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Te Mana Kounga Kai – Ahitereiria me Aotearoa

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Supporting document 1

Food technology, microbiological, nutrition, toxicology and dietary exposure assessment report

Application A1175 Rapeseed protein isolate as a novel food

Executive summary

FSANZ has assessed an application from DSM Nutritional Products Asia Pacific to amend the Australia New Zealand Food Standards Code (the Code) to permit the use of rapeseed protein isolate as a novel food.

Rapeseed protein isolate is intended to be used as a protein source and ingredient in a range of foods. It is intended as a replacement for existing protein sources. The applicant provided typical use levels from 2 to 10% in order for FSANZ to undertake a more refined risk assessment. The isolate, which is at least 90% protein, also provides desirable functional and sensory properties in food.

The rapeseed protein isolate comes from the following *Brassica* species: *Brassica napus, Brassica rapa* and *Brassica juncea.* These species are naturally low in the anti-nutritional factors erucic acid and glucosinolates, which can cause adverse effects at sufficiently high doses so the *Brassica* species for sourcing rapeseed protein isolate from is an important consideration for addition to food. The anti-nutritional factors are reduced further during the manufacture of the isolate. The collective term canola is also used for *Brassica* species that are low in erucic acid and glucosinolates and can be used in place of the term rapeseed.

The food technology assessment concluded that rapeseed protein isolate when used as protein source as a replacement for other protein sources in a range of foods is technologically justified in the quantities and form proposed. It is suitable for addition at typical use levels from 2 to 10% in a range of foods and up to maximum use levels of 30% in meat analogues and protein-based products only. The typical use levels reflect those more likely to be used by food manufacturers.

FSANZ concludes that as rapeseed protein isolate is a low moisture food it may pose a microbiological risk for *Salmonella* spp. if used in some types of manufactured convenience foods with no subsequent microbiocidal step. The applicant has certification in relevant food safety management systems to control foodborne hazards, however there are no microbial reduction steps in the manufacturing process of the rapeseed protein isolate. A screening method was used to assess the risk for *Salmonella* spp. and *Bacillus cereus* when consuming the product when used in manufactured convenience foods. The risk assessment

concluded that the risk levels were low-medium for *Salmonella* spp. and low for *B. cereus*. For cooked foods, such as bakery products, where rapeseed protein isolate is used as an ingredient, the risk level will be low for both *Salmonella* spp. and *B. cereus.*

An assessment was undertaken to estimate dietary exposure to rapeseed protein isolate based on the most recent consumption data from national nutrition surveys for Australians (2 years and above) and New Zealanders (children 5-14 years and adults 15 years and above) and information provided by the applicant on proposed foods and maximum and typical use levels. Results based on typical use levels better reflect longer term or chronic risk, and therefore these were used by FSANZ for risk characterisation purposes.

Rapeseed protein isolate, when used as a protein source in foods at the proposed maximum or typical use levels, does not raise nutritional concerns in relation to the protein adequacy of the diet. The protein quality of rapeseed protein isolate, as determined from its amino acid profile and digestibility, is comparable to that of the milk protein casein and slightly higher than that of soy protein isolates.

Rapeseed protein isolates contain phytates and high phytate intakes can reduce mineral bioavailability. At a typical use level of rapeseed protein isolate of 10% (w/w) in foods, and with a proposed maximum phytate level in rapeseed protein isolate of 1.5% (w/w), manufactured foods would have maximum levels of phytate of 0.15% (w/w), which is close to the lower end of the range reported for commonly consumed foods such as cereals, beans and nuts. Also, the maximum phytate level of 1.5% is similar to the maximum levels reported for soy protein isolates (1.5–1.7%) which are the most widely used plant protein isolates. On this basis, phytate levels of up to 1.5% in rapeseed protein isolate do not raise concerns regarding mineral bioavailability.

For erucic acid, estimated dietary exposures from rapeseed protein isolate based on typical use levels at the mean (0.03-0.06 mg/kg bw/day) and 90th percentile (0.05-0.11 mg/kg bw/day) were higher than the dietary exposures from *Brassica* vegetables at both the mean (0.003-0.01 mg/kg bw/day) and 90th percentile (0.01-0.03 mg/kg bw/day) for all population groups assessed in Australia and New Zealand. The exposure to erucic acid from rapeseed protein isolate was not considered to represent a public health concern as dietary exposure estimates were well below the provisional tolerable daily intake (PTDI) established by FSANZ in 2003 of 7.5 mg/kg bw per day.

Australians could be exposed to more glucosinolates from rapeseed protein isolate based on typical use levels (mean 0.30 mg/kg bw/day; 90th percentile 0.61 mg/kg b/day) than from *Brassica* vegetables (mean 0.21 mg/kg bw/day; 90th percentile 0.56 mg/kg bw/day). However, New Zealand dietary exposures to glucosinolates from *Brassica* vegetables (mean 0.66-0.92 mg/kg bw/day; 90th percentile 1.40-2.17 mg/kg bw/day) were higher than exposures from rapeseed protein isolate based on typical use levels (mean 0.25-0.57 mg/kg bw/day; 90th percentile 0.50-1.04 mg/kg bw/day). The additional exposure to glucosinolates from rapeseed protein isolate based on typical use levels of around 20 mg/day is equivalent to the consumption of around 30 g/day of *Brassica* vegetables (one large broccoli floret or one medium cauliflower floret) and therefore not a public health concern.

Potential contaminants that may be bioaccumulated by the rapeseed plant include arsenic, lead, cadmium, zinc, copper and chromium. Increased dietary exposures to these contaminants from the addition of rapeseed protein isolate to the diet are estimated to be low based on typical use levels and the small market update estimated by the applicant, and are not of toxicological concern.

Rapeseed plants contain allergenic proteins which show cross-reactivity with proteins from related mustard species due to the high amino acid sequence similarity between the

proteins. An allergy assessment concluded that rapeseed protein isolate has the potential to induce allergic responses in individuals who are allergic to mustard.

In conclusion, the approval for the use of rapeseed protein isolate as a novel food in the food classes noted at the proposed typical use levels would not represent a public health and safety concern for many of the areas assessed. The aspects identified as potential public health and safety concerns included the microbiological risk from *Salmonella* spp., the potential allergic responses to individuals who are allergic to mustard, and the need to ensure levels of substances such as phytates and certain metal contaminants are retained as low as reasonably achievable.

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1. Introduction

FSANZ received an application from DSM Nutritional Products Asia Pacific to amend the Code to permit the use of rapeseed protein isolate as a novel food. The application was submitted following FSANZ's Advisory Committee on Novel Foods (ACNF) view in May 2017 that rapeseed protein isolate was a non-traditional and novel food (FSANZ, 2017a). This means that for rapeseed protein isolate to be permitted as a novel food, assessment and an amendment to the Code is required.

Rapeseed protein isolate is intended for use as a protein source and ingredient in a range of food classes. It is intended to be used as a replacement for proteins sourced from animal (e.g. whey) or other plants (e.g. soy, pea), including for use in new product development. The applicant did not seek permission for use of rapeseed protein isolate in infant formula products (includes infant formula, follow-on formula and infant formula products for special dietary uses) and infant foods so the scope of the assessment does not include these food classes. The applicant provided typical use levels from 2 to 10% for FSANZ to undertake a more refined risk assessment. The application also noted maximum proposed use levels of up to 30% in protein based products and meat analogues.

Throughout this document the substance is referred to as rapeseed protein isolate. However, it is also known as rapeseed protein. The terms rapeseed and canola are often used interchangeably, with canola being the collective name for rapeseed cultivars that have been developed to be low in the anti-nutritional factors erucic acid and glucosinolates. The applicant's rapeseed protein isolate is commercially known and available as CanolaPRO and is derived from low erucic acid cultivars of *Brassica napus*, *Brassica rapa or Brassica juncea* (OECD 2011).

Rapeseed protein isolate is derived via extraction from rapeseed press cake that is retained after oil pressing from the seeds of one or more of *Brassica napus*, *Brassica rapa or Brassica juncea.* Rapeseed protein isolate has a good sensory profile for use up to levels of 30% (noting that typical use levels are up to 10%) as an ingredient in food. The protein content of at least 90% also provides a range of functional properties in food applications.

2. Food technology assessment

2.1 Objectives for the food technology assessment

To determine whether rapeseed protein isolate functions as a replacement protein source, including for use in new product development and ingredient in the form and quantities proposed for a range of foods.

2.2 Chemical and physical properties

Rapeseed protein isolate contains at least 90% protein and is derived from rapeseed press cake, which is retained from edible rapeseed oil production. DSM Nutritional Products Asia Pacific's (DSM's) rapeseed protein isolate is sourced from the following *Brassica* species: *Brassica napus, Brassica rapa* and *Brassica juncea.* These species are low in the anti-nutritional factors; erucic acid and glucosinolates.

The applicant's rapeseed protein isolate is a tan powder that is readily soluble in water and stable at ambient temperature under dry storage conditions for up to 2 years. Shelf life testing at 20°C and 40°C showed that protein content and solubility remained constant throughout this time.

2.2.1 Composition

The protein content in rapeseed protein isolate is at least 90%, with levels from 96.3% to 99.1% for the applicant's rapeseed protein isolate. The isolate consists of two main proteins: cruciferin and napins. Cruciferins are globulins, the main storage protein in the seed with a molecular weight of approximately 300 kDa. The napins are albumins and low molecular weight storage proteins of 14 kDa. The composition of the applicant's product, CanolaPRO consists of 40-65% cruciferins and 35-60% napins.

The protein in rapeseed protein isolate includes all the essential amino acids. Refer to section 4.3 and table 4.1 for the nutrition risk assessment and details on the amino acid profile.

The application included the analysis for five batches of rapeseed protein isolate. There is no carbohydrate and the fibre content is below limits of detection. The moisture content ranges from 2.3 to 4.3 % w/w. Ash accounts for the remaining composition with levels ranging from 0.06 to 0.71 % w/w.

2.2.1.1 Impurity profile

Anti-nutritional factors

Rapeseed sourced from *Brassica* species contains anti-nutritional factors, specifically erucic acid, glucosinolates, phytic acid and phenolic compounds. The applicant's product CanolaPRO is sourced from *Brassica* species that are low in erucic acid and glucosinolates. The manufacturing steps help reduce these further. Analysis of five batches of CanolaPRO showed that in all five batches glucosinolates were at levels of < 0.1 µmol/g, and below the level of quantification. Analysis of five batches of the applicant's rapeseed protein isolate showed erucic acid levels of <0.005% w/w.

The International Codex Standard 210-1999 for Named Vegetable Oils includes a definition for rapeseed oil, including for low-erucic acid rapeseed oil which must not contain more than 2% erucic acid (as % of fatty acids) (Codex, 2019). The table to subsection S19-6(2) in the Code also includes a limit for erucic acid of 20,000 mg/kg (i.e. 2%) in edible oils, which could include rapeseed oil. Rapeseed protein isolate is extracted from the "de-fatted" cake/meal that remains after pressing the rapeseed to remove the oil, so any erucic acid that remains in the "de-fatted" cake/meal will be reduced further during the subsequent manufacturing steps and isolation of the protein. These levels are significantly lower than the 2% erucic acid in the Codex Standard 210-1999 for vegetable oils and in the Code. In a research paper that looked at the effect of processing on anti-nutritional factors in rapeseed protein it was shown that up to 95% of glucosinolates, 92% of phytic acid and 100% of tannic acid can be removed (Mansour et al. 1993). By selecting *Brassica* species low in erucic acid and glucosinolates together with processing the levels of anti-nutritional factors can be reduced.

Metals

The maximum and mean levels for various metals in 5 batches of the applicant's rapeseed protein isolate and in the Code are summarised in Table 2.1. The maximum levels for arsenic, cadmium, lead and mercury provided by the applicant are lower than the default maximum limits for metals listed in S3-4 in the Code. The only metal for which there is an additional and lower maximum limit is for lead at 0.5 mg/kg. This information was proposed by the applicant for the product specification in Table 2.5. This is also consistent with the maximum limit in the 2013 EFSA assessment and US GRAS notifications for DSM's and other rapeseed protein isolates (EFSA NDA Panel, 2013; US FDA 2010 and 2017).

Table 2.1 *Metal maximum and mean levels in rapeseed protein isolate compared with maximum limits in S3-4 of the Code*

Metal	Maximum level for Rapeseed Protein Isolate (mg/kg)	Mean level for Rapeseed Protein Isolate (mg/kg)	S3-4 Maximum Limits (mg/kg)
Arsenic	*	*	1.00
Cadmium	0.028	0.017	1.00
Lead**	0.024	0.013	2.00
Mercury	*	*	1.00

* These results are N/A as limit of detection for arsenic is <0.01 and for mercury is <0.02.
** There were 8 batches and results that contributed to the maximum and mean levels for the metal lead.

Mycotoxins

The level of mycotoxins during processing in the rapeseed press cake (from which rapeseed protein isolate is then obtained) were below limits of quantification except for deoxynivalenol (DON) which was found at a level if 39 mg/kg in one batch. However DON was not detected in the applicant's final product, CanolaPRO.

Pesticides

Screening the applicant's rapeseed protein isolate for over 600 pesticides from an independent laboratory using gas chromatography-mass spectrometry and liquid chromatography–mass spectrometry indicated there were no pesticide residues of concern. Results were also below levels of quantification of 0.01 mg/kg, the default maximum residue limit for the EU in Regulation (EC) No 396/2005. The applicant's suppliers also provided confirmation of compliance with relevant requirements for pesticide residues.

Microbiological

The rapeseed press cake, a co-product from the oil extraction process from which rapeseed protein isolate is obtained, is monitored for microbiological contaminants. Although the manufacturing process contains filtration steps and temperature control to assist with managing the microbiological load, there are no microbiological reduction steps in the manufacturing process. The final product is tested against microbiological parameters and meets the applicant's product specification in Table 2.5. Refer to section 3 for further details on the microbiological assessment.

2.3 Technological purpose

The technological purpose of rapeseed protein isolate is to replace protein from animal and other plant sources, including for use in new product development in a range of food classes. Proposed typical use levels range from 2% to 10% and proposed maximum use levels are up to 30%.

The Schedule 15 food classes the applicant is proposing to use rapeseed protein isolate in as a protein source, together with examples and the typical and maximum use levels are included in Table 2.2.

Table 2.2 *Rapeseed protein isolate use as a protein source in food classes, typical and maximum use levels (%)*

Food class	Examples	Proposed typical use level (%)	Proposed maximum use level (%)
Bakery products	Bread, pastries, biscuits, cakes, pies, muffins, cereal bars, ready to eat cereals	3 - 5	5
Beverages	Fruit juice, fruit juice blends, soft drinks, formulated beverages, dairy and plant based milks, energy drinks.	2 - 5	5
Dairy products	Yoghurt, cheese, cheese products, milks, creams, desserts, dairy and plant based dips/desserts/toppings, dairy based ice blocks and sorbets	3 - 5	5
Mixed foods	Ready to eat meals, soup, pasta, extruded snacks including cookies	5	10
Meat analogues	Patties, fillets, strips	5	30
Protein based products	Bars, energy bars, pasta, protein powders, beverages	10	30

2.3.1 Functional properties

Rapeseed protein isolate has a protein content of at least 90%, providing a range of technological functions including thickening, water binding, emulsifying, gelling, foaming or providing texture.

The functional properties of rapeseed protein isolate are influenced by its structure, including the cruciferin and napin content. In CanolaPRO all the proteins are present in their native state. The manufacturing process is carried out in such a way as to maintain the native state of the protein. This is because only the native protein exhibits the desired functional properties of CanolaPRO. Compared with other proteins derived from animal (e.g. whey) or other plants (e.g. soy, pea) the use of rapeseed protein isolate as an ingredient can provide improved functional properties including in bread dough as an emulsifier, in meringue improved volume and foam stability and in meat patties improved water holding capacity and yield (Wanasundara et al. 2016). Some of the functional properties for rapeseed protein isolate also provide sensory benefits. For example gelling gives good mouthfeel, including texture when used in meat analogues.

In the form of a powder rapeseed protein isolate is readily soluble in water, which also influences its functional properties. The solubility of cruciferin and napin is different and is influenced by pH, temperature and salt level. At pH levels between 3 and 4 cruciferin is insoluble while napin is soluble from pH levels 2 to 10 (Wanasundara et al. 2016). Salts such as sodium chloride used in the applicant's rapeseed protein isolate can assist in stabilising napin. Limitations with water binding capacity and also bitterness from rapeseed protein isolate at higher use levels mean the maximum use levels in foods is unlikely to exceed 30%, noting that typical use levels are a maximum of 10%.

Examples for the applicant's rapeseed protein isolate's functional properties and use in food applications are included in Table 2.3 below.

