STATUTORY DECLARATION

Statutory Declarations Act 1959

I, *Alexander Fleminglaan 1, 2613 AX Delft, Netherlands, Group lead Global Regulatory Affairs DSM Food Specialties,*
make the following declaration under the *Statutory Declarations Act 1959*:

1. The information provided in this application fully sets out the matters required
2. The information provided in this application is true to the best of my knowledge and belief
3. No information has been withheld that might prejudice this application, to the best of my knowledge and belief

I understand that a person who intentionally makes a false statement in a statutory declaration is guilty of an offence under section 11 of the *Statutory Declarations Act 1959*, and I believe that the statements in this declaration are true in every particular.

_____
Signature of person making the declaration

Declared at *Delft* on 12th of November 2018
Place Day Month and Year

Before me,

DSM Nutritional Products Asia Pacific

19 February 2019

_____
Signature of person before whom the declaration is made

Senior Regulatory Affairs Manager
DSM Nutritional Products Asia Pacific
Mapletree Business City, 30 Pasir Panjang Road #13-31, Singapore 117440

Full name, qualification and address of person before whom the declaration is made (in printed letters)

A statutory declaration provided on behalf of a body corporate must be made by a senior officer of that body corporate who is authorised to make the declaration on its behalf. The senior officer must state their name and source of knowledge and authority in making the statutory declaration and include a sufficient explanation of who they are (name, address, organisation/employer, position).

Dossier for Application of Rapeseed (Canola) Protein (Isolate) as a Novel Food in Australia/New Zealand

DSM Food Specialties B.V.
Global Regulatory Affairs
Nutrition Cluster

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Date
21-2-2019

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EXECUTIVE SUMMARY

Purpose of the application

The purpose of this application is to amend *Schedule 25—2 to clause 3 (a) of Australia New Zealand Food Standards Code (the Code) Standard 1.5.1 Novel Foods*, to permit the introduction of rapeseed protein, known commercially as CanolaPRO™, as novel food in Australia and New Zealand. The rapeseed protein is intended to be used as protein source in a wide range of food applications, similar to the use of for instance animal, soy or pea proteins.

Throughout this dossier, the substance to be registered can be referred to as "CanolaPRO™", "rapeseed protein" or "rapeseed protein isolate". These terms are used interchangeably. The nomenclature "rapeseed" and "canola" refers to the same substance.

The world's population is projected to increase within the next few decades from 6 billion to 9 billion by 2050[1]. Consequentially, demand for high quality proteins will increase. There will not be enough land available for the livestock to meet the increasing demand for animal proteins. At the same time, the carbon footprint needs to be lowered to mitigate the impact on global warming due to our agricultural output. Common belief is that plant-based rather than animal-based proteins will be more sustainable and therefore favorable for human consumption. Rapeseed protein is one of the promising protein sources. This was also recognized by the Australian Grain Research and Development Corporation[2] who funded a project at the Charles Sturt University, Wagga Wagga NSW, on improving food functionality of canola proteins. This resulted amongst others in a scientific review by Tan et al. (2011) on Canola Proteins for Human Consumption. It can be extracted from rapeseed cake, a by-product from rapeseed oil production. Globally, consumer demand in plant-based proteins is growing significantly. The Australia Institute of Food Science and Technology[3] similarly identified these trends on "flexitarianism and alternate protein sources" in an article "Top Four Trends for 2018". This is coupled with a strong consumer demand for protein across a broad age and demographics. New protein sources are becoming increasingly important as more consumers make a conscious decision to eat less meat. There is a market gap in finding sources of sustainable plant proteins that have the nutritional value and functionality of dairy proteins.

Given these trends and growing demand, CanolaPRO™ meets these market needs by providing a sustainable source of high nutritional value, a good taste profile and desirable functional properties enabling its inclusion in a broad range of food products. The benefits of offering CanolaPRO™ are further elaborated in the main application section "D.1.1 Costs and benefits

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[1] UN DESA report, "World Population Prospects: The 2015 Revision", https://esa.un.org/unpd/wpp/publications/files/key_findings_wpp_2015.pdf

[2] https://grdc.com.au/research/projects/project?id=1943

[3] Food Files: Top Four Trends For 2018 https://www.aifst.asn.au/food-files-top-four-trends-2018

of the application".

