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

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Supporting document 1

Risk and technical assessment – Application A1246

Phospholipase A1 from GM *Aspergillus oryzae*

Executive summary

Novozymes Australia Pty Ltd (Novozymes) submitted an application to permit the use of the enzyme phospholipase A1 (EC 3.1.1.32) from a genetically modified (GM) strain of *Aspergillus oryzae* containing the phospholipase A1 gene from *Valsaria rubricosa*. The phospholipase A1 enzyme preparation is proposed for use as a processing aid in the manufacture of bakery products, in accordance with Good Manufacturing Practice (GMP) conditions.

FSANZ has undertaken an assessment and concludes that the proposed use of the enzyme as a processing aid to produce bakery products is technologically justified. Analysis of the evidence supplied by the applicant provides adequate assurance that the enzyme achieves its technological function in the quantity and form proposed. The enzyme meets international purity specifications.

No public health and safety concerns were identified in the assessment of phospholipase A1 from GM *A. oryzae* under the proposed use conditions. *A. oryzae* 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. oryzae* host is neither pathogenic or toxigenic. The assessment confirmed both presence and genetic stability of the inserted DNA.

Toxicology studies conducted with the phospholipase A1 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. A no observed adverse effect level (NOAEL) of 957 mg total organic solids (TOS)/kg bw/day was established in rats. The theoretical maximum daily intake (TMDI) based on FSANZ's calculations for solid food is 0.12 mg TOS/kg body weight/day. Comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of around 8000. No evidence of genotoxicity was found in either genotoxicity assay.

Recent bioinformatics searches were conducted by comparing the sequence of the phospholipase A1 to those of known allergens. No significant matches with food allergens were found. A match with an occupational respiratory allergen was identified, with 36.4% identity. However, there is good evidence that respiratory allergens do not pose an allergic hazard when consumed. Wheat flour is used as a stabilizing agent in the commercial enzyme preparation and the enzyme preparation therefore includes wheat and gluten.

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 for this phospholipase A1 from GM *A. oryzae*.

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1 Introduction

Novozymes Australia Pty Ltd applied to Food Standards Australia New Zealand (FSANZ) to permit the use of the enzyme phospholipase A1 (EC 3.1.1.32) as a processing aid in the manufacture of bakery products. This enzyme is sourced from a genetically modified (GM) strain of *Aspergillus oryzae* containing the phospholipase A1 gene from *Valsaria rubricosa.*

Currently, Schedule 18 of the Australia New Zealand Food Standards Code (the Code) includes permission for one other phospholipase A1 enzyme produced by *Aspergillus oryzae* containing the phospholipase A1 gene from *Fusarium venenatum.* Therefore, this particular phospholipase A1 enzyme produced by a GM *A. oryzae* containing the phospholipase A1 gene from *V. rubricosa* needs pre-market assessment before permission can be given for its use as a processing aid. If permitted, the enzyme will provide an option for manufacturers of bakery products.

1.1 Objectives of the assessment

The objectives of this risk and technical assessment were to:

- determine whether the proposed purpose is clearly stated and that the enzyme achieves its technological function in the quantity and form proposed to be used as a food processing aid.

- evaluate potential public health and safety concerns that may arise from the use of this enzyme, produced by a GM microorganism, as a processing aid, specifically by considering the:

  - history of use of the gene donor and production microorganisms
  - characterisation of the genetic modification(s), and
  - safety of the enzyme.

2 Food technology assessment

2.1 Characterisation of the enzyme

2.1.1 Identity and properties of the enzyme

The production microorganism of the enzyme is a GM stain of *A. oryzae.* The donor microorganism of the phospholipase A1 gene is *V. rubricosa* (further details contained in Section 3). The applicant provided relevant information regarding the identity of the enzyme, and this has been verified using the IUBMB enzyme nomenclature database (McDonald et al 2009). Details of the identity of the enzyme are provided below.

Accepted IUBMB[1] name:	Phospholipase A1
Systematic name:	Phosphatidylcholine 1-acylhydrolase
Other names:	PS-PLA1 Phosphatidylserine-specific phospholipase A1

[1] International Union of Biochemistry and Molecular Biology

IUBMB enzyme nomenclature:	EC 3.1.1.32
CAS number[2]:	9043-29-2
Reaction:	Phosphatidylcholine + H_2O = 2-acylglycerophosphocholine + a carboxylate Hydrolysis of the sn-1 ester bond of diacylphospholipids.