Table 2.3 *Examples of rapeseed protein isolate's functional properties in food applications*

Functional property	Example of food application	Typical use level (%)
Thickener/Water binder	In dairy and plant based proteins	3 to 10
Emulsifier	Replacement for egg yolk in mayonnaise Replacement for mono-diglycerides in baked goods and sauces	0.5 to 3
Foaming agent	In ice cream to provide a light foamy product Replacement for egg white in baked products	1 to 4
Texturiser	A replacement for gluten in baked products	5 to 10

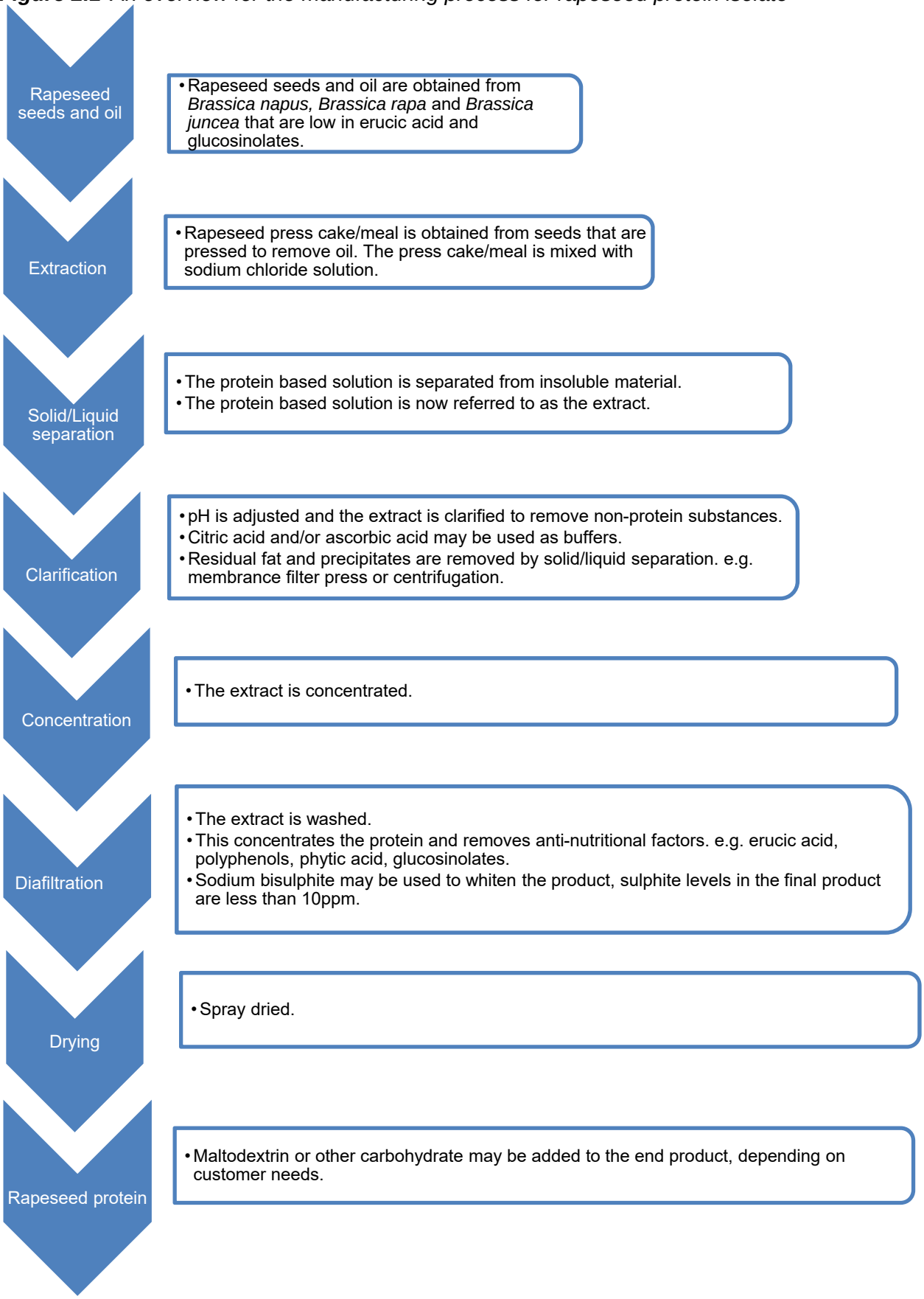
2.4 Technological justification

Rapeseed protein isolate can be used to replace protein from animal and other plant sources, including for use in new product development in a range of food classes at proposed typical use levels up to 10%.

2.5 The manufacturing process

Rapeseed protein isolate is derived via extraction from rapeseed press cake that is retained after oil pressing from the seeds of one or more of *Brassica napus*, *Brassica rapa or Brassica juncea.* The rapeseed protein isolate is produced from rapeseed press cake that remains after the cleaned, flaked and conditioned seeds have been pressed to separate the oil. Any ingredients, food additives and processing aids used in the manufacturing process are food grade and must be permitted in the relevant standards and schedules in the Code. The manufacturing process is summarised in Figure 2.1.

Figure 2.1 *An overview for the manufacturing process for rapeseed protein isolate*



Rapeseed protein isolate is considered stable under dry and ambient temperature storage conditions for up to 15 months. Protein content and solubility remained constant for samples stored up to 16 weeks at 20°C and 40°C. The shelf life for the applicant's product is 2 years from the date of production.

2.6 Analytical method for detection

Rapeseed protein isolate can be detected using common protein analytical methods such as sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). The applicant has described this process in their application and provided an example of an SDS-PAGE gel for a typical rapeseed protein isolate batch.

2.7 Product specification

There is not a primary or secondary source for a specification in section S3-3 of the Code. As rapeseed protein isolate is a novel food, a product specification needs to be developed. There is a Codex General Standard 174-1989 (Codex Standard) for vegetable protein products which is summarised in Table 2.4. The applicant's proposed product specification for rapeseed protein isolate is consistent with the relevant parameters in this Codex standard.

Table 2.4 *Rapeseed protein isolate and comparison with Codex General Standard 174-1989 for vegetable protein products*

Parameter	**Proposed product specification for rapeseed protein isolate (%)**	**Codex Standard**
Moisture	≤7	Low to ensure microbiological stability
Crude protein	≥90	≥40%
Ash	≤4	<10%
Fat	≤2	Consistent with levels of good manufacturing practice

A product specification with parameters and specifications proposed by the applicant and amended by FSANZ is included in Table 2.5. The application also contains analytical results for older (2015) and more recently manufactured (2018) batches of the applicant's rapeseed protein isolate. All are within limits listed in the product specification shown in Table 2.5.

Table 2.5 *Product specification for rapeseed protein isolate*

Parameter	Specification
Appearance	Tan powder
Composition*: Total protein Carbohydrates Fat Ash Moisture	 ≥ 90 % w/w ≤ 7 % w/w ≤ 2 % w/w ≤ 4 % w/w ≤ 7 % w/w
Purity: Erucic acid Glucosinolates Phytates	 ≤ 0.005% ≤1 µmol/g ≤1.5 % w/w
Metals: Lead	 ≤ 0.5 mg/kg
Microbiological: Total plate count *E. coli* *Salmonella* spp. Yeast and moulds	 ≤ 10 000 cfu/g** Absent/10g Absent/25g ≤ 100 cfu/g**

*Note that there is no fibre present as the levels in 5 batches of the applicants rapeseed protein are below detection limits of <0.5% and 0.6% for the two laboratories and test methods used.
**cfu = colony forming unit.

2.8 Food technology conclusion

FSANZ concludes that rapeseed protein isolate, when used as a novel food ingredient and as a replacement protein source, including for use in new product development in a range of foods is technologically justified at typical use levels of up to 10% and in the form proposed. For use in food, rapeseed protein needs to be sourced from *Brassica* species that are low in the anti-nutritional factors; erucic acid and glucosinolates.

It also provides various technological functions in foods, including thickening, water binding, emulsifying, gelling, foaming and providing texture. At typical use levels bitter notes will not be apparent.

3. Microbiological assessment

3.1 Objectives for the microbiological assessment

The objectives for the microbiological risk assessment were:

- to identify microbiological hazards for public health and safety in permitting rapeseed protein isolate
- to review the manufacturing process and controls for microbiological hazards
- to consider the risk associated with food permissions.

3.2 Microbiological hazards in rapeseed protein isolate

Rapeseed protein isolate is a low moisture food and as such, *Salmonella* spp. and *Bacillus cereus* are the primary pathogens of concern.

As a novel protein, there are currently no requirements in the Code for rapeseed protein isolate. For this reason the microbiological assessment referred to the Codex General Standards for Vegetable Protein Products (VPP) (CXS 174-1989) (Codex Alimentarius Commission, 2019) which applies to vegetable protein products (including rapeseed protein) intended for use in foods. The standard covers composition, quality and nutrition factors as well as packaging and labelling requirements. Section 6 of the standard covers hygiene and recommends that VPP should be prepared in accordance to the Codex General Principles of Food Hygiene (CXC 1-1969) (Codex Alimentarius Commission, 2003) and that products shall be free of micro-organisms which may represent a hazard to health. The moisture content of VPP must be low enough to ensure microbiological stability under the recommended conditions of storage.

The Codex General Principles of Food Hygiene sets out the necessary hygiene conditions for producing food which is safe and suitable for consumption. The control of food hazards can be achieved through the use of systems such as Hazard Analysis and Critical Control (HACCP) which should:

• identify any steps in their operations which are critical to the safety of food;
• implement effective control procedures at those steps;
• monitor control procedures to ensure their continuing effectiveness; and
• review control procedures periodically, and whenever the operations change.

A Critical Control Point (CCP) is defined as a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

Codex has also developed specific guidance for low moisture foods through the Code of Hygienic Practice for Low-Moisture Foods (CXC 75-2015) (Codex Alimentarius Commission, 2018) which outlines Good Manufacturing Practices (GMPs) and Good Hygienic Practices (GHPs) that will help control microbial hazards during the manufacture of low moisture foods. The Code applies to a wide range of low moisture foods including dried fruits and vegetables (e.g. desiccated coconut), cereal-based products (e.g. breakfast cereals), dry protein products (e.g. dried dairy products and soy protein), confections (e.g. chocolate and cocoa), snacks (e.g. spice-seasoned chips/crisps), tree nuts, seeds for consumption (e.g. sesame seeds and sesame seed paste), spices and dried culinary herbs. Rapeseed protein isolate would fit in the dry protein products group. Low moisture foods often have water activity values of 0.85 or below which is too low for foodborne pathogens to grow (ICMSF, 1996). Even though growth of *Salmonella* spp. is prevented in low moisture foods, cells can survive and remain viable for long periods of time (Codex Alimentarius Commission, 2018). The water activity for the applicant's rapeseed protein isolate is 0.2 (refer to section 3.3).

The Code identifies *Salmonella* spp. and *B. cereus* are the primary pathogens of concern in low-moisture foods. Epidemiological evidence highlights the importance of *Salmonella* spp. as the cause of many outbreaks associated with low moisture foods. Factors that contribute to *Salmonella* spp. causing outbreaks are the small number of cells needed to cause illness (WHO/FAO, 2002) and that cells are capable of remaining viable in low moisture foods for long periods of time (Podolak et al. 2010).

3.3 Manufacturing process and controls

Rapeseed protein isolate is derived via extraction from rapeseed press cake that is retained after oil pressing from the seeds of one or more of *Brassica napus*, *Brassica rapa or Brassica juncea.* The rapeseed press cake that remains after the cleaned, flaked and conditioned seeds have been pressed to separate the oil is the raw material for protein production. The manufacturing process is summarised in Figure 2.1. No solvent extraction step is included in

this process.

Food Safety System Certification (FSSC) 22000 (Version 4.1) approval has been achieved by the applicant and certified by Lloyd's Register. The certification includes ISO 22000:2005, ISO/TS 22002-1:2009 and additional FSSC 22000 requirements for Food Chain (Sub) Category: C IV. FSSC 22000 is a food safety management system to control foodborne hazards. The certification is applicable for the production of proteins (from non-animal sources) for food use. The applicant has performed a HACCP analysis on the manufacturing process of rapeseed protein isolate, according to EU Regulation EC/852/2004. This has been certified by an external auditor with FSSC22000 certification.

Supplier approval, environmental monitoring and verification programs are incorporated into the GMP practices at both production locations. *E. coli* is monitored in the rapeseed press cake. In-process monitoring of microbial hygiene after each unit operation is by analysis of Total Plate Count. *B. cereus* levels are also determined in the final product according to the method ISO 7932: 2004. The product specification for *B. cereus* is ≤100 cfu/g. The water activity of the final product is 0.2 (n = 3 samples). Process parameters including temperature and throughput time are monitored to control microbial growth.

The applicant states that whilst GMP practices are used throughout manufacture, there are no microbial reduction steps in the manufacturing process of the rapeseed protein isolate.

3.4 Microbiological risk assessment

The acknowledgement from the applicant that the manufacturing process does not have a control point for microorganisms, including *Salmonella* spp., raises concerns about the scope of the requested permissions.

The food permissions cover a range of food classes including bakery products, beverages, dairy products, mixed foods, meat analogues and protein-based products including protein-enriched powders and bars (Table 2.2). From a risk perspective, the greatest concern for *Salmonella* spp. is for manufactured convenience foods which are consumed without additional control steps, such as cooking.

Foods such as breads and biscuits which have been baked will have received a temperature-time combination sufficient to inactivate *Salmonella* spp. present in the rapeseed protein isolate. Amongst the other foods that the product could be used in, protein enriched powders with typical use levels of up to 10% rapeseed protein isolate may fall into the manufactured convenience food group. Protein-enriched powders may be used in the home or gym, for example, as an ingredient for foods which are cooked or added to drinks and smoothies which are consumed without cooking. The potential for extended storage times after preparation and poor temperature control would further raise the risk from consuming these particular types of foods. Without specific information on the preparation and consumption patterns of rapeseed protein isolates it is not possible to quantitatively establish the risk of illness for *Salmonella* spp. or *Bacillus cereus*.

FSANZ screened the risk from consuming the rapeseed protein isolate using its *Imported food risk advice framework* (FSANZ, 2018). The framework uses a semi-quantitative approach to assess the risk from imported food using information on infectivity, disease severity and the likelihood of exposure. Information on the characteristics, symptoms of disease and epidemiology of *Salmonella* spp. and *B. cereus* can be found in the FSANZ Agents of Foodborne illness technical series (FSANZ, 2017b).

The hazard impact assessment considers the effects of exposure to a hazard on an individual. The assessment takes into account the infectivity, i.e. the dose likely to cause

illness and the severity of the consequences of that illness. The results of these two components are then applied in a matrix and expressed as a hazard impact score. The microbiological infectivity for *Salmonella* spp. was assigned a rating of Low/Medium to account for uncertainty in the infective dose. A 'Very Low' microbiological infectivity was assigned for *B. cereus* as very high numbers of cells are required in foods before toxins are produced which may cause illness. The disease severity was assigned as 'Serious' for *Salmonella* spp. and 'Mild' for *B. cereus*. Subsequently the hazard impact scores were determined to be 'Medium/High' for *Salmonella* spp. and 'Very Low' for *B. cereus*.

The exposure assessment considers five likelihood categories ranging from 'Very High' to 'Very low'. Within each category, graded descriptors allow a determination of the type and level of evidence required to allocate a likelihood estimate.

These descriptors are based on:

- evidence that supports the hazard has caused foodborne illness
- evidence that supports the hazard is present in the food and at levels sufficient to cause illness
- evidence that supports the food is consumed
- effects of food processing on the hazard (increase, reduce, no effect)
- post-processing contamination
- whether the characteristics of the food will support the growth of any contaminating pathogen.

No evidence was found that *Salmonella* spp. or *B. cereus* is present in unprocessed rapeseed or rapeseed protein isolate at levels sufficient to cause foodborne illness. Using information for other types of seeds may be a useful proxy for unprocessed rapeseed prior to oil extraction. The FAO/WHO (2014) ranking of low moisture foods report includes statistical analysis of the prevalence of bacteria, including *Salmonella* spp. and *B. cereus* in seeds. A low prevalence of *Salmonella* spp. was found in seeds for consumption (alfalfa, flax, hemp, karela, melon, poppy, pumpkin, and sunflower) and mixed/unspecified seeds (0.5%) (FAO/WHO, 2014). The average prevalence of *B. cereus* in seeds (flax, karela, poppy, pumpkin, sunflower) was 7.0% based on three studies (FAO/WHO, 2014). These results suggest that a low prevalence of *Salmonella* spp. and *B. cereus* could be expected in unprocessed rapeseed prior to oil extraction.

The effect of processing on the levels of the hazards was assigned as 'No effect', noting that there are no microbial reduction steps in the manufacturing process of the rapeseed protein isolate. Post-processing contamination was also assigned as 'No effect'. The low moisture level and water activity of the product inhibits the growth for both *Salmonella* spp. and *B. cereus*.

The final risk characterisation estimate is a rating on a three point scale: Low, Medium and High. As the rapeseed protein isolate is a novel food (there is no current consumption data for Australia or New Zealand), information required to determine the risk rating is limited or not available and proxy information was used. The available information for *Salmonella* spp. and *B. cereus* were put through the risk framework to determine risk levels for the final product prior to use. The resulting risk characterisation estimate was low-medium risk for *Salmonella* spp. and low risk for *B. cereus*.

3.5 Microbiological risk assessment conclusions

FSANZ concludes that rapeseed protein isolate may pose a microbiological risk for *Salmonella* spp. in some types of manufactured convenience foods that do not undergo a

final microbiocidal step. Rapeseed protein isolate is a low moisture food (as defined by the Codex Alimentarius Commission) and the water activity of the product inhibits the growth of pathogens including *Salmonella* spp. and *B. cereus*, provided suitable storage conditions are maintained. The applicant has certification in relevant food safety management systems to control foodborne hazards however there are no microbial reduction steps in the manufacturing process of the rapeseed protein isolate. A screening method was used to assess the risk for *Salmonella* spp. and *B. cereus* when the product is used in manufactured convenience foods. The risk levels determined were low-medium for *Salmonella* spp. and low for *B. cereus*. For cooked foods, such as bakery products, where rapeseed protein isolate is used as an ingredient, the risk will be low for both *Salmonella* spp. and *B. cereus*.

4. Nutrition risk assessment

4.1 Objectives for the nutrition risk assessment

The application states that rapeseed protein isolate can be used in foods as a replacement for proteins derived from animal sources (e.g. whey) or other plant sources (e.g. soy, pea), including for use in new product development.

The objectives for the nutrition risk assessment were to:

- Identify whether there are nutritional concerns for any population groups if rapeseed protein isolate is used as a protein source in the proposed foods at the proposed use levels. Potential nutritional concerns include risks related to protein quality and anti-nutritional factors.

- Compare the protein quality of rapeseed protein isolate to other protein sources from animal (e.g. whey) and other plant (e.g. soy, pea) protein.

- Evaluate whether anti-nutritional factors (protease inhibitors, phytate, and phenolic substances) in rapeseed protein isolate pose a risk to meeting the nutritional requirements of the general population.

4.2 Approach for the nutrition risk assessment

The application relates to the use of rapeseed protein isolate in a range of food applications. The applicant states that maximum use levels are not expected to exceed 30% in any one food, with typical use levels being up to 10%. The application does not request or provide information for the use of rapeseed protein isolate in infant formula products (infant formula, follow-on formula and infant formula products for special dietary uses) and infant foods. The scope of the nutrition risk assessment, therefore, does not include these food classes. FSANZ has assumed that the macronutrient composition of food classes and products using rapeseed protein isolate as an ingredient will not substantially alter and, thus, usual dietary protein intake will remain unchanged.

Five anti-nutritional factors are found in canola:
- Erucic acid
- Phenolics (expressed as sinapic acid, the predominant phenolic)
- Phytic acid
- Glucosinolates
- Protease inhibitors.

