assumed that the enzyme remains in the final food where as it is likely to be removed during processing and that dairy foods produced using the β-galactosidase enzyme are unlikely to contribute as much as 15% as a proportion of all solid foods consumed.

A comparison of the NOAEL with the TMDI's estimated by FSANZ indicates that the Margin of Exposure is more than 100 and 200 respectively.

3.6 Assessments by other regulatory agencies

A letter of approval was provided by the applicant obtained from the Ministry of Environment and Food in Denmark, outlining the permitted use of *L. delbrueckii bulgaricus* β-galactosidase produced in *B. subtilis* as dairy processing aid. Additionally, a letter from the Ministère de l'Économie et des Finances de la République française (Ministry of Economy and Finances, Republic of France) allowing the use of *L. delbrueckii bulgaricus* β-galactosidase in France for processing dairy products.

The applicant provided an externally produced GRAS opinion on the safety of *L. delbrueckii bulgaricus* β-galactosidase produced in *B. subtilis*. This opinion has not been submitted to the FDA as a GRAS notification and is not recognised by FSANZ as an assessment by other international agencies.

4 Conclusion

There are no public health and safety concerns associated with the use of β-galactosidase produced by the genetically modified *B. subtilis*, as a food processing aid at GMP levels in dairy foods.

The safety assessment did not identify any concerns associated with the host organism, *B. subtilis,* or the gene donor organism, *L. delbrueckii subsp. bulgaricus*. The host is neither pathogenic nor toxigenic and has a long history of safe use in food. Characterisation of the GM production strain confirmed both presence and stable inheritance of the inserted β-galactosidase gene. Bioinformatic analyses found no similarity of the enzyme protein to known toxins or allergens.

The NOAEL in a 13-week repeated dose oral toxicity study in rats was the highest dose tested and corresponds to 1000 mg/kg bw/day TOS. This is more than 100-fold higher than the TMDI estimated by FSANZ when using worst case assumptions (9.7 mg TOS/kg body weight/day), and more than 200-fold higher than FSANZ's estimate of exposure over a long period of time or a lifetime (4.8 mg TOS/kg body weight/day), based on the proposed use, as stated in the Application.

Based on the reviewed toxicological and dietary exposure data, it was concluded that an acceptable daily intake (ADI) 'not specified' is appropriate.

5 References

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