2.2 Manufacturing process

2.2.1 Production of the enzyme

The enzyme is produced by submerged fermentation of the *A*. *oryzae,* carrying the phospholipase A1 gene from *V*. *rubricosa.* The fermentation processes are consistent with the scientific literature and references provided by Novozymes (Aunstrup 1979). All preparations are completed aseptically in accordance with Good Manufacturing Practices (GMP). Novozymes have provided certificates for compliance with ISO 9001:2015.

The fermentation process starts with the preparation of the medium including carbon, nitrogen, vitamins and minerals. The pH is adjusted and additional processing aids are used as antifoaming agents. This is then followed by inoculum, seed fermentation, main fermentation and the recovery stage to separate the enzyme from the biomass and to purify, concentrate and stabilize it. Ultrafiltration and/or evaporation are used for additional concentration and purification. The final enzyme preparation will depend on the intended use, for example, remain as a single enzyme preparation or be blended with other enzymes to form a granulate.

The final quality control processes involve reviewing the analytical results of samples from before inoculation, intervals during cultivation and before harvest/transfer for microbial contamination. If two samples are contaminated, fermentation is ceased.

The application states that all raw materials used in the fermentation and recovery processes are standard ingredients of food grade quality that meet predefined quality standards. The raw materials conform to either specifications set out in the Food Chemical Codex, 12th edition, 2020 or regulations applying in the European Union. The applicant has advised that wheat flour is used for stabilisation and has confirmed that wheat is present in the final enzyme preparation[3]. The Product Data Sheet lists the presence of cereals containing gluten (i.e. wheat, rye, barley, oats spelt, kamut). It is worth noting that the enzyme will be used in bakery products largely containing wheat. Section 3.3.4 provides more information on the allergenicity associated with the enzyme and is further discussed in Section 2.2.3 of the Call for Submissions document.

Details on the manufacturing process, raw materials and ingredients used in the production of the phospholipase A1 enzyme preparation were provided in the application or as Confidential Commercial Information.

[2] Chemical Abstracts Service

[3] On page 18 of the application, the applicant states that the preparation does not contain known food allergens. However, through subsequent correspondence between FSANZ and the applicant, the applicant has been able to clarify that the preparation does contain wheat.

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 (FAO/WHO 2006) and in the Food Chemicals Codex (FCC) (The United States Pharmacopeia 2020). 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 arsenic and heavy metals (section S3—4) if they are not already detailed within specifications in sections S3—2 or S3—3.

Table 1 provides a comparison of the analysis of three batch of the phospholipase A1 enzyme 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 meets all relevant specifications.

Table 1 *Analysis of enzyme phospholipase A1 compared to JECFA, Food Chemicals Codex, and Code specifications for enzymes (three batches)*

Analysis	Results from Applicant	JECFA	Food Chemicals Codex	Australia New Zealand Food Standards Code (section S3-4)
Lead (mg/kg)	Not detected	≤ 5	≤ 5	≤2
Arsenic (mg/kg)	Not detected	-	-	≤1
Cadmium (mg/kg)	Not detected	< 0.5	-	≤1
Mercury (mg/kg)	Not detected	< 0.5	-	≤1
Coliforms (cfu/g)	<4	≤30	≤30	-
Salmonella (in 25 g)	Not detected	Absent	Negative	-
E. coli (in 25 g)	Not detected	Absent	-	-
Antimicrobial activity	Not detected	Absent	-	-

2.3 Technological purpose of the enzyme

The enzyme is intended to be used in the manufacture of bakery products. Phospholipase A1 is a lipase which works on the endogenous lipid portion of dough. The endogenous portion of the dough makes up 2-4% of wheat and contains the lipids (fats) that the phospholipase acts on (Morrison as cited in Papantoniou et al 2003). It is a small but important part of the dough. Phospholipase A1 converts phospholipids into lysophospholipids and free fatty acids (Richmond and Smith, 2011). It is this activity that makes phospholipases useful as emulsifiers. In early enzyme development, the lipases would hydrolyse the apolar triacylglycerol lipids leaving a free fatty acid and a diacyl- or monoacyl-glycerol. These enzymes were effective in improving the crumb structure and softening but provided little benefit for dough stability. Dough stability relates to the gas bubbles formed during processing which creates dough with greater volume and consistency (Papantoniou et al 2003).