Protease inhibitors, phytic acid and phenolics are evaluated in the nutrition risk assessment.

Erucic acid and glucosinolates are assessed in the toxicological assessment (refer to Section 5) because they have been associated with overt signs of toxicity or a health-based guidance value has been established by FSANZ.

4.3 Protein quality

The application:

- states that rapeseed protein isolate contains ~40-65% cruciferins and 35-60% napins
- presents the protein content from analysis of five batches of CanolaPRO produced in 2015 and 3 batches produced in 2018, and a nitrogen conversion factor of 6.25. Total protein range: 96.3–99.1%.
- proposes a specification for protein content: ≥ 90%
- presents an amino acid profile using five batches of CanolaPRO (Table 4.1)
- states that the Protein Digestibility Corrected Amino Acid Score (PDCAAS) of rapeseed protein isolate is 100%.

Protein quality reflects the ability of dietary protein to provide adequate essential amino acids and depends on the amino acid composition and digestibility of the protein. Protein quality can be quantified using the PDCAAS. Rapeseed protein contains all of the essential amino acids as shown by the amino acid profile of CanolaPRO in Table 4.1. The PDCAAS of three rapeseed protein isolates and the values used to derive the PDCAAS (amino acid score and protein digestibility) are presented in Tables 4.2, 4.3a and 4.3b.

The PDCAAS of CanolaPRO has been measured to be 100% (Table 4.2). The PDCAAS of other rapeseed protein isolates approved overseas (Isolexx and Vitalexx) have been reported as 86% and 87%, respectively (Table 4.3a) and both as 100% when assessed in a different laboratory (Table 4.3b). The PDCAAS values of the rapeseed protein isolates are consistently higher than soy protein isolate comparators. In conclusion, the protein quality of rapeseed protein isolates (specifically CanolaPRO, Isolexx and Vitalexx) has been reported to be similar to that of the milk protein casein and slightly higher than that of soy protein isolates.

Table 4.1 *Amino acid profile of CanolaPRO*

Amino acid	Amino acid content (g/100 g)
Alanine	4.17
Arginine	6.32
Aspartate	5.68
Glutamate	22.40
Glycine	4.83
Histidine*	3.05
Hydroxyproline	<0.05
Isoleucine*	3.52
Leucine*	6.84
Lysine*	6.08
Ornithine	<0.05
Phenylalanine*	3.65
Proline	6.57
Serine	3.91
Threonine*	3.73
Tyrosine	1.96
Valine*	4.69
Cysteine	3.51
Methionine*	2.06
Tryptophan*	1.36

* Essential amino acids.

Table 4.2 *Protein quality of rapeseed protein isolate (CanolaPRO), soy protein isolate and a casein control*[1]

	Rapeseed protein isolate (CanolaPRO)	Soy protein isolate (Supro 515™)	Casein control
Amino acid score	1.18	1.00	1.34
True protein digestibility (%)	94.0	96.2	98.2
Actual PDCAAS (%)[2]	110	96	131
PDCAAS (%)	100	96	100

PDCAAS, protein digestibility corrected amino acid score (= amino acid score x true protein digestibility).
[1] DSM study number 44697.
[2] Prior to truncation (PDCAAS values greater than 100% are truncated to 100%).

Table 4.3a *Protein quality of rapeseed protein isolate (Isolexx™ and Vitalexx™), soy protein isolate and a casein control*[1]

	Rapeseed protein isolate (Isolexx™)	**Rapeseed protein isolate (Vitalexx™)**	**Soy protein isolate (Dunasoy 90™)**	**Casein control**
Amino acid score	0.90	0.89	0.87	1.12
True protein digestibility (%)	94.8	98.0	94.9	97.4
Actual PDCAAS (%)[2]	86	87	83	109
PDCAAS (%)	86	87	83	100

PDCAAS, protein digestibility corrected amino acid score.
[1] Source: GRAS Notice No. GRN 000386 Table 9a and 10a (analysis conducted in manufacturer's lab).
[2] Prior to truncation (PDCAAS values greater than 100% are truncated to 100%).

Table 4.3b *Protein quality of rapeseed protein isolate (Isolexx™ and Vitalexx™), soy protein isolate and a casein control*[1]

	Rapeseed protein isolate (Isolexx)	**Rapeseed protein isolate (Vitalexx)**	**Soy protein isolate (Dunasoy 90)**	**Casein control**
Amino acid score	1.09	1.10	0.99	1.51
True protein digestibility (%)	94.8	98.0	94.9	97.4
Actual PDCAAS (%)[2]	104	108	94	147
PDCAAS (%)	100	100	94	100

PDCAAS, protein digestibility corrected amino acid score.
[1] Source: GRAS Notice No. GRN 000386 Table 9b and 10b (analysis conducted using at LUFA Nord-West, Oldenburg, Germany).
[2] Prior to truncation (PDCAAS values greater than 100% are truncated to 100%).

4.4 Anti-nutritional factors

The application:

- presents the concentration of the following anti-nutritional factors from analysis of five batches of CanolaPRO:
    - Total phenolics (expressed as sinapic acid, %): 0.060–0.088
    - Phytic acid (%): all < 0.14
    - Trypsin inhibitor activity (mg/g): 18.5–25.5
- proposes a specification for CanolaPRO for phytate: ≤ 1.5 % w/w.

The concentrations of anti-nutritional factors in two other rapeseed protein isolates are available. A cruciferin-rich rapeseed protein isolate (Mejia et al. 2009a & GRAS Notice No. GRN 000327) contains:

- Phytic acid (%): 0.32
- Total phenolics (%): 0.40.

A napin-rich rapeseed protein isolate (Mejia et al. 2009b) contains:

- Phytic acid (%): 3.34
- Total phenolics (%): 0.26.

4.4.1 Protease inhibitors

Protease inhibitors, such as trypsin inhibitors, are found in many plants including the seeds of most legumes and cereals. Inhibition of proteases in the gut can reduce protein digestion. The application reports the trypsin inhibitor activity of CanolaPRO batches ranging from 18.5 to 25.5 mg/g protein. At the proposed typical use level of CanolaPRO up to 10%, this equates to a maximum trypsin inhibitor activity of 1.9–2.6 mg/g food. At the proposed maximum use level of CanolaPRO of 30%, this equates to a maximum trypsin inhibitor activity of 5.6–7.7 mg/g food.

Protease inhibitors can be denatured by heating which inactivates them. As many of the food categories proposed to contain rapeseed protein isolate may be heat processed, the final trypsin inhibitor activity in the food is likely to be lower. No guidance value for usual dietary intake of protease (trypsin) inhibitors or limits for its concentration in individual products have been established. The protease inhibitor activity of CanolaPRO can be compared with that of other foods. Xiao et al. (2012) reported that soybeans have a high protease inhibitor content, although the final content in soy foods after manufacturing could vary. Doell et al. (1981) reported trypsin inhibitor activities for raw soybeans, raw tofu, and cooked tofu as 49.6, 9.2 and 5.5 mg/g protein, respectively.

As described in Section 4.3, the protein quality of rapeseed protein isolate is high and favourable when compared to, for example, soy protein isolate. Therefore, any potential inhibition of protein digestion due to the presence of protease inhibitors in CanolaPRO does not raise nutritional concerns.

4.4.2 Phytate

Phytates are salt forms of phytic acid, a phosphorus storage compound present in plants. Phytates can reduce mineral bioavailability by chelating (binding) mineral ions, particularly iron and zinc, but also calcium and manganese (Hurrell 2003). Mineral deficiency due to phytate intake depends on the extent of consumption of phytate-rich foods in the total diet, in particular the contribution of cereal proteins (Gemede and Ratta 2014). Phytate has also been associated with beneficial health outcomes, which are not considered in this report. The negative impact of phytates on mineral absorption may be attenuated by the consumption of enhancers of mineral bioavailability, such as vitamin C which enhances the bioavailability of non-haem iron.

4.4.2.1 Phytate concentration of rapeseed protein isolates

The applicant reported phytate levels of <0.14% in batches of CanolaPRO and has proposed a specification of ≤1.5%. The GRAS Notice No. 000386 reports the concentration of phytate in other rapeseed protein isolates to be <1.25% (Isolexx from *Brassica juncea*), and <1.0% (Vitalexx from *Brassica juncea* and *Brassica napus*). GRAS Notice No. 000327 reports phytic acid concentrations for rapeseed protein isolates of 0.32% in Puratein™, and 3.34% in Supertein. The specification for phytate in the EC 2014/424/EU regulation is ≤1.5%. This threshold may have been chosen because the novel food ingredient (Isolexx) that EFSA (2013) assessed contained ≤1.5% phytate.

4.4.2.2 Adverse effects

Phytate in foods can reduce the bioavailability of minerals such as zinc and iron, so high intakes of phytate may pose a risk to individuals at higher risk of low iron and zinc status, such as those following vegan or vegetarian diets. Phytates can inhibit non-haem iron absorption (Beck et al. 2014), and the bioavailability of non-haem iron is generally lower than that of haem iron, and is more variable (Carpenter and Mahoney 1992).

4.4.2.3 Phytate content in foods

The calculated phytate concentration in products proposed to contain rapeseed protein isolate is compared to that of foods without added rapeseed protein isolate (Table 4.4). The use of rapeseed protein isolate containing 1.5% w/w phytate (the maximum level proposed in the application) at the proposed typical and maximum use levels of rapeseed protein isolate, was used to calculate the phytate content in various foods. A comparison using the phytate concentration of 3.34% in Supertein is also provided. At the proposed typical use level of CanolaPRO, the range of resulting phytate concentrations derived from rapeseed protein isolate across food categories is 0.075–0.15% (using a phytate content of 1.5% w/w) and 0.17–0.33% (using a phytate content of 3.34% w/w). The respective phytate concentrations at the proposed maximum use level are 0.075–0.45% and 0.17–1.0%.

Three food categories can contain relatively high levels of phytate: whole beans (0.2–9.2%); bean-based foods (e.g. tofu: 2.9%); and nuts (0.2–9.4%). High bran ready-to-eat cereal is also high in phytate (3.3%). Variation in phytate levels within food categories and sub-categories is apparent. For breads, differences in the extent of fermentation and extraction rate of flour may contribute to the variation. Within one type of bean, differences in growing condition and cultivars may account for variation (Reddy 2002). Differences in phytate or phytic acid content may also be attributed to the stage of maturation and method of analysis used (Schlemmer et al. 2009).

Table 4.4 *Phytate content in foods proposed to contain rapeseed protein isolate and other dietary sources*

Source	**Food category**	**Examples**	**Proposed typical use level of rapeseed protein isolate[3] (%)**	**Phytate/phytic acid content[4] (%)**	
DSM CanolaPRO application	Bakery products, Cereals and cereal products	Breads and rolls; doughnuts; cookies; crackers; cakes; pies; batters; muffins; cereal and granola bars; breakfast cereals	5	0.075[1]	0.17[2]
	Beverages	Fruit juices and juice blends; soft drinks; energy drinks	5	0.075[1]	0.17[2]
	Dairy products	Yogurt; cheese and cheese products; desserts and dessert toppings; ice pops and sorbets, jellies	5	0.075[1]	0.17[2]
	Mixed foods	Prepared food such as ready-to-eat meals, soups, and pasta; and, snacks (e.g. extruded)	5[5]	0.075[1,5]	0.17[2,5]
	Meat analogues	Meat analogues	5[5]	0.075[1,5]	0.17[2,5]
	Protein based products	Protein-enriched, bars, and pasta; protein-enriched powder and ready-to-drink beverages; energy bars	10[5]	0.15[1,5]	0.33[2,5]
Macfarlane et al. (1988)	Nuts	Walnut, almond, peanut, hazelnuts, Brazil nuts, coconut	n/a	0.36–1.7	
Schlemmer et al. (2009)	Cereals	Cereals (excluding bran and germ)	n/a	0.06–2.22 (sorghum: 3.35)	
	Legumes	Whole legumes	n/a	0.22–2.90	
	Nuts	Nuts	n/a	0.15–9.42	
Reddy (2002)	Bakery products, Cereals and cereal products	Cereals	n/a	0.06–2.22	
		Ready-to-eat cereal products	n/a	0.05–1.83 (Wheat cereal, 100% Bran: 3.29)	
		Infant cereals	n/a	0.06–1.38	

Source	Food category	Examples	Proposed typical use level of rapeseed protein isolate[3] (%)	Phytate/phytic acid content[4] (%)
		Breads	n/a	0.03–1.38 (Iranian flat bread, Tanok, unleavened: 2.41)
	Beans and bean products	Whole beans	n/a	0.17–2.93 (pigeon bean: 0.22–7.00; dolique beans: 5.92–9.15)
		Bean-based foods	n/a	0.05–2.90 (khaman: 5.20)
	Tubers, fruits, leafy vegetables, nuts, and other foods	Tubers	n/a	0.01–0.32
		Fruit		0.10–0.18 (avocado: 0.51)
		Leafy products and vegetables	n/a	0.01–0.31
		Nuts	n/a	0.63–3.22 (Brazil nut: 1.97–6.34)

[1] Phytate content derived from the isolate only (not total phytate). Calculated using the maximum phytate concentration for rapeseed protein isolate (1.5% w/w) from DSM's proposed phytate specification, at the typical use level proposed by the applicant for CanolaPRO.

[2] Phytate content derived from the isolate only (not total phytate). Using the highest phytate concentration reported for a rapeseed protein isolate (3.34% w/w, Supertein), at the typical use level proposed by DSM for CanolaPRO.

[3] Where the typical use level proposed by DSM is a range, the highest value has been used.

[4] Phytate or phytic acid content is reported, depending on the data published.

[5] The proposed maximum use levels of rapeseed protein isolate for 'Mixed foods' (10%), 'Meat analogues' (30%) and 'Protein based products' (30%), are higher than the proposed typical use levels. The phytate content using the maximum use levels and a phytate concentration of 1.5% w/w is: 0.15% ('Mixed foods'); 0.45% ('Meat analogues'); and, 0.45% ('Protein based products'). The phytate content using the maximum use levels and a phytate concentration of 3.34% w/w is: 0.33% ('Mixed foods'); 1.0% ('Meat analogues'); and, 1.0% ('Protein based products').

4.4.2.3.1 Phytate from products containing rapeseed protein isolate versus dietary sources without protein isolates

A direct comparison of the phytate concentration of products proposed to contain rapeseed protein isolate and dietary sources without added rapeseed protein isolate is most relevant when using similar food categories. This is possible for the breads and ready-to-eat cereals groups. Phytate concentration is 0.03–1.83% for most breads and ready-to-eat cereal products (Table 4.4.2.1). For bakery and cereal products using rapeseed protein isolate, the phytate concentration derived from the isolate alone could be up to 0.075–0.17% (the variation reflects the range of isolates' phytate content). The total phytate content will be higher than this range, however, since non-isolate components of the product will also contribute phytate. The expected total phytate content is not known but the range could be 0.1–2% (the sum of 0.03–1.83% and 0.075–0.17%) which represents a relatively small change in phytate concentration. This reflects a 'worst-case scenario' that assumes there is an addition, with no substitution, of phytate to the original product. The phytate concentration of breads and ready-to-eat cereals using rapeseed protein isolate is expected to be lower than, or in line with, that of many current sources outlined in Table 4.4.2.1. A phytate content of up to 2% (reflecting the breads and ready-to-eat cereals groups containing the rapeseed protein isolate) does not exceed the upper phytate concentration of food categories with naturally high levels of phytate (e.g. whole beans, bean-based foods and nuts) or individual products (e.g. tofu: 2.9% and high bran ready-to-eat cereal: 3.3%). The volume of breads and ready-to-eat cereals consumed by the Australian and New Zealand populations may, however, differ to these other foods.

4.4.2.3.2 Phytate from the applicant's rapeseed protein isolate compared with other protein isolates

The application states that rapeseed protein isolate may be used to replace other protein sources, such as soy, whey, or pea, including for use in new product development. The phytate content of soy protein isolates, which are the most widely used plant protein isolates (Singh et al. 2008), is presented in Table 4.5. Minimal information was located on the phytate content of other protein isolates.

The reported range of phytate levels in various rapeseed protein isolates (<0.14% for CanolaPRO to 3.34% for Supertein™) is wider than that reported for soy protein isolates. Honig et al. (1984) reported the phytic acid content of commercial soy protein isolates to be 1.6–2.0% before dialysis and 1.1–1.8% after dialysis. Hurrell et al. (1992) reported 0.9–1.7% phytate in commercial soy protein isolates. Analyses done by others report between 1.5% and 1.7% (De Boland et al. 1975; Thompson and Erdman 1982; Naczk et al. 1986). Koletzko et al. (2006) quote a range of 1–2% but no source for this data is cited.

Assuming that rapeseed protein isolate were to replace, not add to, the existing total protein isolate intake, maintaining a maximum level of phytate similar to the upper levels present in soy protein isolates (1.5–1.7%) would minimise any risk of increased total phytate intake. Since the phytate content of rapeseed protein isolates is highly variable, and contingent on factors such as genotype and environmental conditions (Prynne et al. 2010), specifying a maximum phytate level is recommended.

Table 4.5 *Phytate content in rapeseed and soy protein isolates*

Source	Protein isolate	Commercial name	Phytate/phytic acid content[1] (%)
A1175 application	Rapeseed protein isolate	CanolaPRO	<0.14
GRAS000327	Rapeseed protein isolate	Vitalexx™, Isolexx™	<1.0 and <1.25
GRAS000386	Rapeseed protein isolate	Puratein™, Supertein™	0.32 and 3.34
Koletzko et al. (2006)	Soy protein isolate	n/a	1–2
Honig et al. (1984)	Soy protein isolate (commercial, before dialysis)	Edipro A™, Supro 710™, and Supro HD90™ (Ralston Purina Company, USA)	1.6–2.0
Honig et al. (1984)	Soy protein isolate (commercial, after dialysis)	Edipro A™, Supro 710™, and Supro HD90™ (Ralston Purina Company, USA)	1.1–1.7
Hurrell et al. (1992)	Soy protein isolate (commercial)	n/a	0.9–1.7
Thompson and Erdman (1982)	Soy protein isolate	n/a	1.63
De Boland et al. (1975)	Soy protein isolate	n/a	1.52
Naczk et al. (1986)	Soy protein isolate	SUPRO 620™ (Ralston Purina Company, USA)	1.69

n/a, not applicable.
[1] Phytate or phytic acid content is reported, depending on the data published.