No exclusive capturable commercial benefit (ECCB) conferred with this application

For this Application, DSM is not seeking exclusive use of this novel food nor deems that it confers an exclusive capturable commercial benefit with respect to rapeseed protein. There are several industrial players active in the field of plant protein production for use in food, including rapeseed protein. This is substantiated by two US GRAS dossiers submitted by two distinctive companies (GRN000327, US FDA, 2010 and GRN000386, US FDA, 2017) and the EU novel food dossier that was submitted by another company than DSM.

It is expected that, in future, more companies active in plant protein will start producing rapeseed protein.

Characteristics

CanolaPRO rapeseed protein has a protein content of ≥ 90% with negligible amounts of carbohydrates or fat. Typical levels are given in Table 7 in Section C.1.1 of the NOVEL FOODS part of this dossier. It is derived from rapeseed press cake from classical or conventional rapeseeds sources, a byproduct of edible rapeseed oil production. The rapeseed used for the rapeseed protein production is from the *Brassica* varieties (*Brassica napus, Brassica rapa* and *Brassica juncea*) that are low in anti-nutrients including erucic acid and glucosinolates.

The protein consists of two major protein fractions: cruciferins and napins. Cruciferins are globulins and are the major storage protein in the seed. They are composed of 6 subunits with a total molecular weight of approximately 300 kDa. Napins are albumins, low-molecular-weight storage proteins (14 kDa) composed of two disulfide-linked polypeptides (Tan, S.H. *et al.*, 2011). CanolaPRO™ rapeseed protein isolate contains approximately 40-65% cruciferins and 35-60% napins. An amino acid profile of rapeseed protein isolate presented in Section "*B.3 Information on the physical and chemical properties of the novel food or novel food ingredient*" of the application confirms the presence of all 9 essential amino acids in appreciable amounts.

Regulatory status

CanolaPRO or Rapeseed Protein Isolate has been evaluated and permitted for use in humans by various Regulatory Authorities and Scientific Bodies (RASBs).

- CODEX

The rapeseed protein as described in this application is within the scope of the VPP, the General Guidelines for the Utilization of Vegetable Protein Products in Foods, as described by Codex Alimentarius (ALIMENTARIUS, C., 1989).

The rapeseed protein is isolated from press cake that remains after pressing oil from the conventional (non-GM) rapeseed cultivars *Brassica napus* or *Brassica rapa* for low erucic acid rapeseed oil as defined in Codex Standard 210-1999 on Vegetable Oils (Alimentarius, C., 1999).

- USA

DSM notified US FDA of the GRAS status of their rapeseed protein (GRN 000683) and received a No Questions letter from the FDA Center for Food Safety and Nutrition in the USA (US FDA, 2017). Rapeseed protein isolate produced by other manufacturers, was the subject of two prior GRAS Notices, GRN000327 in 2010 and GRN000386 in 2011. Both Notices received no questions letters (US FDA 2010, US FDA 2011).

- European Union

The EU authorized the use of rapeseed protein isolate from a competitor as a novel food ingredient in 2014 (EU/424/2014) (EC, 2014). And since March 8, 2017 the use of DSM's CanolaPRO™ is also approved in the EU as being substantial equivalent to EU/424/2014 (EC, 2017).

Intended use of CanolaPRO Rapeseed Protein

DSM intends to market CanolaPRO™ rapeseed (canola) protein isolate to food product manufacturers as a direct protein replacement of animal- or vegetable-based protein such as soy, whey, and pea protein. CanolaPRO™ has broad functionality and can be used in a wide range of food applications, as a protein source, thickener, water binder, emulsifier, gelling agent, foaming agent, or texturizer.