Phospholipase A1 acts as an emulsifier and creates better dough stability and improved crumb grain by producing by-products that work with gluten proteins, leading to the improved dough (Vogel and May 2019). The hydrolysis action of phospholipase A1 is depicted below in Figure 1. Phospholipase A1 hydrolyses the sn-1 ester bond of diacylphospholipids.

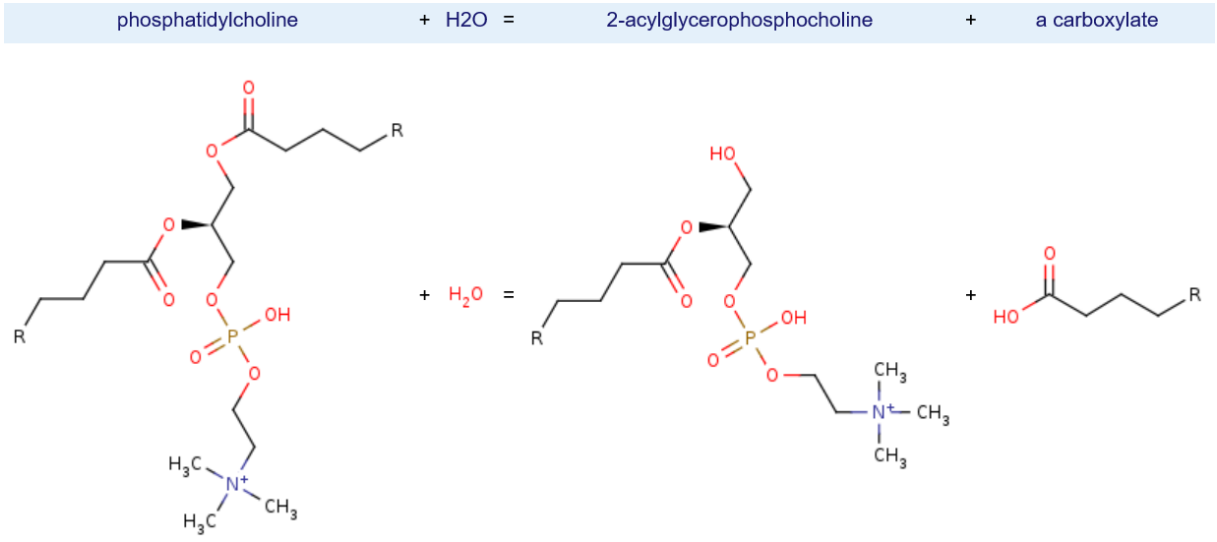

***Figure 1**. Phospholipase A1 hydrolyses a phospholipid to produce a lysophospholipid and a free fatty acid*

The stated technological purpose of phospholipase A1 in bakery products is consistent with that described in the scientific literature (De Maria et al 2007).

Table 2 includes a summary of the physical and chemical properties of the enzyme.

***Table 2** Phospholipase A1 enzyme physical/chemical properties*

Physical/chemical properties	
Enzyme activity	1000 PLA-B/g (Phospholipase A1 activity for baking applications)
Appearance	Granulate off white to light brown
Temperature optimum	~30°C
Temperature stability/storage	0 – 100°C (32 – 500°F)
pH optimum	4
pH stability	2 – 9

Phospholipase A1 will be used as a processing aid where it is present in negligible amounts in the final food and having no ongoing technical function. Information provided by Novozymes states that the enzyme is inactivated at 80°C. Using the enzyme in bakery products at high oven temperatures, the enzyme will be inactivated.

Novozymes claims and FSANZ confirms that phospholipase A1 performs its technological function during food processing and, as such, meets the definition of a processing aid.

2.4 Technological justification of the enzyme

Novozymes has indicated that the this enzyme is effective in improving dough strength, structure and stability, and this results in increased fermentation tolerance during baking (Gerits et al as cited in Borrelli and Trono 2015). The enzyme also helps in producing a uniform and improved crumb structure, which may otherwise be impaired by industrial

processing of the dough. Novozymes notes that these benefits are not exclusively obtained through enzyme treatment however the enzyme does provide an alternative to physical industrial processing.