4.4.2.4 Conclusion

At the highest typical use level of 10% in foods, the maximum phytate level in rapeseed protein isolate proposed in the application (1.5% w/w) equates to maximum levels in foods of 0.15%, which is close to the lower end of the range reported for commonly consumed foods such as cereals, beans, and nuts. Also, the maximum phytate level of 1.5% proposed in the specifications for rapeseed protein isolate is similar to the maximum levels reported for soy protein isolates (1.5–1.7%) which are the most widely used plant protein isolates. Therefore, phytate levels of up to 1.5% in rapeseed protein isolate do not raise concerns regarding mineral bioavailability.

4.4.3 Phenolic compounds

Phenolic compounds, which include flavonoids, phenolic acids and tannins are common to many plants and are therefore abundant in the human diet. The predominant phenolic substance in rapeseed is 3,5-dimethoxy-4-hydroxycinnamic acid (sinapic acid). No references were found to adverse effects of sinapic acid, and EFSA (2013) cited references reporting that the concentration of sinapic acid in rapeseed protein isolate is lower than in commonly consumed foods including apple, pear, broccoli and potato flour. Sinapic acid in rapeseed protein isolate is therefore not considered to be of health concern.

4.5 Nutrition risk assessment conclusions

FSANZ concludes that rapeseed protein isolate, when used as a protein source in foods at the proposed maximum or typical use levels, does not raise nutritional concerns.

The protein quality of rapeseed protein isolate, as determined from its amino acid profile and digestibility, is comparable to that of the milk protein casein and slightly higher than that of soy protein isolates.

At the highest typical use level of 10% in foods, the maximum phytate level in rapeseed protein isolate proposed in the application (1.5% w/w) equates to maximum levels in foods of 0.15%, which is close to the lower end of the range reported for commonly consumed foods such as cereals, beans and nuts. Also, the maximum phytate level of 1.5% is similar to the maximum levels reported for soy protein isolates (1.5–1.7%) which are the most widely used plant protein isolates. Therefore, phytate levels of up to 1.5% in rapeseed protein isolate do not raise concerns regarding mineral bioavailability.

As rapeseed protein isolate will be used as an ingredient in processed foods as a replacement for other protein sources, including for use in new product development, usual protein intakes are not expected to change if rapeseed protein isolate is approved as a protein source.

5. Toxicological assessment

5.1 Objective and scope of the toxicological assessment

Rapeseed protein isolate and other members of the *Brassicaceae* family contain anti-nutritional compounds including erucic acid, phenolic compounds, phytic acid, glucosinolates, and protease inhibitors. The objective of this section is to assess whether the levels of erucic acid or glucosinolates in rapeseed protein isolate pose any toxicological concerns. The potential allergenicity or rapeseed protein isolate has also been assessed.

5.2 Toxicological data

5.2.1 Erucic acid

Erucic acid is a 22–carbon monounsaturated fatty acid with a single double bond at the omega 9 position. It constitutes about 30–60% of the total fatty acids of rapeseed, mustard seed and wallflower seed and up to 80% of the total fatty acids of nasturtium seeds. Erucic acid has also been found in some marine animal oils.

FSANZ conducted a toxicological review and risk assessment of erucic acid in 2003 and established a provisional tolerable daily intake for erucic acid of 7.5 mg/kg bw on the basis of myocardial lipidosis observed in neonatal piglets. The PTDI was based on a no observed effect level (NOEL) of 750 mg/kg bw/day and the application of a 100-fold uncertainty factor. FSANZ noted at the time that the effect appeared to be transient and reversible, and that epidemiological studies show no association between cardiac disease in humans and a diet high in erucic acid. The full report is available at: https://www.foodstandards.gov.au/publications/Documents/Erucic%20acid%20monograph.pdf.

EFSA (2016) identified a TDI for erucic acid of 7 mg/kg bw/day, also based on lesions of myocardial lipidosis in rats and in neonatal piglets, but noted that in human beings, an inverse relationship between erucic acid in erythrocytes and coronary heart disease has

been observed in two independent cohorts (EFSA 2016).

A literature search did not identify any new studies that would warrant a review of the FSANZ PTDI.

FSANZ has conducted a Dietary Exposure Assessment (refer to section 6) to estimate the dietary exposure to erucic acid of Australian and New Zealand consumers from rapeseed protein isolate. The exposure was not considered to represent a public health concern as dietary exposure estimates based on both the proposed maximum and typical use levels were well below the PTDI established by FSANZ in 2003 of 7.5 mg/kg bw.

5.2.2 Glucosinolates

Glucosinolates are a class of *S*-β-thioglucoside *N*-hydroxysulphate structures. More than 120 glucosinolates have been identified, with most isolated from *Brassicaceae* (cruciferous plants). In intact plants, glucosinolates are accompanied by, but physically separated from, β-thioglucosidase enzymes (myrosinases). When plant tissue is damaged, the enzymes come in contact with the glucosinolates and rapid hydrolysis occurs, with the production of reactive metabolites including isothiocyanates. Conversion of glucosinolates to isothiocyanates and other metabolites is also mediated by bacterial flora in human intestines (Dinkova-Kostova and Kostov 2012).

In human beings, metabolites of glucosinolates including isothiocyanates, thiocyanates and 5-vinyloxazolidine-2-thione inhibit iodine absorption by the thyroid gland (Downey 2005). Similar anti-thyroidal effects have been demonstrated in rats fed rapeseed proteins containing high levels of glucosinolates, but these effects were not observed in rats fed rapeseed protein isolates containing 30 ppm glucosinolates (Loew et al. 1976).

FSANZ has considered glucosinolates in genetically modified canola species in a number of previous applications (A363, A372, A388, A1071, A1089, A1140, A1143) and has cited a regulatory limit (AOF 2015) for glucosinolates in canola meal for feeding to livestock of <30 µmol/g in oil-free, air-dried meal. This limit is based on goitrogenic properties of glucosinolates. Analysis of five independent, representative batches of CanolaPRO showed that in all five batches, concentrations of glucosinolates were < 0.1 µmol/g, and below the level of quantification.

FSANZ has conducted a Dietary Exposure Assessment (Refer to section 6) to estimate the dietary exposure to glucosinolates of Australian and New Zealand consumers from *Brassica* vegetables in the diet, and to compare that to exposure from rapeseed protein isolate (refer to section 6). The assessment found that additional exposure to glucosinolates from rapeseed protein based on typical use levels is low and equivalent to the consumption of around 30 g/day of *Brassica* vegetables, and therefore is not a health concern.

5.2.3 Allergenicity

5.2.3.1 Relationship between rapeseed and mustard plant species

The applicant has requested the use of rapeseed protein isolate produced from three *Brassica* species: *Brassica napus, Brassica rapa and Brassica juncea.* These three species are also used for the production of rapeseed oil, as defined in the Codex Standard 210-1999 on vegetable oils (Codex 1999). Two of the *Brassica* species from which rapeseed protein isolate is derived (*Brassica rapa and Brassica juncea*) are also referred to as types of mustard, and allergy to foods derived from the seeds of mustard species is well documented (see section 5.2.3.3).

Brassica juncea is commonly known as Indian mustard, Chinese mustard, brown mustard or oriental mustard and is used as a spice, as is white (yellow) mustard (*Sinapis alba*, also known as *Brassica alba*) and black mustard (*Brassica nigra*) (Sharma et al. 2019). *Brassica napus* is commonly referred to as rapeseed and oilseed rape, and *Brassica rapa* as field mustard and turnip rape (Table 5.1).

Table 5.1 *Scientific and common names of some Brassica and Sinapis species**

Scientific name	Common names
Brassica napus	Rapeseed, oilseed rape
Brassica rapa	Field mustard, turnip rape
Brassica juncea	Indian mustard, Chinese mustard, brown mustard, oriental mustard
Brassica nigra	Black mustard
Sinapis alba (also known as *Brassica alba*)	White mustard, yellow mustard

* Note that the term canola refers to seeds of the genus *Brassica* (*Brassica napus*, *Brassica rapa* or *Brassica juncea*) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of several specified glucosinolates per gram of air-dry, oil-free solid (https://www.canolacouncil.org/oil-and-meal/what-is-canola/#OfficialDefinition).

5.2.3.2 Bioinformatics study on proteins from Brassica species

The applicant submitted a bioinformatics study investigating the potential allergenicity of several proteins from *Brassica* species:

Bioinformatics Analysis of Potential Allergenicity and Celiac Disease of Six Storage Proteins from Rapeseed for Food Safety Evaluation (Goodman 2016; unpublished report). Regulatory status: GLP; conducted in accordance with Codex Alimentarius (2003) guidelines.

The amino acid sequences of six proteins from *Brassica* species (five proteins from *Brassica napus*, and one protein from *Brassica oleracea*), submitted to the Food Allergy Research and Resource Program (FARRP), were evaluated using AllergenOnline.org version 16, using a full-length FASTA search and a sliding 80 amino acid FASTA search. A search of the NCBI-Protein database using BLASTP 2.4.0+ with keywords “allergen” or “allergy” was also conducted. Sequences were also run against the Celiac Database.

There were no exact matches to peptides in the Celiac Database, and FASTA alignments had scores well below the limits considered to indicate risk of celiac disease.

High identity amino acid sequence matches were found between proteins from *Brassica* species and proteins from the mustard species *Sinapis alba*. The author concluded that closely related species in the mustard family (in the genera *Brassica* and *Sinapis*) are likely to elicit cross-reactivity in those sensitised and allergic to any member of those genera, and that cross-reactivity could be due to sensitisation to cruciferins, napins or lipid transfer proteins.

Moderate to high sequence identities to seed storage allergens of more distantly related plants were identified, however these were not considered likely to be clinically relevant, because according to Goodman (2016) there is a lack of evidence for clinical cross-reactivity between mustard seed proteins and homologues outside the family *Brassicaceae*.

5.2.3.3 Human allergenicity data on Brassica species

Allergy to foods derived from the seeds of mustard species is well documented (Sharma et al. 2019) with a cause and effect relationship confirmed in single and double-blind placebo controlled food challenges (Rancé et al. 2000; Morisset et al. 2003; Figueroa et al. 2005). Mustard allergy was reported to be the fourth leading cause of food allergy in France (Rancé et al. 1999). The EU and Canada have included mustard as a priority allergen for labelling purposes (CEC 2007; Health Canada 2010). The US FDA does not include mustard as a major food allergen.

Rapeseed plants contain allergenic storage proteins and allergy to rapeseed proteins has been described. A study in Finland, where rapeseed oils are commonly consumed undertook screening of 1887 children with atopic dermatitis using skin prick tests (SPT) for sensitization to crushed seeds from turnip rape (*Brassica rapa*) and oilseed rape (*Brassica napus*). Two hundred and six (11%) tested positive for at least one species. Of 28 children that underwent an open lip challenge with seeds from turnip rape, 17 tested positive, and of the remaining 11 individuals 8 returned a positive oral food challenge (Poikonen et al. 2006).

Cross-reactivity of rapeseed allergens with related *Brassica* species have been observed, due to the high sequence similarity between the seed storage proteins. Cross-reactivity between the rapeseed (*Brassica napus*) allergen Bra n 1 (previously referred to as BnIII) and mustard (*Sinapis alba*) allergen Sin a 1 was reported in a study of a single rapeseed-allergic patient that developed respiratory symptoms on inhalation of rapeseed flour, and oedema and pruritus of lips, oral mucosa and pharynx, and facial urticaria after ingestion of mustard sauce. An inhibition assay indicated that Sin a 1 inhibited 60% of binding of antibodies to Bra n 1 present in the serum of the rapeseed-allergic patient, whereas Bra n 1 completely inhibited IgE binding to Sin a 1 (Monsalve et al. 1997).

A study by Poikonen et al. (2009) performed allergy testing on fourteen Finnish and fourteen French children with atopic dermatitis, allergies to either wheat, egg, peanut or milk and who previously had a positive SPT to turnip rape (*Brassica rapa*). In contrast to France, consumption of mustard is low in Finland and it is not consumed in significant amounts by children. All of the children had positive SPT to mustard (*Sinapis alba*); nine Finnish (64%) and seven French children (50%) had positive Sin a 1 IgE tests and five children (36%) from each cohort had a positive lip or oral food challenge (Table 5.2).

All of the Finnish children had positive SPT and lip or oral challenge to turnip rape. Although all of the French children had positive SPT to turnip rape, 8 children (57%) had positive Bra r 1 IgE tests and 5 children (36%) had a positive lip or oral challenges. Twelve Finnish children (86%) had positive tests for oilseed rape (*Brassica napus*) and ten children (71%) had positive Bra n 1 IgE tests, however lip or oral challenges were not conducted. Ten French children (71%) had positive SPTs for turnip rape and eight (57%) had positive Bra n 1 IgE tests (Table 5.2).

To assess IgE cross-reactivity between the three allergens, inhibition experiments using 2S albumins at concentrations of 0 - 1000 ng/mL were performed on sera from four children. Dose dependent IgE inhibition from patient sera by all inhibitors was observed.

Table 5.2 *Challenge, skin prick test and immunoassay results from Poikonen et al. (2009)*

	No. with +ve SPT			**No. with +ve 2S albumin IgE test and *IgE ImmunoCAP****			**No. with +ve lip or oral food challenge**	
	Mustard *Sinapis alba*	**Oilseed rape *Brassica napus***	**Turnip rape *Brassica rapa***	**Mustard**	**Oilseed rape**	**Turnip rape**	**Mustard**	**Turnip rape**
Finnish children (n = 14)	14 (100%)	12 (86%)	14 (100%)	9 (64%) *13 (93%)*	10 (71%) *14 (100%)*	10 (71%)	5 (36%)	14 (100%)
French children (n = 14)	14 (100%)	10 (71%)	14 (100%)	7 (50%) *10 (71%)*	8 (57%) *10 (71%)*	8 (57%)	5 (36%)	5 (36%)

* IgE antibodies to oilseed rape and mustard were tested using ImmunoCAP assay. IgE antibodies to 2S albumins of turnip rape (Bra r 1), oilseed rape (Bra n 1) and mustard (Sin a 1) allergens were examined using enzyme-linked immunosorbent assay (ELISA).

Palomares et al. (2002) described an ELISA inhibition assay in which human sera of mustard-allergic patients inhibited binding of recombinant precursor napin protein Bn 1 b to polyclonal antiserum to that protein.

Poikonen et al. (2008) found that 97% (62/64) of children that were sensitized to turnip rape and/or oilseed rape had positive SPT to mustard (*Sinapis alba*).

Six entries for food allergens from *Brassica* species are present in the WHO/International Union of Immunological Societies (IUIS) Allergen Nomenclature database (http://www.allergen.org/). These are the proteins Bra n 1 (from *Brassica napus*), Bra r 1, Bra r 2 and Bra r 5 (from *B. rapa*), Bra j 1 (from *B. juncea*), and Bra o 3 (from *B. oleracea* - cabbage). Information from the database on the allergenicity of these proteins is as follows:

> Bra n 1: “In ELISA approximately 80% of the patients had IgE to purified Bra n 1. In SPTs purified Bra n 1 caused positive reactions in all 6 children tested.” (Puumalainen et al. 2006).
>
> Bra r 1: “In ELISA, approximately 80% of the 72 patients had IgE binding to purified napin from turnip rape. The 72 patients have atopic dermatitis and clearly positive reactions to seeds of oilseed rape and turnip rape, or both on SPTs.” (Puumalainen et al. 2006).
>
> Bra r 2: “Of 60 natural rubber latex-sensitive patients, 51 (82%) showed IgE binding to purified Bra r 2 in ELISA. In 4 out of 6 patients tested purified Bra r 2 (100 µg/mL) induced a positive skin reaction.” (Hänninen et al. 1999).
>
> Bra r 5: No text is present in the database.
>
> Bra j 1: “Of 11 mustard-sensitive patients tested, 7 (64%) showed IgE binding on immunoblot to a synthetic peptide (13.8 kD) of the large chain of Bra j 1.” (Monsalve et al. 1993).

Bra o 3: “Skin prick test with purified Bra o 3 showed positive results in 12 of 14 patients (86%) with cabbage immediate hypersensitivity.” (Palacín et al. 2006).

Health Canada undertook a systematic review of the evidence relating to the allergenicity of mustard to determine the scientific validity for inclusion as a priority allergen for labelling. It noted the potential cross-reactivity between mustard and rapeseed, and as a major producer of both crops concluded that mustard should be included in the list of priority allergens (Health Canada 2010).

EFSA (2013) reviewed the potential allergenicity of rapeseed protein isolate produced from two *Brassica* species – *Brassica napus* and *Brassica rapa*. EFSA concluded that the risk of sensitisation to rapeseed, as well as the risk of cross-reactivity in subjects allergic to mustard, cannot be excluded.

Data from the 2011-2012 Australian National Nutrition and Physical Activity Survey (NNPAS) indicated that 51% of Australians aged 2 years and above consumed mustard over a two day period and 33% were consumers on any single day (ABS, 2014). From the 2008/09 New Zealand Adult Nutrition Survey (NNS) (Ministry of Health, 2011a; Ministry of Health, 2011b), 40% of New Zealanders aged 15 and above consumed mustard. From the 2002 New Zealand Children’s Nutrition Survey (Ministry of Health, 2003; Ministry of Health, 2005), 43% of those aged 5-14 consumed mustard. These figures were derived using the survey data and FSANZ’s recipe database and included mustard powder, liquid style mustards or foods containing mustard. FSANZ is not aware of any case studies of mustard allergy in the Australian or New Zealand population and notes that the Australasian Society of Clinical Immunology and Allergy website does not discuss mustard allergy.

In summary, rapeseed plants contain allergenic proteins which show cross-reactivity with proteins from related mustard species due to the amino acid high sequence similarity between the proteins. FSANZ considers that rapeseed protein isolate has the potential to induce allergic responses in individuals who are allergic to mustard.

5.2.4 Contaminants

According to a review by van der Spiegel et al. (2013), *Brassica napus* can accumulate a number of metals. Metals that have been shown to be absorbed by the roots of the plant and translocated to other parts of the plant include lead, cadmium, zinc, copper and chromium.