CanolaPRO™ will be used in a variety of food products for the general population. Examples of the intended foods and the proposed maximum levels of use are provided in Table 9, NOVEL FOOD Section "D.1 A list of the foods or food groups proposed to or which might contain the novel food ingredient or substance" of this Application. Due to potential unpalatability (bitterness) and/or technological limitations associated with its water-binding capacity, use levels are not expected to exceed 30% in any one food.

All information provided in this application, to the best of our abilities, has been obtained, described and referenced as indicated in "*Section E.1 Data Requirements of the FSANZ Application Handbook (FSANZ, 1-3-2016)*" and in accordance with the items provided in Checklist For General Requirements (3.1.1) and Checklist for applications for New Foods, Novel Foods (3.5.2) provided in the Application Handbook aforementioned.

-End of Executive Summary-

GENERAL REQUIREMENTS

B Applicant details

(a) applicant (individual or organisation's) name:

DSM Nutritional Products Asia Pacific

(b) name of contact person

Regulatory Affairs Manager

(c) address (street and postal)

DSM Nutritional Products Asia Pacific

Mapletree Business City, 30 Pasir Panjang Road #13-31, Singapore 117440

(d) telephone number

(e) email address

(f) nature of applicant's business
Royal DSM is a global science-based company active in health, nutrition and materials. The DSM Innovation Center is supporting innovation in DSM's core businesses and venturing activities and to develop its Emerging Business Areas (EBAs).

(g) details of other individuals, companies or organisations associated with the application.

Not applicable

C Purpose of the application

The purpose of this application is to amend *Schedule 25—2 to clause 3 (a) of the Australia New Zealand Food Standards Code (the Code) Standard 1.5.1 Novel Foods*, to permit the introduction of rapeseed protein, known commercially as CanolaPRO™, as novel food in Australia and New Zealand. The rapeseed protein is intended to be used as protein source in a wide range of food applications, similar to the use of for instance animal, soy or pea proteins.

D Justification for the application

There is expectation that the world's population will rapidly increase within the next few decades from 6 billion people now to a projected 9 billion in 2050[4]. Consequentially, demand for high quality proteins will increase. There will not be enough land available for the livestock to meet the increasing demand for animal proteins. At the same time, the carbon footprint needs to be lowered to mitigate the impact on global warming due to our agricultural output. Common belief is that plant-based rather than animal-based proteins will be more sustainable and therefore favorable for human consumption. Rapeseed protein is one of the promising protein sources. It can be extracted from rapeseed cake, a by-product from the globally growing rapeseed oil industry, that is currently only used in animal feed. The big challenge however is to maintain the beneficial protein functionality during processing while at the same time minimizing the level of antinutritional factors (Tan et al., 2011). DSM succeeded in this challenge with the development of CanolaPRO™.

Globally, consumer demand in plant-based proteins is growing significantly. The Australia Institute of Food Science and Technology[5] similarly identified these trends on "flexitarianism and alternate protein sources" in an article "Top Four Trends for 2018". This is coupled with a strong consumer demand for protein across a broad age and demographics. New protein sources are becoming increasingly important as more consumers make a conscious decision to eat less meat. There is a market gap in finding sources of sustainable plant proteins that have the nutritional value and functionality of dairy proteins. CanolaPRO™ meets these market needs by providing a sustainable source of high nutritional value, a good taste profile and desirable functional properties enabling its inclusion in a broad range of food products.

As a testament, CanolaPRO™, was awarded the "Most Novel Protein Ingredient Award 2017" at the 10th Bridge2Food Protein Summit in Reims, France (Bridge2Food, 2017).

Although rapeseed oil has been a recognized food ingredient for many years[6], rapeseed protein is a relatively new food ingredient. With respect to the definitions in Standard 1.5.1 in the Australian New Zealand Food Standards Code, rapeseed protein can be considered as a non-traditional and novel food in Australia and New Zealand. This was confirmed by the Advisory Committee on Novel Foods (ACNF, 26-5-2017).