Phospholipases have been used to increase softness of bakery products. This is due to the improvement in dough stability and volume which leads to greater uniformity, softness and crumb structure (Borrelli and Trono 2015).

Shelf-life and flavour are enhanced by the esterification of short-chain fatty acids (Aravindan, Anbumathi and Viruthagiri 2006). By removing the short-chain fatty acids, the 'off flavour' is reduced that can occur with this reaction.

The enzyme has already been approved for use in Denmark and France and this application for approval seeks to address international customer interest.

2.5 Food technology conclusion

FSANZ concludes that the proposed use of phospholipase A1 from GM *A. oryzae* in the manufacture of bakery products is consistent with its known technological function of converting phospholipids into lysophospholipids and free fatty acids. Analysis of the evidence provides adequate assurance that the use of this enzyme, in the quantity and form proposed to be used, produced under GMP controls and processes, is technologically justified. The enzyme meets international purity specifications.

Phospholipase A1 performs its technological purpose during the production of food and is not performing a technological purpose in the final food. It is therefore appropriately categorised 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 assessment

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

3.1 History of use

3.1.1 Host organism

The *A. oryzae* parental strain (IFO 4177) was obtained from the Institute for Fermentation in Osaka, Japan. The strain is also known by a synonym A1560. Novozymes has used this parental strain for the development of production strains for the manufacture of enzyme processing aids for many years. For example, FSANZ has assessed the safety of this organism in previous applications including A561 (2006) and A606 (2008). No safety concerns were noted in these assessments.

It is recognised that certain strains of *A. oryzae* are capable of producing toxic secondary metabolites including cyclopiazonic acid, Kojic acid and β-nitropropionic acid (Frisvad et al 2018). The host strain has been genetically modified to remove genes required for the production of these metabolites. Analytical analysis of the enzyme product did not detect the presence of the three metabolites. The production strain was not present in the enzyme product.

The identity of the parental strain was confirmed using molecular techniques.

3.1.2 Gene donor organism

The gene donor organism is a fungus known as *Valsaria rubricosa*. The strain was sourced from a recognised culture collection.

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 the phospholipase A1 gene was introduced into the *A. oryzae* host strain's genome, producing the production strain. The phospholipase A1 gene is derived from *V. rubricosa* genomic DNA and is under the control of a hybrid *Aspergillus* promoter and *A. niger* terminator. Data provided by the applicant and assessed by FSANZ confirmed the identity of the phospholipase A1 enzyme.

A vector containing the phospholipase A1 expression cassette was used to transform the host strain. The expression cassette was integrated at specific integration sites in the host's genome. The final production strain was selected based on rapid growth and high phospholipase A1 activity.

3.2.2 Characterisation of inserted DNA

The applicant provided data to characterise the inserted DNA in the production strain. These data confirm the presence of the expression cassette in the genome of the production strain.

The applicant also provided the results of genome sequencing which confirmed the absence of antibiotic resistance genes in the production strain.

3.2.3 Genetic stability of the inserted gene

The assessment confirmed the inserted DNA is integrated into the production organism's genome 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 phospholipase A1 gene, the applicant provided phenotypic data from large-scale fermentation of the production strain. These data confirmed that the phospholipase A1 gene is expressed over multiple generations and is stable.

3.3 Safety of phospholipase A1

3.3.1 History of safe use of the enzyme

The phospholipase A1 that is the subject of this application was approved for use in France in 2019, and in Denmark in 2020. It is also used in unspecified countries in which there are no restrictions on enzyme processing aids, or in which it is covered by a positive list.

3.3.2 Bioinformatics concerning potential for toxicity

A recent (February 2021) bioinformatics search was conducted by comparing the sequence of the phospholipase A1 that is the subject of this application, to those of known toxins in the

UNIPROT database[4]. No matches to known toxins were found. The largest homology identified was 19.3%.

3.3.3 Toxicology data

Toxicology studies conducted with the phospholipase A1 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.