Analysis of three batches of CanolaPRO showed zinc levels ranging from 7.59 to 8.24 mg/kg, copper levels ranging from 6.51 to 8.65 mg/kg, and chromium levels ranging from 0.176 to 0.285 mg/kg. On the basis of a maximum daily intake of 1.9 g/kg bw of rapeseed protein isolate as estimated by the applicant, overall exposure to zinc, copper and chromium from rapeseed protein isolate would be orders of magnitude lower than the recommended daily intakes of these metals, all of which are essential micronutrients although toxic in excess. Increased dietary exposure to zinc, copper and chromium from the addition of rapeseed protein isolate to the diet is not, therefore, considered to be of safety concern.

FSANZ noted in the 25th Australian Total Diet Survey (FSANZ, 2019) that arsenic and cadmium are environmental contaminants and that eliminating them from the diet is not practical, but that the As Low As Reasonably Achievable (ALARA) principle should be applied with regard to both. Preliminary dietary exposure estimates suggest that the inclusion of rapeseed protein estimate in the Australian diet will not significantly increase exposure to these elements.

Based on the dietary exposures to rapeseed protein isolate as estimated by FSANZ (based

on typical use levels for Australians 2 years and above, mean 0.67 g/kg bw/day and 90th percentile of 1.33 g/kg bw/day) and the analysed lead concentration in rapeseed protein isolate (0.013 mg/kg, the mean concentration from eight batches), FSANZ estimated an exposure to lead from the isolate of 0.009 µg/kg bw/day at the mean and 0.017 µg/kg bw/day at the 90th percentile. From the 25th Australian Total Diet Study (ATDS) (FSANZ, 2019), the mean dietary exposure to lead for the same age group (2 years and above) was 0.02 – 0.18 µg/kg bw/day (lower to upper bound).

These results demonstrate that the mean exposure to lead from rapeseed protein isolate (which is most likely to be reflective of chronic dietary exposure), is much lower than the mean amount of lead exposure from all other foods in the diet. The mean lead dietary exposure from the isolate when considered together with the mean lead exposures from the 25th ATDS results in only a small increase in the total micrograms of lead exposure per kilogram of body weight per day.

Lead exposures from rapeseed protein isolate are likely to be much lower in reality compared to that predicted as it was assumed that 100% of foods within each of the food classes requested to contain the isolate did so at the proposed typical use levels. In addition, the applicant indicated that the uptake of rapeseed protein isolate into the market is expected to be <0.1% therefore indicating that these estimates are even more likely to be much lower in reality and the overall impact on the total diet long term is going to be negligible. FSANZ's estimated mean lead exposure from rapeseed protein isolate would be similar for adults but would be approximately doubled for children, whose mean lead exposures from the isolate are around double those of the 2 years and above group (see Table 6.3). From rapeseed protein isolate the mean two day average exposures to lead for Australians 2 years and above of 0.009 µg/kg bw/day and for children 2-14 years of 0.017 µg/kg bw/day (again assuming 100% foods in all classes at the typical use level) are much lower than the 0.3 µg/kg bw/d that is associated with a drop of 0.5 IQ points in children as noted by JECFA (JECFA, 2011). Based on all of these factors combined, increased dietary exposure to lead from the addition of rapeseed protein isolate to the diet at the mean analysed lead concentration is therefore not considered to be of a safety concern.

5.3 Assessments by other agencies

Rapeseed protein isolate was assessed and authorized as a novel food ingredient by EFSA in 2014, and CanolaPRO was assessed as being substantially equivalent to the rapeseed protein isolate that was the subject of the 2014 assessment in 2017 (FSAI 2017). The EFSA Panel noted in their discussion (EFSA 2013) that anti-nutritional effects could not be excluded if rapeseed protein isolate was the main protein source in the diet, but considered that such a scenario is extreme and unrealistic, and implies the consumption of an unbalanced diet, which is not recommended. EFSA also concluded that it is likely that rapeseed protein isolate can trigger allergic reactions.

EFSA (2013) reviewed a small number of dietary studies in rats that have not been reviewed by FSANZ. Two of the studies, a teratogenicity study in rats and a 28-day toxicity study in rats, lacked sufficient information on the composition of the test article for EFSA to draw conclusions on their relevance to EFSA's review. For this reason, FSANZ did not consider retrieval of the papers to be justified. EFSA also reviewed two articles concerning 90-day dietary studies in rats. The isolates were not comparable to the rapeseed protein isolate that is the subject of this application, because one of them consisted primarily (>80%) of cruciferin, and the other consisted primarily (>80%) of napin. FSANZ did not retrieve these studies because of these differences in composition but notes that no adverse effects were observed in association with the cruciferin-rich isolate at up to 20% of the diet, and that adverse effects of the napin-rich isolate were consistent with anti-nutritional effects, such as low palatability and/or gastrointestinal discomfort, rather than toxicological effects.

Rapeseed protein isolates including the applicant's CanolaPRO have been the subjects of three GRAS notices, and the US FDA responded with no questions letters to all three GRAS notices.

Information on allergenicity assessments by other agencies is provided in Section 5.2.3.

5.4 Toxicological Assessment conclusions

Of the plant metabolites in rapeseed protein isolate that are considered to be have anti-nutritional potential, two are considered to have toxic effects, erucic acid and glucosinolates.

Erucic acid is not considered to represent a risk because the dietary exposure assessment shows that the addition of rapeseed protein isolate to the diet at both proposed maximum and typical use levels does not result in an exceedance of the PTDI of 7.5 mg/kg bw/day for erucic acid established by FSANZ in 2003. No new information was located to suggest that the TDI established in 2003 should be revised downward.

There is no health-based guidance value (HBGV) for glucosinolates, but the dietary exposure assessment has shown that the addition of rapeseed protein isolate to the diet is comparable to the addition of amounts of *Brassica* vegetables that are within normal daily consumption. Glucosinolates in rapeseed protein isolate are therefore not considered to represent a risk.

Rapeseed proteins show high cross-reactivity with allergenic proteins in mustard, and have the potential to induce allergic responses, including cross-reactivity responses in individuals who are allergic to mustard.

Increased dietary exposures to certain metal contaminants (arsenic, lead, cadmium, zinc, copper and chromium) from the addition of rapeseed protein isolate to the diet are estimated to be negligible based on typical use levels and the small market update estimated by the applicant, and are not of toxicological concern.

6. Dietary exposure assessment

6.1 Objectives for the dietary exposure assessment

The objective for the dietary exposure assessment was to estimate population dietary exposure to rapeseed protein isolate as a novel food and protein source in a range of foods as a replacement for other animal, soy, or pea proteins in order to assess the public health impact should the requested permissions be granted.

In addition, the assessment also aimed to estimate the dietary exposure to erucic acid and glucosinolates from the consumption of rapeseed protein isolate and put that in the context of naturally occurring intake from the diet, specifically from *Brassica* vegetables as a source of these substances. The dietary exposure assessment also fed into other parts of the hazard assessment, such as that for the metal contaminant lead.

6.2 Methodology and approach for the dietary exposure assessment

6.2.1 Approach for the dietary exposure assessment

Dietary exposure assessments require concentration data for the chemical of interest in food and consumption data for the foods collected through national nutrition surveys.

The dietary exposures to rapeseed protein isolate were estimated based on the maximum proposed use levels (or concentrations) noted in the application and typical use levels or concentrations in the requested food classes as provided by the applicant. The applicant provided typical use levels in order for FSANZ to undertake a more refined risk assessment. Results based on typical use levels better reflect longer term or chronic risk, and therefore these were used by FSANZ for risk characterisation purposes and form the basis of the discussion in this supporting document. The estimated dietary exposures are also provided in Appendix 1 for completeness.

The dietary exposure assessments were primarily undertaken using FSANZ's dietary modelling computer program, Harvest. This was the case for the estimates for rapeseed protein isolate, using a semi-probabilistic approach (individual dietary records and single concentrations for each food group). Harvest wasn't used for the dietary exposure assessment calculations for erucic acid and glucosinolates, except to extract relevant summary consumption data for *Brassica* vegetables (from the range of consumption amounts for individuals). These summary consumption data were used with single concentration data points for *Brassicas* in a deterministic calculation. As novel food permissions in the Code apply to both Australia and New Zealand, dietary exposure assessments were undertaken for both countries.

A summary of the general FSANZ approach to conducting the dietary exposure assessment for this Application is at Appendix 1. A detailed discussion of the FSANZ methodology and approach to conducting dietary exposure assessments is set out in *Principles and Practices of Dietary Exposure Assessment for Food Regulatory Purposes* (FSANZ 2009).

Further details regarding the assumptions and limitations of the dietary exposure assessment can be found in Appendix 1.

6.2.2 Estimating dietary exposure to rapeseed protein isolate

A Harvest food additive model was the most appropriate for the dietary exposure assessment for rapeseed protein isolate as nutrition survey foods are grouped as per the food classes in Schedule 15 of the Code and concentrations of rapeseed protein isolate are assigned to these relevant classes. Food classes in Schedule 15 are primarily processed food based, which is where the novel food would be added. The food classes in Schedule 15 of the Code in some instances vary from the codes in Harvest. Therefore, to match the proposed uses to the specific consumption data the corresponding codes needed to be matched. The Schedule 15 food class code and the corresponding Harvest code are listed in Appendix 1.

6.2.3 Estimated dietary exposures to erucic acid and glucosinolates

A dietary exposure assessment was undertaken for erucic acid and glucosinolates in *Brassica* vegetables to estimate how much of each of these substances people are exposed to from Australian and New Zealand diets. These substances were identified as part of the hazard assessment of rapeseed protein isolate and will be compared to the amount of erucic acid and glucosinolates people would be exposed to from consuming rapeseed protein

isolate. *Brassica* vegetables were the only food included for this assessment because they were identified as a source of these substances in the food supply.

A literature search was undertaken to locate concentration data for erucic acid and glucosinolates in *Brassica* vegetables. The data were evaluated to determine representative concentrations to use in the dietary exposure assessment. These concentrations were combined with consumption data for *Brassica* vegetables extracted from Harvest to estimate dietary exposure. Further details of the literature search, the evaluation of the studies and selection of the representative concentration data can be found in Appendix 1.

The dietary exposure to erucic acid and glucosinolates that would come from the addition of rapeseed protein isolate to foods was estimated. This was done by multiplying the amount of erucic acid or glucosinolates in the specification by the dietary exposure to rapeseed protein isolate. The maximum amount erucic acid in rapeseed protein isolate according to the specification was 0.005%. For glucosinolates the specification was 1 µmol/g. This was converted using a molecular weight of 455 g/mol (VanEtten *et. al.* 1980) to 0.0455% for the dietary exposure calculation.

6.2.4 Food consumption data used

Dietary exposure assessments based on food consumption data from national nutrition surveys provide the best estimation of actual consumption of a food and the resulting estimated dietary exposure for the Australian and New Zealand populations. Further details about the consumption data including the design of the nutrition surveys and the key attributes, including survey limitations, are set out in Appendix 1.

The food consumption data used for the dietary exposure assessments were:

- 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)
- 2008–09 New Zealand Adult Nutrition Survey (2008 NZ ANS)
- 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS).

Further details regarding the food consumption data used in the assessment can be found in Appendix 1.

Two days of consumption data were averaged for Australia using the 2011-12 NNPAS, while consumption amounts for New Zealand were based on a single day of nutrition survey data.

6.2.5 Population groups assessed

No specific target or at risk population groups were identified as requiring separate dietary exposure assessments. Therefore the population groups assessed were based on the those for the nutrition surveys: Australians aged 2 years and above, New Zealand children 5-14 years and New Zealand adults 15 years and above.

The use of rapeseed protein isolate in infant formula products (includes infant formula, follow-on formula and infant formula products for special dietary uses) and infant foods was not requested by the applicant and so the dietary exposure assessment does not include this population group.

6.2.6 Concentration data

6.2.6.1 Concentration data for rapeseed protein isolate

The proposed maximum use levels, typical use levels and the food groups where rapeseed protein isolate could be used were provided by the applicant (Table 6.1). These proposed

food groups were matched to the appropriate food category in FSANZ's Harvest program. The list of categories and concentrations used in the dietary exposure assessment are presented in Appendix 1. Where a range of concentrations is provided for the typical use levels, the highest concentration in the range was used in the dietary exposure assessment as a worst case for the typical use scenario.

Table 6.1 *Maximum and typical proposed use levels of rapeseed protein isolate and the proposed food groups as provided by the applicant*

Food Group	Examples	Excludes*	Proposed maximum use level (%)	Proposed typical use level (%)
Cereals and cereal products	Breads and rolls; doughnuts; cookies; crackers; cakes; pies; batters; muffins; cereal and granola bars; breakfast cereals	Hot porridge type cereals, flour, whole single grains (e.g. rice)	5	3-5
Beverages	Fruit juices and juice blends; soft drinks; energy drinks	Waters and flavoured waters, coffee, tea	5	2-5
Dairy products	Yogurt; cheese and cheese products; desserts and dessert toppings; ice pops and sorbets, jellies	Water based ice blocks	5	3-5
Other	Ready-to-eat meals, soups, and pasta. Extruded snacks including cookies		10	5
	Meat analogues		30	5
	Protein-enriched, bars, and pasta; protein-enriched powder and ready-to-drink beverages; energy bars		30	10

* Applicant specified and FSANZ assumed.

6.2.5.2 Concentration data for erucic acid and glucosinolates

Table 6.2 shows concentrations of erucic acid and glucosinolates in the selected *Brassica* vegetables. The erucic acid and glucosinolates concentrations of 8 mg/kg and 646 mg/kg respectively in broccoli on fresh weight basis were selected as representative of *Brassica* vegetables and used in the dietary exposure calculations.

Due to the small amount of concentration data available, there were not enough data points to enable a dietary exposure assessment to be undertaken that separated out each individual type of *Brassica*. Therefore, one representative concentration was selected and used to represent all *Brassicas*. Similarly, consumption data were not separated by type of *Brassica*, and were extracted for all *Brassicas* combined to allow combination with the concentration data.

Table 6.2 *Erucic acid and glucosinolates concentration data in selected Brassica vegetables used for the dietary exposure assessment*

Components	Food	Number of samples	Concentration (mg/kg fw)	Source	Comments
Erucic acid	Broccoli florets	42	8	West et al. (2002)	Reliable data estimate, large sample number
Glucosinolates	Green broccoli	3	646	Schonhof et al. (2004)	Reliable worst case scenario concentration values, sample no. >1

Due to the limited information in the literature regarding the concentrations of glucosinolates and erucic acid in specific *Brassica* vegetables, the calculations of dietary exposure will contain a degree of uncertainty.

The concentrations of erucic acid and glucosinolates can be affected by variety, genetic and environmental factors of *Brassica* species (Ciska et al. 2000; Pereira et al. 2002; Wallace et al. 2016). Different analytical methods used by different studies could also be in part responsible for some of the wide variations in the concentration data reported for glucosinolates and erucic acid.

6.3 Dietary exposure assessment results

6.3.1 Estimated dietary exposures to rapeseed protein isolate

Dietary exposures to rapeseed protein isolate were estimated based on the proposed maximum use levels and typical use levels. The results based on the typical use levels were deemed appropriate for the risk characterisation given these levels represent more likely concentrations that consumers will eat over a chronic or long period of time. The results based on the typical use levels are therefore discussed below. The results based on the proposed maximum use levels are provided in Appendix 1.

Estimated daily dietary exposures to rapeseed protein isolate were calculated as mean and 90th percentile in g/day and g/kg body weight/day for Australian and New Zealand population groups as shown in Table 6.3.

Mean dietary exposure to rapeseed protein isolate based on typical use levels for Australians aged 2 years and above was 40.2 g/day. Whereas for New Zealand children 5-14 years and adults 15 years and above mean dietary exposures were 45.2 g/day and 42.1 g/day respectively. On a body weight basis, estimated mean daily exposure to rapeseed protein isolate for Australian aged 2 years and above was 0.67 g/kg bw/day and for New Zealand children and adults respectively were 1.25 g/kg bw/day and 0.55 g/kg bw/day.

Estimated 90th percentile dietary exposure to rapeseed protein isolate for Australians aged 2 years and above was 74.2 g/day. Estimated 90th percentile dietary exposures to rapeseed protein isolate for New Zealand children 5-14 years and adults 15 years and above were 77.7 g/day and 82.8 g/day. When expressed on a body weight basis, estimated 90th percentile daily exposure to rapeseed protein isolate for the Australian aged 2 years and above, was 1.33 g/kg bw/day and 2.28 g/kg bw/day for New Zealand children and 1.09 g/kg bw/day for the adults.

Table 6.3 *Estimated mean and high percentile dietary exposure to Rapeseed Protein Isolate (g/day; g/kg bw/day) based on proposed typical use levels*

Country*	Population age (years)	Number of consumers	Consumers as a proportion of respondents (%)	Mean		90th Percentile	
				g/day	g/kg bw/day	g/day	g/kg bw/day
Australia	2 years and above	7735	100	40.2	0.67	74.2	1.33
New Zealand	Children 5-14 years**	3275	100	45.2	1.25	77.7	2.28
	15 years and above	4703	99.6	42.1	0.55	82.8	1.09

* Estimated dietary exposures are based on the average of two days of data for Australia, and only one day of data for New Zealand.

** As a comparison, estimated 2-day average dietary exposures for Australian children aged 5-14 years (to match the age range for New Zealand) were 1.16 g/kg bw/day at the mean and 1.91 g/kg bw/day at the 90th percentile; and for children 2-14 years were 1.27 g/kg bw/day at the mean and 2.18 g/kg bw/day at the 90th percentile.

6.3.2 Estimated dietary exposures to erucic acid and glucosinolates

Estimated daily dietary exposures to erucic acid and glucosinolates were calculated as mean and 90th percentile in mg/day and mg/kg body weight/day for Australian and New Zealand population groups.

6.3.2.1 Erucic acid

6.3.2.1.1 Dietary exposure to erucic acid from Brassica vegetables

Table 6.4 shows the mean and 90th percentile dietary exposure to erucic acid based on daily consumption of all *Brassicas* combined (broccoli, Brussels sprouts, cabbage head and cauliflower).

Estimated mean dietary exposures to erucic acid from *Brassicas* for Australians aged 2 years and above was 0.18 mg/day. Estimated mean dietary exposure to erucic acid from *Brassicas* by New Zealand children 5-14 years and adults 15 years and above were 0.46 mg/day and 0.64 mg/day respectively. When expressed on a body weight basis, estimated mean daily exposure to erucic acid for the Australian aged 2 years and above was 0.003 mg/kg bw/day, while for New Zealand children and adults, it was the same at 0.01 mg/kg bw/day.

Estimated 90th percentile dietary exposures to erucic acid from *Brassicas* for Australians aged 2 years and above was 0.48 mg/day. Estimated 90th percentile dietary exposure to erucic acid from *Brassicas* by New Zealand children 5-14 years and adults 15 years and above were 1.07 mg/day and 1.37 mg/day respectively. When expressed on a body weight basis, estimated 90th percentile daily exposure to erucic acid was 0.01 mg/kg bw/day for Australians aged 2 years and above, and for New Zealand children 0.03 mg/kg bw/day and adults was 0.02 mg/kg bw/day.

Table 6.4 *Estimated mean and 90th percentile dietary exposure to erucic acid from Brassica vegetables*

Country*	Population age (years)	Mean		90th Percentile	
		mg/day	mg/kg bw/day	mg/day	mg/kg bw/day
Australia	2 years and above	0.18	0.003	0.48	0.01
New Zealand	Children 5-14 years	0.46	0.01	1.07	0.03
	15 years and above	0.64	0.01	1.37	0.02

* Estimated dietary exposures are based on the average of two days of data for Australia, and only one day of data for New Zealand.

6.3.2.1.2 Dietary exposure to erucic acid from rapeseed protein isolate with a comparison to dietary exposures from Brassica *vegetables*

Table 6.5 shows the estimated exposure to erucic acid from rapeseed protein isolate based on proposed typical use levels.

Estimated mean dietary exposures to erucic acid from rapeseed protein isolate based on proposed typical use levels for Australians aged 2 years and above was 2.01 mg/day. Mean dietary exposure to erucic acid from rapeseed protein isolate for New Zealand children 5-14 years and adults 15 years and above were 2.26 mg/day and 2.11 mg/day respectively. When expressed on a body weight basis, the estimated mean daily exposure for Australian aged 2 years and above was 0.03 mg/kg bw/day and for New Zealand children and adults were 0.06 mg/kg bw/day and 0.03 mg/kg bw/day respectively.

Estimated 90th percentile dietary exposures to erucic acid from rapeseed protein isolate for Australians aged 2 years and above was 3.71 mg/day. Estimated 90th percentile dietary exposure for New Zealand children 5-14 years and adults 15 years and above were 3.88 mg/day and 4.14 mg/day respectively. When expressed on a body weight basis, estimated 90th percentile daily exposure to erucic acid from rapeseed protein isolate was 0.07 mg/kg bw/day for Australian aged 2 years and above, and for New Zealand was 0.11 mg/kg bw/day for children and 0.05 mg/kg bw/day for adults.

Table 6.5 *Estimated dietary exposure to erucic acid from rapeseed protein isolate based on proposed typical use levels**

Country	Population age (years)	Mean		90th Percentile	
		mg/day	mg/kg bw/day	mg/day	mg/kg bw/day
Australia	2 years and above	2.01	0.03	3.71	0.07
New Zealand	Children 5-14 years	2.26	0.06	3.88	0.11
	15 years and above	2.11	0.03	4.14	0.05

* Based on 0.005% concentration of erucic acid in rapeseed protein isolate according to the specifications. This is the limit of detection for the analytical method for erucic acid.

Mean and 90th percentile dietary exposures to erucic acid from rapeseed protein isolate are higher than mean and 90th percentile dietary exposure from *Brassica* for all population groups assessed in Australia and New Zealand.

6.3.2.2 Glucosinolates

6.3.2.2. Dietary exposure to glucosinolates from Brassica vegetables

Table 6.6 shows the mean and 90th percentile dietary exposure to glucosinolates based on daily consumption of all *Brassicas* combined (broccoli, Brussels sprouts, cabbage head and

cauliflower).

Estimated mean dietary exposure to glucosinolates from *Brassicas* for Australians aged 2 years and above was 14.9 mg/day. Estimated mean dietary exposure to glucosinolates from *Brassicas* by New Zealand children 5-14 years and adults 15 years and above were 36.8 mg/day and 51.9 mg/day respectively. When expressed on a body weight basis, mean dietary exposure to glucosinolates from *Brassicas* was estimated for Australians aged 2 years and above at 0.21 mg/kg bw/day, for New Zealand children at 0.92 mg/kg bw/day, and for New Zealand adults at 0.66 mg/kg bw/day.

Estimated 90th percentile dietary exposure to glucosinolates from *Brassicas* for Australians aged 2 years and above was 39.1 mg/day. Estimated 90th percentile dietary exposure to glucosinolates from *Brassicas* by New Zealand children 5-14 years and adults 15 years and above were 86.6 mg/day and 110.3 mg/day respectively. When expressed on a body weight basis, 90th percentile dietary exposure to glucosinolates from *Brassicas* was estimated for Australian aged 2 years and above at 0.56 mg/kg bw/day, for New Zealand children 2.17 mg/kg bw/day, and for New Zealand adults at 1.4 mg/kg bw/day.

Table 6.6 *Estimated mean and 90th percentile dietary exposure to glucosinolates from Brassica vegetables*

Country*	Population age (years)	Mean		90th Percentile	
		mg/day	mg/kg bw/day	mg/day	mg/kg bw/day
Australia	2 years and above	14.9	0.21	39.1	0.56
New Zealand	Children 5-14 years	36.8	0.92	86.6	2.17
	15 years and above	51.9	0.66	110.3	1.40

* Estimated dietary exposures are based on the average of two days of data for Australia, and only one day of data for New Zealand.

6.3.2.2.2 Dietary exposure to glucosinolates from rapeseed protein isolate with a comparison to dietary exposures from Brassica vegetables

Table 6.7 shows the estimated dietary exposure to glucosinolates from rapeseed protein isolate based on proposed typical use levels.

Estimated mean dietary exposures to glucosinolates from rapeseed protein isolate based on proposed typical use levels for Australians aged 2 years and above was 18.3 mg/day. Mean dietary exposure for New Zealand children 5-14 years and adults 15 years and above were 20.6 mg/day and 19.2 mg/day respectively. When expressed on a body weight basis, estimated mean daily exposure for Australian aged 2 years and above was 0.30 mg/kg bw/day and for New Zealand children and adults were 0.57 mg/kg bw/day and 0.25 mg/kg bw/day respectively.

Estimated 90th percentile dietary exposures to glucosinolates from rapeseed protein isolate for Australians aged 2 years and above was 33.8 mg/day. Estimated 90th percentile dietary exposure for New Zealand children 5-14 years and adults 15 years and above were 35.3 mg/day and 37.7 mg/day respectively. When expressed on a body weight basis, estimated 90th percentile daily exposure to glucosinolates from rapeseed protein isolate was 0.61 mg/kg bw/day for Australian aged 2 years and above and 1.04 mg/kg bw/day for New Zealand children and 0.50 mg/kg bw/day for the adults.

Table 6.7 *Estimated dietary exposure to glucosinolates from rapeseed protein isolate based on proposed typical use levels**

Country	Population age (years)	Mean		90th Percentile	
		mg/day	mg/kg bw/day	mg/day	mg/kg bw/day
Australia	2 years and above	18.3	0.30	33.8	0.61
New Zealand	Children 5-14 years	20.6	0.57	35.3	1.04
	15 years and above	19.2	0.25	37.7	0.50

* Based on 1 µmole/g concentration of glucosinolates in rapeseed protein isolate according to the specifications. Calculations for total glucosinolates based on molecular weight of 455 g/mol (VanEtten et al. 1980).

From the available evidence in the literature, glucosinolate intakes from *Brassica* vegetables are variable across the world. Agudo et al. (2008) reported 6.53 mg/day as the mean exposure to glucosinolates in the population aged 35–64 years of the EPIC-Spain cohort (n=40,684). The average daily exposure to total glucosinolates in the UK from *Brassica* vegetables was estimated to be about 50 mg (Wattenberg et al. 1986), whereas in the Netherlands, the estimated mean exposure to glucosinolates from *Brassica* vegetables was 22 mg/day (Jongen, 1996; Kistenmaker et al. 1998). The exposure to glucosinolates from *Brassica* vegetables estimated for Australia and New Zealand (mean 15-52 mg/day) are within the range of international estimates.

Australians aged 2 years and above were exposed to more glucosinolates from rapeseed protein isolate than from the *Brassica* vegetables based on mean and 90th percentile dietary exposures both in mg/day and on a body weight basis. However, for New Zealand children 5-14 years and adults 15 years and above, mean and 90th percentile dietary exposures to glucosinolates from *Brassica* vegetables were higher than exposures from rapeseed protein isolate both in mg/day and on a body weight basis.

From the dietary exposure assessment it was estimated that around 20 mg of glucosinolates would be consumed on average from the addition of rapeseed protein isolate to foods based on typical use levels. Dietary exposure results indicate that around 30 g of *Brassicas* would need to be consumed to give 20 mg of glucosinolates, if 1 kg of broccoli has 646 mg of glucosinolates (the concentration used in the dietary exposure assessment). This estimate of around 30 g/day of *Brassica* would be equivalent to around 1 large floret of broccoli or 1 medium floret of cauliflower per day (based on gram weights from 2011-13 AUSNUT Measure file (FSANZ, 2016)). This level of consumption would be well within normal daily intake, and not much additional to the diet. The 30 g is also less than the mean daily consumption amount estimated and reported in the Table A1.1 of around 57- 80 grams for any single day from the New Zealand national nutrition surveys and similar to the 33 g for day 1 for Australia.

The additional amount of equivalent *Brassica* would be less than 30 g/day in reality. This is because the consumption amount is based on the estimates of glucosinolate exposure from dietary exposure to rapeseed protein isolate which in itself is a conservative estimate. The estimates of dietary exposure to rapeseed protein isolate are based on the typical concentrations of use for rapeseed protein isolate, assuming 100% of foods in all of the categories include it. It also assumes that consumers always chose the food that contains rapeseed protein isolate every time they eat it over a lifetime. Also, the glucosinolates content of the rapeseed protein isolate was assumed to be at the level in the specification (1 µmol/g) but analytical results from the rapeseed protein isolate show much lower levels (< 0.1 µmol/g).

6.4 Dietary exposure assessment conclusions

An assessment was undertaken to estimate dietary exposure to rapeseed protein isolate based on the most recent consumption data from national nutrition surveys for Australians (2 years and above) and New Zealanders (children 5-14 years and adults 15 years and above) and information provided by the applicant on proposed foods and use levels.

For erucic acid, estimated dietary exposures from rapeseed protein isolate based on typical use levels at the mean (0.03-0.06 mg/kg bw/day) and 90th percentile (0.05-0.11 mg/kg bw/day) were higher than the dietary exposures from *Brassica* vegetables at both the mean (0.003-0.01 mg/kg bw/day) and 90th percentile (0.01-0.03 mg/kg bw/day) for all population groups assessed in Australia and New Zealand.

Australians could be exposed to more glucosinolates from rapeseed protein isolate based on typical use levels (mean 0.30 mg/kg bw/day; 90th percentile 0.61 mg/kg b/day) than from *Brassica* vegetables (mean 0.21 mg/kg bw/day; 90th percentile 0.56 mg/kg bw/day). However, New Zealand dietary exposures to glucosinolates from *Brassica* vegetables (mean 0.66-0.92 mg/kg bw/day; 90th percentile 1.40-2.17 mg/kg bw/day) were higher than exposures from rapeseed protein isolate based on typical use levels (mean 0.25-0.57 mg/kg bw/day; 90th percentile 0.50-1.04 mg/kg bw/day). The additional exposure to glucosinolates from rapeseed protein isolate based on typical use levels of around 20 mg/day is equivalent to the consumption of around 30 g/day of *Brassica* vegetables (one large broccoli floret or one medium cauliflower floret).

7. Risk assessment conclusions

Food technology, microbiological, nutrition, toxicology and dietary exposure assessments were undertaken to evaluate any potential risks associated with the approval for the addition of rapeseed protein isolate as a novel food. In conclusion, the approval for the use of rapeseed protein isolate in the food classes noted at the proposed typical use levels would not represent a public health and safety concern for many of the areas assessed. The aspects identified as potential public health and safety concerns include the microbiological risk from *Salmonella* spp., the potential allergic responses to individuals who are allergic to mustard, and the need to ensure levels of substances such as phytates and certain metal contaminants are retained as low as reasonably achievable.

8. References

ABS (2014) National Nutrition and Physical Activity Survey, 2011–12, Basic CURF. Australian Government, Canberra. Accessed 2 July 2108. http://www.abs.gov.au/AUSSTATS/abs@.nsf/Latestproducts/4324.0.55.002Main%20Features652011-12?opendocument&tabname=Summary&prodno=4324.0.55.002&issue=2011-12&num=&view=

Aires A, Rosa E, Carvalho R. (2006). Effect of nitrogen and sulphur fertilization on glucosinolates in the leaves and roots of broccoli sprouts (*Brassica oleracea* var. italica), J. Sci. Food Agric, 86:1512–1516.

Agudo A, Ibanez R, Amiano P, et al. (2008). Consumption of cruciferous vegetables and glucosinolates in a Spanish adult population. Eur J Clin Nutr, 62:324-331.

AOF (2015) Section 1: Quality standards, technical information and typical analysis 2015/19 Australian Oilseeds Federation Inc.

https://www.graintrade.org.au/sites/default/files/file/Commodity%20Standards/2015_2016/201516%20AOF%20Standards%20V14%20-%20August%201%202015.pdf Accessed February 2020.

Beck KL, Conlon CA, Kruger R and Coad J (2014). Dietary determinants of and possible solutions to iron deficiency for young women living in industrialized countries: a review. Nutrients 6:3747-76.

Carpenter CE and Mahoney AW (1992). Contributions of heme and nonheme iron to human nutrition. Crit Rev Food Sci Nutr 31(4):333-67.

Center for Food Safety and Applied Nutrition (CFSAN) / United States Food and Drug Administration (FDA). (2011). Office of Food Additive Safety 2010 GRN 00327.

Charron CS, Saxton AM, Sams CE (2005). Relationship of climate and genotype to seasonal variation in the glucosinolates –myrosinase system. I. Glucosinolate content in ten cultivars of *Brassica* oleracea grown in fall and spring seasons. J. Sci. Food Agric. 85, 671–681.

Ciska E, Martyniak-Przybyszewska B, Kozlowska H (2000). Content of glucosinolates in cruciferous vegetables grown at the same site for two years under different climatic conditions. J. Agric. Food Chem., 48, 2862 –2867.

Codex Alimentarius Commission (1999). Standard for Named Vegetable Oils CXC 210 – 1999. Accessed 23 April 20.

Codex Alimentarius Commission (2003). General Principles of Food Hygiene CXC 1-1969. Adopted 1969. Revised 2003.

Codex Alimentarius Commission (2018). Code of Hygienic Practice for Low-Moisture Foods CXC 75-2015. Accessed 22 April 20.

Codex Alimentarius Commission (2019). General Standard for Vegetable Protein Products (VPP) CXS 174-1989. Adopted in 1989. Amended in 2019.

Commission of the European Communities (CEC) (2007). Commission directive 2007/68/ec. Official Journal of the European Union. 28.11.2007. L 310/14. Website: http:/ eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:31 0:0011:0014:EN:PDF Accessed 19 May 2020.

De Boland AR, Garner GB and O'Dell BL (1975). Identification and properties of 'phytate' in cereal grains and oilseed products. J Agric Food Chem 23:1188.

Dinkova-Kostova AT and Kostov RV (2012). Glucosinolates and isothiocyanates in health and disease. Trends in Molecular Medicine 18(6): 337-347.

Doell BH, Ebden CJ and Smith CA (1981). Trypsin inhibitor activity of conventional foods which are part of the British diet and some soya products. Qual Plant Foods Hum Nutr 31:139-50.

Downey K (2005). Rapeseed to Canola: Rags to Riches. Agriculture and Agri-food Canada Research Centre, Saskatoon. Agriculture Biotechnology: Economic Growth through New Products, Partnerships and Workforce Development. NABC Report 18: 67-75.

European Food Safety Authority (EFSA) NDA Panel on Dietetic Products, Nutrition and Allergies) 2013. Scientific opinion on the safety of "rapeseed protein isolate" as a Novel Food

ingredient. EFSA Journal 11(10): 3420, 23 pp. doi: 10.2903/j.efsa.2013.3420.

European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (2016). Erucic acid in feed and food. EFSA Journal 14(11). https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2016.4593

European Commission (2014). Commission Implementing Decision of 1 July 2014 authorising the placing on the market of rapeseed protein as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council, EU/424/2014. http://data.europa.eu/eli/dec_impl/2014/424/oj. Accessed April 2020.

Figueroa J, Blanco C, Dumpiérrez AG, Almeida L, Ortega N, Castillo R, Carrillo T (2005). Mustard allergy confirmed by double-blind placebo-controlled food challenges: clinical features and cross-reactivity with mugwort pollen and plant-derived foods. Allergy 60(1): 48-55.

Food and Agriculture Organization of the United Nations, World Health Organization. 2014. Ranking of low moisture foods in support of microbiological risk management. Report of an FAO/WHO consultation process. Available at http://www.fao.org/tempref/codex/Meetings/CCFH/CCFH46/LMF Part 1-3 30Oct 2014.pdf Accessed 20 April 2020.

FSAI (2017) Substantial Equivalence Opinion. DSM Rapeseed protein. https://www.fsai.ie/science_health/novel_food_applications/substantial_equivalence_opinions.html Accessed February 2020.

FSANZ (2016). 2011-13 Australian Food and Nutrient Database (AUSNUT). https://www.foodstandards.gov.au/science/monitoringnutrients/ausnut/pages/default.aspx Accessed April 2020.

FSANZ (2017a). Record of views from the Advisory Committee on Novel Foods (ACNF) or Novel Foods Reference Group (NFRG) to inquiries on Standard 1.5.1 Novel Foods. https://www.foodstandards.gov.au/industry/novel/novelrecs/pages/default.aspx Accessed April 2020.

FSANZ (2017b) Agents of foodborne illness. https://www.foodstandards.gov.au/publications/Pages/agentsoffoodborneill5155.aspx. Accessed 22 May 2020.