3.3.3.1 Short term study in animals

Thirteen-week repeat-dose oral gavage study of phospholipase A1 in Crl:WI(Han) rats (Holalagoudar et al 2018; unpublished study). Regulatory status: GLP; in compliance with OECD test guideline 408

This study was conducted using phospholipase A1, batch PPW50972, as the test article. The vehicle and negative control article was water. Dose formulation stability and homogeneity were confirmed prior to study and concentrations were analysed and confirmed during weeks 1, 6 and 13 of study. The test system comprised Crl:WI(Han) rats, 10/sex/group. Rats were obtained as weanlings (29 to 36 days old) and acclimatised to standard laboratory environmental and husbandry conditions for 13 days prior to study start. During the in-life phase, rats were gavaged once daily with 0, 95.7, 315.9 or 957.3 mg total organic solids (TOS)/kg bw/day phospholipase A1, at a dose volume of 10 mL/kg bw.

Parameters assessed during the in-life phase included survival, clinical observations, ophthalmology, body weights and bodyweight changes, food consumption, water consumption (qualitative), and performance on a functional observational battery (FOB). At the end of the in-life phase, blood was collected for haematology, clinical chemistry and measurement of coagulation factors before rats were killed and subject to gross necropsy and histopathological examination of a comprehensive list of organs and tissues.

All rats survived to the end of the in-life phase. There were no dose-related effects on clinical observations, ophthalmological findings, body weights or bodyweight changes, food consumption, water consumption, clinical pathology findings, gross necropsy findings, or histopathology. Male performance on the FOB showed no dose-related changes. Group mean values for total activity counts, total mobile counts, and total rears of females treated with phospholipase A1 were increased relative to those of female controls, and the difference reached statistical significance for the high dose group. In the absence of any other effects on the health of these rats, and the lack of similar observations in males, these increases were considered to be unrelated to treatment.

It was concluded that the No Observed Adverse Effect Level (NOAEL) was 957.3 mg TOS/kg bw/day, the highest dose tested.

3.3.3.2 Genotoxicity studies

Bacterial reverse mutation assay (Ballantyne et al 2018; unpublished study). Regulatory status: GLP; in compliance with OECD Guideline 471

The test article for this assay was phospholipase A1, batch PPW50972. The test system comprised the *Salmonella* Typhimurium strains TA98, TA100, TA1535 and TA1537, and the *Escherichia coli* strain WP2 uvrA pKM101. Assays were conducted with and without the presence of S9 mix for metabolic activation. The tests were conducted using the ‘treat and

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

plate' method. The vehicle and negative control article was water. In the preliminary dose-range finding assay, concentrations of the test article were 16, 50, 160, 500, 1600 and 5000 µg TOS/mL. Evidence of toxicity was observed at 5000 µg TOS/mL in TA100,TA1535 and TA1537 in both the absence and presence of S-9. In the definitive assay, all treatments were performed in duplicate, and with and without S9 mix. Concentrations of test article were 160, 300, 625, 1250, 2500 and 5000 µg TOS/mL. Evidence of toxicity was observed at ≥2500 µg TOS/mL in all the *S.* Typhimurium strains in both the absence and presence of S9. No notable, concentration-related increases in numbers of revertant colonies were observed in any bacterial strains, with or without S9 mix, treated with phospholipase A1, when compared to negative controls. Negative (vehicle) and positive control treatments were conducted for all strains in both experiments. The mean numbers of revertant colonies fell with acceptable ranges for the negative control treatments and were elevated as expected by the positive control treatments, confirming the validity of the assay.

It was concluded that the test article showed no evidence of mutagenic activity in this assay system.

In vitro *micronucleus assay (Whitwell et al 2018; unpublished study). Regulatory status: GLP; in compliance with OECD Guideline 487*

The test article for this study was phospholipase A1, batch PPW50972. The vehicle and negative control article was water. The test system comprised human lymphocyte cultures from the pooled blood of two male donors. Tests were conducted in duplicate, and with and without S9 mix for metabolic activation. Mitomycin C (MMC) and vinblastine (VIN) were used as clastogenic and aneugenic positive control articles respectively in the absence of S9 mix, and cyclophosphamide (CPA) was used as a clastogenic positive control article in the presence of S9 mix . Concentrations of the test article ranged from 0 to 5000 µg TOS/mL. Concentrations for the definitive assay were based on preliminary observations of the effects of the test article on the replication index (RI) and also on the presence or absence of precipitate. The maximum concentrations analysed were either 5000 µg TOS/mL (3+21 hour -S-9), or, limited by post treatment precipitate (3+21 hour +S-9), or, limited by cytotoxicity (24+24 hour -S-9) in line with regulatory guidance for the *in vitro* micronucleus assay.