FSANZ (2018) Imported food risk assessment: How FSANZ assesses food safety risks from imported foods. https://www.foodstandards.gov.au/consumer/importedfoods/Documents/Imported%20food%20risk%20assessment%20document.pdf. Accessed April 2020.

FSANZ (2019) 25th Australian Total Diet Study. June 2019. https://www.foodstandards.gov.au/publications/Pages/25th-Australian-Total-Diet-Study.aspx

Gemede HF and Ratta N (2014). Anti-nutritional factors in plant foods: potential health benefits and adverse effects. Int J Nutr Food Sci *3*(4):284-9.

Goodman RE (2016) Bioinformatics analysis of potential allergenicity and celiac disease of six storage proteins from rapeseed for food safety evaluation. Unpublished report.

Hänninen AR, Mikkola JH, Kalkkinen N, Turjanmaa K, Ylitalo L, Reunala T, Palosuo T (1999). Increased allergen production in turnip (*Brassica rapa*) by treatments activating defense

mechanisms. J Allergy Clin Immunol 104(1):194-201.

Health Canada (2010). Mustard: A Priority Food Allergen in Canada. A systematic review. https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/fn-an/alt_formats/pdf/pubs/label-etiquet/mustard-moutarde/index-eng.pdf Accessed 19 May 2020.

Herrmann, K., Inhaltsstoffe des Chinakohl und Pak Choi. (1999). Die industrielle Obst- und Gem severwertung 2, 40–44.

Honig DH, Wolf WJ and Rackis JJ (1984). Phytic acid and phosphorus content of various soybean protein fractions. Cereal Chem. 61(6):523-526.

Hurrell FD, Juillerat MA, Reddy MB, Lynch SR, Dassenko SA and Cook JD (1992). Soy protein, phytate, and iron absorption in humans. Am J Clin Nutr 56(3):573-8.

Hurrell RF (2003). Influence of vegetable protein sources on trace element and mineral bioavailability. J Nutr 133(9)2973S-7S.

ICMSF (1996). Salmonellae. Ch 14 In: Microorganisms in food 5: Microbiological specifications of food pathogens. Blackie Academic and Professional, London, p. 217–264.

JECFA (2011) *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series: 64 (Lead addendum). Joint FAO/WHO Expert Committee on Food Additives, Geneva. http://apps.who.int/food-additives-contaminants-jecfa-database/document.aspx?docID=9003. Accessed on May 18 2017.

Jongen WMF (1996). Glucosinolates in *Brassica*: occurrence and significance as cancer-modulating agents. Proc Nutr Soc 55:433–446.

Kang, JY, Ibrahim KE, Juvik JA, Kim DH, et al. (2006). Genetic and environmental variation of glucosinolate content in Chinese cabbage. J. Hortic. Sci. 41:1382 –1385.

Kistenmaker C, Bouman M, Hulshof KFAM (1998). Food Consumption Standard 1997–1998, report no 98812 Zeist. TNO Nutrition and Food Research: The Netherlands.

Koletzko B, Agostoni C, Axelsson I, Goulet O, Michaelsen KF, Puntis J, Rieu D, Rigo J, Shamir R, Szajewska H, Turck D (2006). Soy protein infant formulae and follow-on formulae: a commentary by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) committee on nutrition. J Pediatric Gastroent Nutr 42:352-361.

Kushad MM, Brown AF, Kurilich AC, Juvik JA, et al. (1999). Variation of glucosinolates in vegetable crops of *Brassica oleracea*, J. Agric. Food Chem. 47:1541–1548.

Lee MK, Chun JH, Byeon DH, et al. (2014). Variation of glucosinolates in 62 varieties of Chinese cabbage (*Brassica rapa* L. ssp. pekinensis) and their antioxidant activity. Food Sci Technol. 58:93–101.

Loew FM, Doige CE, Manns JG, Searcy GP, Bell JM and Jones JD (1976). Evaluation of dietary rapeseed protein flours in rats and dogs. Toxicology and Applied Pharmacology 35: 257-267.

Macfarlane BJ, Bezwoda WR, Bothwell TH, Baynes RD, Bothwell JE, MacPhail AP, Lamparelli RD and Mayet F (1988). Inhibitory effect of nuts on iron absorption. Am J Clin Nutr 47:270-4.

Mansour E, Dworschak E, Lugasi A, Gaal O, Barna E and Gergely A (1993). Effect of processing on the antinutritive factors and nutritive value of rapeseed products. Food Chemistry 47(3):247-252.

Mejia LA, Korgaonkar CK, Schweizer M, Chengelis C, Marit G, Ziemer E, Grabiel R and Empie M (2009a). A 13-week sub-chronic dietary toxicity study of a cruciferin-rich canola protein isolate in rats. Food and Chemical Toxicology 47:2645-54.

Mejia LA, Korgaonkar CK, Schweizer M, Chengelis C, Novilla M, Ziemer E, Williamson-Hughes PS, Grabiel R and Empie M (2009b). A 13-week dietary toxicity study in rats of a napin-rich canola protein isolate. Regulatory Toxicology and Pharmacology 55:394-402.

Ministry of Health (2003) NZ Food NZ Children: Key results of the 2002 National Children's Nutrition Survey. Ministry of Health, Wellington.

Ministry of Health (2005) 2002 National Children's Nutrition Survey: National Confidentialised Unit Record File (CURF) User Document. Ministry of Health, Wellington.

Ministry of Health (2011a) Methodology report for the 2008/09 New Zealand Adult Nutrition Survey. Ministry of Health, Wellington.

Ministry of Health (2011b) A focus on nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey. Ministry of Health, Wellington.

Monreal P, Botey J, Pena M, Marin A, Eseverri JL (1992) Mustard allergy. Two anaphylactic reactions to ingestion of mustard sauce. Ann Allergy 69(4):317-320.

Monsalve RI, Gonzalez de la Peña MA, Menendez-Arias L, C Lopez-Otin C, Villalba M, Rodriguez R (1993). Characterization of a new Oriental-mustard (*Brassica juncea*) allergen, Bra J IE: detection of an allergenic epitope. Biochem J 293:625-632.

Monsalve RI, Gonzalez de la Peña MA, Lopezotiin C, Fiandor A, Fernandez C, Villalba M, Rodriguez R (1997). Detection, isolation and complete amino acid sequence of an aeroallergenic protein from rapeseed flour. Clinical & Experimental Allergy 27(7):833-841.

Morisset M, Moneret-Vautrin DA, Maadi F, Fremont S, Guenard L, Croizier A, Kanny G (2003). Prospective study of mustard allergy: first study with double-blind placebo-controlled food challenge trials (24 cases). Allergy 58(4): 295-299.

Naczk M, Rubin LJ and Shahidi F (1986). Functional properties and phytate content of pea protein preparations. J Food Sci 51:1235-1237.

OECD (2011) Revised consensus document on compositional considerations for new varieties of low erucic acid rapeseed (canola): key food and feed nutrients, anti-nutrients and toxicants. ENV/JM/MONO(2011)55. Organization for Economic Co-operation and Development, Paris.

Palacín A, Cumplido J, Figueroa J, Ahrazem O, Sánchez-Monge R, Carrillo T, Salcedo G, Blanco C (2006). Cabbage lipid transfer protein Bra O 3 is a major allergen responsible for cross-reactivity between plant foods and pollens. J Allergy Clin Immunol 117(6):1423-1429.

Palomares O, Monsalve RI, Rodríguez R, Villalba M (2002). Recombinant pronapin precursor produced in *Pichia pastoris* displays structural and immunologic equivalent properties to its mature product isolated from rapeseed. European Journal of Biochemistry 269(10): 2538-2545.

Pereira FMV, Rosa E, Fahey JW, Stephenson KK, et al. (2002). Influence of temperature and ontogeny on the levels of glucosinolates in broccoli (*Brassica oleracea* Var. italica) sprouts and their effect on the induction of mammalian phase 2 enzymes, J. Agric. Food Chem. 50: 6239–6244.

Podolak R, Enache E, Stone W, Black DG, Elliott PH (2010). Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. J Food Protect 73(10):1919–1936.

Poikonen S, Puumalainen TJ, Kautiainen H, Burri P, Palosuo T, Reunala T, Turjanmaa K (2006). Turnip rape and oilseed rape are new potential food allergens in children with atopic dermatitis. Allergy 61(1):124-127.

Poikonen S, Puumalainen TJ, Kautiainen H, Palosuo T, Reunala T, Turjanmaa K (2008). Sensitization to turnip rape and oilseed rape in children with atopic dermatitis: a case-control study. Pediatric Allergy and Immunology 19(5):408-411.

Poikonen S, Rance F, Puumalainen TJ, Le Manach G, Reunala T, Turjanmaa K (2009). Sensitization and allergy to turnip rape: a comparison between the Finnish and French children with atopic dermatitis. Acta Paediatrica 98(2):310-315.

Prynne CJ, McCarron A, Wadsworth MEJ and Stephen AM (2010). Dietary fibre and phytate; a balancing act: results from three time points in a British Birth Cohort. Brit J Nutr 103:274-80.

Puumalainen TJ, Poikonen S, Kotovuori A, Vaali K, Kalkkinen N, Reunala T, Kristiina Turjanmaa K, Palosuo T (2006). Napins, 2S albumins, are major allergens in oilseed rape and turnip rape. J Allergy Clin Immunol 117(2):426-432.

Rancé F, Kanny G, Dutau G, Moneret-Vautrin DA (1999). Food hypersensitivity in children: clinical aspects and distribution of allergens. Pediatric Allergy and Immunology 10(1):33-38.

Rancé F, Dutau G, Abbal M. (2000) Mustard allergy in children. Allergy 55(5):496-500.

Reddy NR (2002) Occurrence, distribution, content, and dietary intake of phytate. In: Reddy NR, Sathe SK (Eds.). Food Phytates. CRC Press, Boca Raton Florida: 25-51.

Schlemmer U, Rolich W, Prieto RM and Grases F (2009). Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. Mol Nutr Food Res 53:S330-S375.

Schonhof I, Krumbein A, Brckner B. (2004). Genotypic effects on glucosinolates and sensory properties of broccoli and cauliflower. Food 48:25–33.

Sharma A, Verma AK, Gupta RK, Dwivedi PD (2019). A comprehensive review on mustard-induced allergy and implications for human health. Clinical Reviews in Allergy & Immunology 57(1):39-54.

Singh P, Kumar R, Sabapathy SN, Bawa AS (2008). Functional and edible uses of soy protein products. Comprehensive Reviews in Food Science and Food Safety 7:14-28.

Thompson DB and Erdman JW (1982). Phytic acid determination in soybeans. J Food Sci 47:513-517.

US Food and Drug Administration (US FDA) (2010) GRN327 Cruciferin-rich canola/rapeseed protein isolate and napin-rich canola/rapeseed protein isolate. Accessed 22 April 2020.

US Food and Drug Administration (US FDA) (2011) GRN386 Canola protein isolate and hydrolysed canola protein isolate. Accessed 22 April 2020.

Vale AP, Santos J, Brito NV, Peixoto V, Carvalho R, Rosa E and Oliveira MBPP (2015). Light influence in the nutritional composition of *Brassica oleracea* sprouts. Food Chemistry 178:292–300.

Van der Speigel M, Noordam MY and van der Fels-Klerx (2013). Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production. Comprehensive Reviews in Food Science and Food Safety 12:662-678.

VanEtten CH, Daxenbichler ME, Tookey HL, Kwolek WF, Williams PH, Yoder OC (1980). Glucosinolates: potential toxicants in cabbage cultivars. J Am Soc Hort Sci 105:710–714.

Wanasundara JPD, McIntosh TC, Perera SP, Withana-Gamage TS, Mitra P (2016). Canola/rapeseed protein-functionality and nutrition. OCL, DOI:10.1051/ocl/2016028. https://www.researchgate.net/publication/304913958_Canolarapeseed_protein-functionality_and_nutrition Accessed January 2020.

Wattenberg LW, Hanley AB, Barany G, Sparrins VL, Lam LKT, Fenwick GR (1986). Inhibition of carcinogenesis by some minor dietary constituents. In: Hayashi Y (ed). Diet, Nutrition and Cancer, pp 193–203. Japan Scientific Society Press: Tokyo.

West L, Tsui I, Balch B, Meyer K and Huth PJ (2002). Determination and health implication of the erucic acid content of broccoli florets, sprouts, and seeds. Journal of Food Science, 67: 2641–2643.

World Health Organization/Food and Agriculture Organization (2002). Risk assessments of Salmonella in eggs and broiler chickens. Geneva: World Health Organization and Food and Agriculture Organization of the United Nations, http://www.who.int/foodsafety/publications/micro/salmonella/en/index.html. Accessed 12 May 2020

Xiao CW, Wood CM, Robertson P and Gilani GS (2012). Protease inhibitor activities and isoflavone content in commercial soymilks and soy-based infant formulas sold in Ottawa, Canada. J Food Comp Anal 25:130-6.

Yang B, Quiros CF. (2010). Survey of glucosinolate variation in leaves of *Brassica rapa* crops. Genet Resour Crop Evol 57:1079–1089.

9. Appendix 1 Details of the dietary exposure assessment

9.1 Food consumption data

The most recent food consumption data available were used to estimate rapeseed protein isolate exposures for the Australian and New Zealand populations. The national nutrition survey data used for these assessments were:

- The 2011-12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS) (ABS, 2014)
- The 2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS) (Ministry of Health, 2003; Ministry of Health, 2005)
- The 2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS) (Ministry of Health, 2011a; Ministry of Health, 2011b).

The design of each of these surveys varies somewhat and key attributes of each are set out below. Further information on the national nutrition surveys used to conduct dietary exposure assessments is available on the FSANZ website at http://www.foodstandards.gov.au/science/exposure/Pages/dietaryexposureandin4438.aspx.

For the dietary exposure assessment for rapeseed protein isolate, consumption data for each food for each individual respondent were used within the Harvest program for the calculations. FSANZ's dietary exposure assessment computer program Harvest was used to calculate the summary consumption data for *Brassicas* (derived from individual consumers' consumption amounts) which was extracted to use in deterministic calculations of dietary exposure for erucic acid and glucosinolates.

2011–12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS)

The 2011–12 Australian National Nutrition and Physical Activity Survey (2011-12 NNPAS), undertaken by the Australian Bureau of Statistics as part of the 2011-13 Australian Health Survey, is the most recent food consumption data for Australia. This survey includes food consumption data from a sample of 12,153 Australians aged from 2 years and above. The survey used a 24-hour recall method for all respondents, with 64% of respondents (n=7,735) also completing a second 24-hour recall on a second, non-consecutive day. The data were collected from May 2011 to June 2012 (with no enumeration between August and September 2011 due to the Census). Only those respondents who had two days of food consumption data were used to estimate dietary exposures for this assessment. The Day 1 and 2 average provides the best estimates of dietary exposures for Australians aged 2 years and above. Consumption and respondent data from the survey were incorporated into the Harvest program from the Confidentialised Unit Record Files (CURF) data set (ABS 2014). These data were weighted during the calculations undertaken in Harvest.

2002 New Zealand National Children's Nutrition Survey (2002 NZ CNS)

The 2002 NZ CNS was a cross-sectional and nationally representative survey of 3,275 New Zealand children aged 5–14 years. The data were collected during the school year from February to December 2002. The survey used a 24-hour food recall and provided information on food and nutrient intakes, eating patterns, frequently eaten foods, physical activity patterns, dental health, anthropometric measures and nutrition-related clinical measures. It was also the first children's nutrition survey in New Zealand to include a second day diet recall data for about 15% of the respondents, and dietary intake from both foods (including beverages) and dietary supplements. Only the Day 1 24-hour recall data for all respondents

(excluding supplements) were used for this assessment. These data were weighted during the calculations undertaken in Harvest.

2008-09 New Zealand Adult Nutrition Survey (2008 NZ ANS)

The 2008 NZ ANS provides comprehensive information on food consumption for a sample of 4,721 respondents aged 15 years and above. The survey was conducted on a stratified sample over a 12-month period from October 2008 to October 2009. The survey used a 24-hour recall methodology with 25% of respondents also completing a second 24-hour recall. The information collected in the 2008 NZ ANS included food and nutrient intakes, dietary supplement use, socio-demographics, nutrition related health, and anthropometric measures. Only the Day 1 24-hour recall data for all respondents (excluding supplements) were used for this assessment. These data were weighted during the calculations undertaken in Harvest.

Consumption data for the assessment of dietary exposure to erucic acid and glucosinolates

Consumption data for *Brassica* vegetables were extracted from the 2011-12 NNPAS, the 2002 NZ CNS, and the 2008–09 NZ ANS.

Brassica vegetables included in dietary exposure assessment were broccoli, Brussels sprouts, cabbage head, cauliflower, Chinese cabbage and kohlrabi because they are the most commonly consumed group of edible plants within *Brassica* genus.

The available consumption data for four *Brassica* vegetables in the Harvest program namely broccoli, Brussels sprouts, cabbage head and cauliflower, were used in determining consumption data for all *Brassica* vegetables to use in the dietary exposure assessment. Consumption of Chinese cabbage and kohlrabi were not reported in the surveys.

Mean and 90th percentile consumption data were extracted (see Table A1.1).

Table A1.1. *Summary consumption data for Brassica for Australia and New Zealand used in the deterministic calculations of dietary exposure to erucic acid and glucosinolates*

Country	Age group	Number of consumers	Proportion of consumers to respondents * (%)	Consumption (grams/consumer/day)**	
				Mean	90th percentile
Australia	2 years and above	5330	69	23	61
New Zealand	5-14 years	763	23	57	134
	15 years and above	1469	31	80	171

* Total number of respondents: Australia 2 years and above = 7735; New Zealand 5-14 years = 3275; New Zealand 15 years and above = 4721.

** Two day average used for Australia; one day only data used for New Zealand. Day 1 only results for Australia are a mean of 33 g/day and 90th percentile of 92 g/day.

9.2 Concentration data

Rapeseed protein isolate

Details of the food categories and concentrations used in Harvest to calculate the dietary exposures to rapeseed protein isolate are shown in Table A1.2.