Small but statistically significant increases in the incidence of micronucleated/binucleate (MNBN) cells were observed in one of two duplicate tests at 3000 µg TOS/mL without S9, and 3500 µg TOS/mL with S9 following 3+21 hour treatments, and also at the highest concentration analysed post 24+24 hour treatment in the absence of S9; 1500 µg TOS/mL. These results were considered to be spurious because they occurred in only one of two duplicate tests and there was no evidence of any concentration-related effects. No other increases in MNBN cells, when compared to vehicle controls, were observed. The proportion of MNBN cells in the vehicle cultures fell within historical vehicle control ranges. All positive control compounds induced statistically significant increases in the proportion of cells with micronuclei, confirming the validity of the assay.

It was concluded that phospholipase A1 did not induce biologically relevant increases in micronuclei in cultured human peripheral blood lymphocytes following treatment in the absence and presence of metabolic activation.

3.3.4 Potential for allergenicity

Recent (February 2021) bioinformatics searches were conducted by comparing the sequence of the phospholipase A1 to those of known allergens in the Food Allergy Research and Resource Program (FARRP) allergen protein database[5]. Searches included 35% identity

[5] http://allergenonline.org

over a sliding window of 80 amino acids (scaled and unscaled), 35% identity over full length alignment, and 100% identity over eight amino acids. No food allergens were matched using the 80 amino acid searches. The only match was to an occupational respiratory allergen produced by the fungus *Shizophyllum commune,* with 36.4% identity. There is good evidence that respiratory allergens do not pose an allergic hazard when consumed (Bindslev-Jensen et al 2006). One phospholipase was matched by the full length alignment search, with only 11.3% identity. No hits were found using the 100% identity over eight amino acids.

Wheat flour is used as a stabilising agent in the commercial enzyme preparation and the enzyme preparation therefore contains wheat and gluten. The enzyme is intended for use in manufacture of baked products, and the quantity of wheat and gluten in the enzyme may be expected to be negligible relative to the wheat and gluten in other ingredients of baked goods.

3.3.5 Assessments by other regulatory agencies

No risk assessments by other regulatory agencies are available.

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 'worse-case scenario' approach to estimating likely levels of dietary exposure, assuming all added phospholipase enzyme 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 processing aid concentration in foods and beverages, and the proportion of foods and beverages that may contain the processing aid. 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, the applicant made the following assumptions:

- the maximum physiological requirement for solid food (including milk) is 25 g/kg body weight/day
- 50% of solid food is processed
- all processed solid food contains 70% flour
- the highest of all proposed uses in final foods for all uses in solid foods was used in the TMDI calculation
- all of the enzyme remains in the final food
- all foods contain the highest use level of 6.6 mg TOS/kg raw material (flour).

Based on these assumptions, the applicant calculated the TMDI of the enzyme to be 0.06 mg TOS/kg body weight/day.

As assumptions made by the applicant differ from those that FSANZ would have made in applying the budget method, FSANZ independently calculated the TMDI using the following assumptions that are conservative and 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).
- FSANZ would generally assume 12.5% of solid foods contain the enzyme based on commonly used default proportions noted in the FAO/WHO Environmental Health Criteria (EHC) 240 Chapter 6 on dietary exposure assessment (FAO/WHO 2009). However, the applicant has assumed a higher proportion of 50% based on the nature and extent of use of the enzyme and therefore FSANZ has also used this proportion for solid foods as a worst case scenario.

All other inputs and assumptions used by FSANZ remained as per those used by the applicant. The TMDI based on FSANZ's calculations for solid food is 0.12 mg TOS/kg body weight/day. Exposure from non-milk beverages was not included in either assessment as the applicant has proposed the enzyme to be used in the manufacture of bakery products.