Table A1.2. *Food classifications and concentrations of rapeseed protein isolate used in the dietary exposure assessment*

Food category in application	Examples	Proposed maximum (typical) use level (%)	Food standards code class	Harvest classification code	Harvest classification name	Concentration used in dietary exposure assessment (g/kg)	
						Maximum use level	Typical use level
Bakery products	Breads and rolls, pastries, biscuits, crackers, cakes, doughnuts, pies, batters, muffins	5 (3-5)	6.4	6.4	Flour products (including noodles and pasta)	50	50
			7	7	Bread & bakery products	50	50
			20.2	20.2.3	Bakery products	50	50
	Cereal bars, ready to eat cereal		6.3	6.3	Processed cereal and meal products	50	50
			20.2	20.2.2	Grains, cereals & cereal products	50	50
Beverages	Fruit juice, fruit juice blends, soft drinks, formulated beverages, energy drinks, dairy and plant based milks.	5 (2-5)	14.1.2	14.1.2	Fruit and vegetable juices and fruit and vegetable juice products	50	50
			14.1.3	14.1.3	Water based flavoured drinks	50	50
			14.1.4	14.1.4	Formulated Beverages	50	50
				14.1.7	Soy beverage	50	50
				14.1.8	Cereal beverages	50	50
			20.1	20.1.1	Beverages, non-alcoholic	50	50
				20.1.3.3.1	Beverages, non-alcoholic, beverages	700	700

Food category in application	Examples	Proposed maximum (typical) use level (%)	Food standards code class	Harvest classification code	Harvest classification name	Concentration used in dietary exposure assessment (g/kg)	
						Maximum use level	Typical use level
					bases, dry powder		
Dairy products	Yoghurt	5 (3-5)	1.2	1.2	Yoghurts	50	50
	Cheese, cheese products		1.6	1.6	Cheese and cheese products	50	50
	Ice blocks and sorbets		3.1	3.1.2.2	Ice confection, sold frozen, dairy	50	50
				3.2	Ice cream	50	50
				3.3	Sherbets & sorbets, frozen	50	50
	Dairy and fat based desserts,		20.2.0.3	20.2.1	Desserts	50	50
	Desserts			20.2.1	Desserts	50	50
	Custard mix, custard powder and blancmange powder		20.2.0.1	20.2.1.3.2	Desserts, dairy, no choc/coffee; custard & blanc mange mix/powd	50	50
	Dessert toppings			20.2.6.1	Sauces & syrups, sweet	50	50
	Jelly		20.2.0.2	20.2.1.4.1	Desserts, non-dairy, jelly	50	50
				20.2.1.4.1.2.1	Desserts, non-dairy, jelly, dry mix, intensely sweetened	1400	1400
				20.2.1.4.1.2.2	Desserts, non-dairy, jelly, dry mix, sugar sweetened	300	300
Mixed foods	Snack foods	10 (5)		20.2.4	Snack foods	100	50
	Ready to eat meals	10 (5)		20.2.5	Prepared dishes	100	50
			20.2.0.5	20.2.8	Soups	100	50
Meat analogues	Patties, fillets, strips	30 (5)		4.3.8.3	Vegetarian meat alternatives	300	50
				12.6	Vegetable protein products	300	50
Protein	Bars,	30 (10)	13.3	13.3	Formula	300	100

Food category in application	Examples	Proposed maximum (typical) use level (%)	Food standards code class	Harvest classification code	Harvest classification name	Concentration used in dietary exposure assessment (g/kg)	
						Maximum use level	Typical use level
based products	energy bars, pasta, powder, beverages				meal replacements & formulated supp foods		
			13.4	13.4	Formulated supplementary sports foods	300	100
			20.1	20.1.3.7	Beverages, non-alcoholic, meal replacement, oral supplement or protein drink based	300	100

Erucic acid and glucosinolates concentrations

A literature search was conducted using google scholar for articles published on glucosinolates and erucic acid concentration data in *Brassica* vegetables. The search terms included: glucosinolates concentration, erucic acid concentration and *Brassica* vegetables. Papers that included quantitative amounts of glucosinolates and erucic acid were selected for further evaluation while the ones that reported a qualitative analysis only were excluded. Papers that only measured total glucosinolates were included. FSANZ was also aware of the EFSA report on erucic acid in food and feed (EFSA 2016) and this was also reviewed for relevant references.

The other criteria by which the studies were assessed were the number of samples and the stage of maturity of the commodity. The plant tissues whose stage of maturity were not stated were not included in the assessment. The states of maturity provided ranged from leaves, florets to sprouts. Only the florets was considered as this is the form that is most commonly consumed by general population. In this assessment, the *Brassica* species designation of the vegetable is as reported in the original articles, while some were mapped to a similar food category.

Twenty studies that reported glucosinolates concentration of *Brassica* were identified. The foods for which concentration data were reported for glucosinolates included cabbage, broccoli, Chinese cabbage, cauliflower, Brussels sprouts and kohlrabi. All data expressed as mg/kg fresh weight were included, while studies with data expressed as µmol /100 g were excluded because they failed to report the molecular weight therefore conversion to the same units was not possible for comparison across all studies. Studies with no units provided were excluded. Moreover, due to wide variations in the values reported for the same vegetable by different studies, the data were further evaluated based on number of samples and reliability of the data. One paper was eventually selected that would be used to be representative of the concentration for *Brassicas*, from which the highest concentration from the range of reported values was selected for the dietary exposure assessment as a worst case scenario (Schonhof et al. 2004). Concentrations noted in the other two papers, one with a small number of samples (n=2) (Schonhof et al. 2004) and one with a larger number of

samples (n=19) (Herrmann et al. 1999), were within a similar range. The representative concentration selected was well within the range of concentrations across all the studies that were identified. A summary of all the papers and reasons for exclusion is included in Table A1.3.

Three papers were initially selected for erucic acid concentrations. The foods where concentration data were available for erucic acid were cabbage, broccoli, cauliflower and Brussels sprouts. For erucic acid, due to limited concentration data available, the three included papers were reviewed and erucic acid concentrations data in green broccoli florets was selected (West et al. 2002). The selected values (see Table 6.2) were used in calculating dietary exposure of consumers to glucosinolates and erucic acid in all *Brassica* vegetables.

Table A1.3. *Evaluation of literature for concentrations of Erucic acid and Glucosinolates in Brassica vegetables*

Chemical	Reference	Foods analysed	Number of samples	Included in final dataset	Reason for exclusion/ inclusion
Erucic acid	West et al. (2002)	Broccoli florets	42	Yes	Reliable data estimate as a worst case scenario, large samples, florets are usually consumed
	West et al. (2002)	Broccoli sprouts	1	No	Unreliable data estimate, only 1 sample
	West et al. (2002)	Broccoli seeds	NS	No	No of samples not provided
	Vale et al. (2015)	Wild Cabbage-sprouts	8	No	Unreliable estimate
	www.efsa.europa.eu/efsajournal	*Brassica* vegetables	1	No	One sample, unreliable estimate
	www.efsa.europa.eu/efsajournal	Cauliflower	24	No	No means nor median values provided
Glucosinolates	Schonhof et al. (2004)	Green broccoli	3	Yes	Reliable worst case scenario conc. values, sample no. >1
	Charron et al. (2005)	Green broccoli	2	No	Low value, not worst case
	Schonhof et al. (2004)	Purple broccoli	1	No	One sample
	Aires et al. (2006)	Green broccoli sprouts	1	No	One sample, unreliable range
	Schonhof et al. (2004)	Green cauliflower	2	No	Low value, not worst case
	Kushad et al.	White	3	No	Low value, not

Chemical	Reference	Foods analysed	Number of samples	Included in final dataset	Reason for exclusion/ inclusion
	(1999)	cauliflower			worst case
	Schonhof *et al*. (2004)	Green cauliflower	1	No	One sample, low value, not worst case
	CFSAN/Office of Food Additive Safety 2010 GRN 00327	Cauliflower	NS	No	Unknown number of samples
	Herrmann et al. (1999)	Chinese cabbage (total)	19	No	Comparable low conc. values, not worst case
	Ciska et al. (2000)	White cabbage	1	No	One sample
	Ciska et al. (2000)	Red cabbage	1	No	One sample
	Ciska et al. (2000)	Savoy cabbage	1	No	One sample
	Yang & Quiros (2010)	Chinese cabbage young leaves	39	No	Low value, not worst case
	Kang et al. (2006)	Chinese cabbage (total)	25	No	Low value, , not worst case
	Lee et al. (2014)	Chinese cabbage leaves	62	No	Comparative lowest value, not worst case
	Kushad et al. (1999)	Brussels sprouts	4	No	Low value, not worst case
	Ciska et al. (2000)	Brussels sprouts	1	No	Unreliable large data value for one sample
	Charron et al. (2005)	Brussels sprouts	2	No	Low value, not worst case
	Ciska et al. (2000)	Kohlrabi	1	No	One sample, low value, not worst case
	CFSAN/Office of Food Additive Safety 2010 GRN 00327	Kohlrabi	NS	No	Unknown number of samples

9.3 Methodology

Dietary exposure to rapeseed protein isolate was calculated for each individual consumer in the national nutrition surveys using their individual food consumption records. The Harvest program multiplied the specified concentrations of rapeseed protein isolate for an individual food by the amount of the food that an individual consumed in order to estimate the exposure to rapeseed protein isolate from each food. Once this had been completed for all of the foods specified to contain rapeseed protein isolate, the total amount of rapeseed protein isolate

consumed from all foods was summed for each individual. Where results are expressed on a body weight basis, each individual's body weight was used. Mean and 90th percentile (P90) dietary exposures were then derived from the individuals' ranked exposures. Estimated dietary exposures for the population on a body weight basis were compared to relevant toxicological endpoints or health based guidance values for risk characterisation purposes.

The applicant did not seek permission for use of rapeseed protein isolate in infant formula products (infant formula, follow-on formula and infant formula products for special dietary uses) and infant foods so the scope for dietary exposure assessment does not include these products.

9.4 Assumptions and limitations of the dietary exposure assessment

The aim of the dietary exposure assessment was to make the most realistic estimation of dietary exposures to rapeseed protein isolate and other anti-nutritional factors as possible. However, where significant uncertainties in the data existed, conservative assumptions were generally used to ensure that the estimated dietary exposure was not an underestimate of exposure.

Assumptions made in the dietary exposure assessment included:

- All foods in a category contain rapeseed protein isolate at the proposed maximum use concentration level concentration.
- Individual foods in the wider group contain the specified concentration of the food chemical.
- There are no reductions in food chemical concentrations from food preparation or due to cooking. The concentration data used for glucosinolates in *Brassica* vegetables are for raw not for cooked vegetables and as such did not account for heat lability of component during food processing.
- Food consumption data from the national nutrition surveys used represent current eating patterns.

In addition to the specific assumptions made in relation to this dietary exposure assessment, there were a number of limitations:

- As only one day of food consumption survey data are available from New Zealand nutrition surveys, the estimated exposures are likely to be over-estimated (particularly at the high percentiles of exposure) given that two days of survey data has been established as more appropriate for estimating long term consumption and therefore dietary exposure.
- Limited and highly varied concentration data (in terms of methods of collection and reporting and range of results) for erucic acid and glucosinolates in *Brassica* vegetables made it difficult to determine representative concentrations for use in the dietary exposure assessment.
- There were insufficient concentration data to enable a baseline dietary exposure assessment to be conducted for erucic acid and glucosinolates from the whole diet.
- National Nutrition Surveys cannot be used to describe an individual's usual intake, or predict how consumers will change their eating patterns if new foods are introduced.
- Age of the food consumption data used in the dietary exposure assessment. Generally, consumption of staple foods such as fruit, vegetables, meat, dairy products and cereal products, which make up the majority of most people's diet, is unlikely to change markedly over time. However, there is uncertainty associated with

the consumption of foods that may have changed in consumption, or that have been introduced to the market since the consumption data were collected.

9.5 Detailed dietary exposure assessment results based on proposed maximum use levels

9.5.1 Estimated dietary exposures to rapeseed protein isolate based on proposed maximum use levels

Estimated daily dietary exposures to rapeseed protein isolate were calculated as mean and 90th percentile in g/day and g/kg body weight/day for Australian and New Zealand population groups as shown in Table A1.4.

Mean dietary exposure to rapeseed protein isolate based on proposed maximum use levels for Australians aged 2 years and above was 58 g/day. Whereas for New Zealand children 5-14 years and adults 15 years and above mean dietary exposures were 60.6 g/day and 59.5 g/day respectively. On a body weight basis, estimated mean daily exposure to rapeseed protein isolate for Australian aged 2 years and above was 0.95 g/kg bw/day and for New Zealand children and adults respectively were 1.68 g/kg bw/day and 0.78 g/kg bw/day.

Estimated 90th percentile dietary exposure to rapeseed protein isolate for Australians aged 2 years and above was 109.9 g/day. Estimated 90th percentile dietary exposures to rapeseed protein isolate for New Zealand children 5-14 years and adults 15 years and above were 107.4 g/day and 121.5 g/day. When expressed on a body weight basis, estimated 90th percentile daily exposure to rapeseed protein isolate for the Australian aged 2 years and above, was 1.9 g/kg bw/day and 3.05 g/kg bw/day for New Zealand children and 1.56 g/kg bw/day for the adults.

Table A1.4 *Estimated mean and high percentile dietary exposure to Rapeseed Protein Isolate (g/day; g/kg bw/day) based on proposed maximum use levels*

Country*	Population age (years)	Number of consumers	Consumers as a proportion of respondents (%)	Mean		90th Percentile	
				g/day	g/kg bw/day	g/day	g/kg bw/day
Australia	2 years and above	7735	100	58.0	0.95	109.9	1.90
New Zealand	Children 5-14 years	3275	100	60.6	1.68	107.4	3.05
	15 years and above	4703	99.6	59.5	0.78	121.5	1.56

* Estimated dietary exposures are based on the average of two days of data for Australia, and only one day of data for New Zealand.

9.5.2 Dietary exposure to erucic acid from rapeseed protein isolate with a comparison to dietary exposures from *Brassica* vegetables

Table A1.5 shows the estimated exposure to erucic acid from rapeseed protein isolate based on proposed maximum use levels.

Estimated mean dietary exposures to erucic acid from rapeseed protein isolate based on proposed maximum use levels for Australians aged 2 years and above was 2.9 mg/day.

Mean dietary exposure to erucic acid from rapeseed protein isolate for New Zealand children 5-14 years and adults 15 years and above were 3.0 mg/day each. When expressed on a body weight basis, the estimated mean daily exposure for Australian aged 2 years and above was 0.05 mg/kg bw/day and for New Zealand children and adults were 0.08 mg/kg bw/day and 0.04 mg/kg bw/day respectively.

Estimated 90th percentile dietary exposures to erucic acid from rapeseed protein isolate for Australians aged 2 years and above was 5.5 mg/day. Estimated 90th percentile dietary exposure for New Zealand children 5-14 years and adults 15 years and above were 5.4 mg/day and 6.1 mg/day respectively. When expressed on a body weight basis, estimated 90th percentile daily exposure to erucic acid from rapeseed protein isolate was 0.10 mg/kg bw/day for Australian aged 2 years and above, and for New Zealand was 0.15 mg/kg bw/day for children and 0.08 mg/kg bw/day for adults.

Mean and 90th percentile dietary exposures to erucic acid from rapeseed protein isolate based on proposed maximum use levels (Table A1.5) are higher than mean and 90th percentile dietary exposure from *Brassica* vegetables (Table 6.4) for all population groups assessed in Australia and New Zealand.

Table A1.5 *Estimated dietary exposure to erucic acid from rapeseed protein isolate**

Country	Population age (years)	Mean		90th Percentile	
		mg/day	mg/kg bw/day	mg/day	mg/kg bw/day
Australia	2 years and above	2.9	0.05	5.5	0.10
New Zealand	Children 5-14 years	3.0	0.08	5.4	0.15
	15 years and above	3.0	0.04	6.1	0.08

* Based on 0.005% concentration of erucic acid in rapeseed protein isolate according to the specifications. This is the limit of detection for the analytical method for erucic acid.

9.5.2 Dietary exposure to glucosinolates from rapeseed protein isolate with a comparison to dietary exposures from *Brassica* vegetables

Table A1.6 shows the estimated dietary exposure to glucosinolates from rapeseed protein isolate based on proposed typical use levels.

Estimated mean dietary exposures to glucosinolates from rapeseed protein isolate based on proposed maximum use levels for Australians aged 2 years and above was 26.4 mg/day. Mean dietary exposure for New Zealand children 5-14 years and adults 15 years and above were 27.6 mg/day and 27.1 mg/day respectively. When expressed on a body weight basis, estimated mean daily exposure for Australian aged 2 years and above was 0.43 mg/kg bw/day and for New Zealand children and adults were 0.76 mg/kg bw/day and 0.35 mg/kg bw/day respectively.

Estimated 90th percentile dietary exposures to glucosinolates from rapeseed protein isolate for Australians aged 2 years and above was 50.0 mg/day. Estimated 90th percentile dietary exposure for New Zealand children 5-14 years and adults 15 years and above were 48.9 mg/day and 55.3 mg/day respectively. When expressed on a body weight basis, estimated 90th percentile daily exposure to glucosinolates from rapeseed protein isolate was 0.87 mg/kg bw/day for Australian aged 2 years and above and 1.39 mg/kg bw/day for New Zealand children and 0.71 mg/kg bw/day for the adults.

Table A1.6 *Estimated dietary exposure to glucosinolates from rapeseed protein isolate based on proposed typical use levels**

Country	Population age (years)	Mean		90th Percentile	
		mg/day	mg/kg bw/day	mg/day	mg/kg bw/day
Australia	2 years and above	26.4	0.43	50.0	0.87
New Zealand	Children 5-14 years	27.6	0.76	48.9	1.39
	15 years and above	27.1	0.35	55.3	0.71

* Based on 1 µmole/g concentration of glucosinolates in rapeseed protein isolate according to the specifications. Calculations for total glucosinolates based on molecular weight of 455 g/mol (VanEtten et al. 1980).

Australians aged 2 years and above were exposed to more glucosinolates from rapeseed protein isolate (based on maximum proposed use levels) (Table A1.6) than from *Brassica* vegetables (Table 6.6) based on mean and 90th percentile dietary exposures both in mg/day and on a body weight basis. However, for New Zealand children 5-14 years and adults 15 years and above, mean and 90th percentile dietary exposures to glucosinolates from *Brassica* (Table 6.6) were higher than exposures from rapeseed protein isolate (Table A1.6) both in mg/day and on a body weight basis.