Both the FSANZ and applicant's estimates of the TMDI will be overestimates of the dietary exposure given the conservatisms in the budget method. This includes that it was assumed that the enzyme remains in the final foods whereas the applicant has stated that it is likely to either be reduced or removed during processing, or would be present in insignificant quantities, and would be inactivated and perform no function in the final food to which the ingredient is added.

4 Discussion and Conclusion

The use of this phospholipase A1 in the manufacture of bakery products is clearly described and is consistent with the known technological functions of phospholipase A1 in converting phospholipids into lysophospholipids and free fatty acids. The use of this phospholipase A1, in the quantity and form proposed to be used, produced under GMP controls and processes, is technologically justified. The enzyme meets international purity specifications.

No public health and safety concerns were identified in the assessment of phospholipase A1 from GM *A. oryzae* under the proposed use conditions. *A. oryzae* 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. oryzae* host is neither pathogenic or toxigenic. Analysis of the modified production strain confirmed the presence and stability of the inserted DNA.

Toxicology studies conducted with the phospholipase A1 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. A NOAEL of 957 mg TOS/kg bw/day was established in rats. The TMDI based on FSANZ's calculations for solid food is 0.12 mg TOS/kg body weight/day. Comparison of the NOAEL and the TMDI results in a Margin of Exposure (MOE) of around 8000. No evidence of genotoxicity was found in either genotoxicity assay.

Recent bioinformatics searches were conducted by comparing the sequence of the phospholipase A1 to those of known allergens. No significant matches with food allergens were found. A match with an occupational respiratory allergen was identified, with 36.4% identity. However, there is good evidence that respiratory allergens do not pose an allergic hazard when consumed. Wheat flour is used as a stabilizing agent in the commercial enzyme preparation and the enzyme preparation therefore includes wheat and gluten.

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 for this phospholipase A1 from GM *Aspergillus oryzae*.

5 References

Aravindan R, Anbumathi P and Viruthagiri T (2006) 'Lipase applications in food industry' *Indian Journal of Biotechnology* 6:141-158.

Bindslev-Jensen C, Skov PS, Roggen EL, Hvass P, Brinch DS (2006) 'Investigation on possible allergenicity of 19 different commercial enzymes used in the food industry' *Food and Chemical Toxicology* 44:1909-1915.

Borrelli GM and Trono D (2015) 'Recombinant lipases and phospholipases and their use as biocatalysts for industrial applications' *International Journal of Molecular Sciences* 16(9): 20774-20840. doi: 10.3390/ijms160920774.

De Maria L, Vind J, Oxenbøll KM, Svendsen A, Patkar S (2007) 'Phospholipases and their industrial applications'. *Applied Microbiology and Biotechnology*. Feb 1;74(2):290-300.

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(8):791-802.

FAO/WHO (2006) Combined compendium of food additive specifications, Food and Agriculture Organization of the United Nations, Rome. http://www.fao.org/docrep/009/a0691e/A0691E03.htm

FAO/WHO (2009) 'Environmental Health Criteria 240. Principles and methods for the risk assessment of chemicals in food' Chapter 6 – Dietary exposure assessment of chemicals in food, WHO, Geneva.

Frisvad JC, Møller LLH, Larsen TO, Kumar R and Arnau J (2018) 'Safety of the fungal workhorses of industrial biotechnology: update on the mycotoxin and secondary metabolite potential of *Aspergillus niger*, *Aspergillus oryzae* and *Trichoderma reesei' Appl Microbio Biotechnol* 102(22):9481-9515.

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

Papantoniou E, Hammond EW, Tsiami AA, Scriven F, Gordon MH and Schofield JD (2003) 'Effects of endogenous flour lipids on the quality of semisweet biscuits' *Journal of Agricultural and Food Chemistry* 51:1057-1063.

Richmond GS and Smith TK (2011) 'Phospholipases A1' *International Journal of Molecular Sciences* 12:588-612.

The United States Pharmacopeia (2020) Food Chemicals Codex 12th Edition, United States Pharmacopeial Convention, Rockville, MD. http://publications.usp.org/

Vogel A and May O (eds) (2019) 'Industrial Enzyme Application' Chapter 2.1 Enzymes used in baking John Wiley and Sons. Pg 106-108.