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[4] UN DESA report, "World Population Prospects: The 2015 Revision", https://esa.un.org/unpd/wpp/publications/files/key_findings_wpp_2015.pdf

[5] Food Files: Top Four Trends For 2018 https://www.aifst.asn.au/food-files-top-four-trends-2018

[6] http://www.foodstandards.gov.au/consumer/generalissues/canola/Pages/default.aspx

Therefore, with this application, DSM would like to propose an amendment to the Code for inclusion of rapeseed protein isolate, CanolaPRO™, as novel food in Australia and New Zealand.

D.1 Regulatory impact information

D.1.1 Costs and benefits of the application

For **Consumers**, the Costs and benefits of rapeseed protein are:

- Sustainability - CanolaPRO™ as protein from a vegetable source is a more sustainable alternative than protein from animal source. It is even more sustainable than other plant-based proteins because it is produced by valorizing the existing side stream of the canola oil production.
- Alternate source of existing supply - As protein demand increases globally, a shortage in existing protein sources can be expected and consequently driving prices up.
- Economically interesting - CanolaPRO™ will be a cost-effective alternative to certain animal and other vegetable-based proteins (depending on the application).
- Good nutrition and taste acceptability - CanolaPRO™ has a high nutritional value, comparable to animal proteins and soy (see PDCAAS study in Appendix 12). It also has a good taste profile.
- Heightened consumer demand for proteins - consumers are actively looking for high protein products to increase their protein intake and also switching more and more to plant proteins (flexitarianism) (AIFST 2017[7]).
- Protein functionality leads to better appreciated products - The high solubility of CanolaPRO™ gives sensorial benefit (mouthfeel) and the gelation properties enable appreciated food texture (e.g. meat analogues).
- Market research[8] has identified the slow and steady rise of vegetarianism in Australia since 2012 the number of Australian adults whose diet is all or almost all vegetarian has risen from 9.7% of the population to almost 11.2%
- CanolaPRO™ offers new opportunities for vegan, soy-free and protein-rich products.

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[7]Food Files: Top Four Trends for 2018 https://www.aifst.asn.au/food-files-top-four-trends-2018
[8] Roy Morgan 15 Aug 2016, Article 6923 "The slow but steady rise of Vegetarianism in Australia" (file:///C:/Users/837084/Downloads/6923-Final-Vegetarianism-on-the-rise.pdf)

For the **Industry**, the costs and benefits of availing rapeseed protein as a food ingredient are:

- Good Functionality - Industry will have an alternative protein source with good functional properties, such as texturing, foaming, emulsifying, which can be applied in a wide range of food products, ranging from weight management products and sports nutrition to ready-to-eat meals.

- Facilitating exports to large global markets - The use of rapeseed protein is already approved in the EU and USA. Permission in Australia and New Zealand will open up new markets for local manufacturers

- Ease of use for local businesses - The approval of rapeseed protein consistent with EU and US regulations will allow global businesses to manufacture the same products for domestic and export markets, reducing barriers to trade

- CanolaPRO™ will be a cost-effective alternative to certain animal and other vegetable-based proteins (depending on the application).

For the Government, the Costs and benefits of rapeseed protein are:

- Diversified supply sources of proteins
- Plant-based protein sources have a relatively lesser impact on terrestrial biodiversity than traditional red meat industries, both in the area covered and nature of the impact. This includes the area of native vegetation cleared for grazing and the impacts of over-grazing and trampling. On a global scale, the impact of meat production on natural systems through land clearing (for both pastures and grain to feed cattle in feedlots), overgrazing, pollution of waterways and greenhouse gas emissions has elicited concern from various avenues. (Williams and Price, 2010).
- Australia, being and important and growing producer of oilseeds (canola), produces increasing amounts of canola meal as by-product of the canola oil production. The use of the high-quality proteins in canola meal in human food instead of the current use in animal feed is an interesting alternative outlet for the increasing amounts of canola meal produced (Tan et al., 2011).

D.1.2 Impact on international trade

There will be a positive impact (less trade barrier) of the proposed change on foods imported into Australia and New Zealand. It will help facilitate international trade by permitting rapeseed containing products in Australia and New Zealand, which were produced in the USA and EU. It may potentially open up new markets or increase market share both domestically and in the USA and EU.

E Information to support the application

E.1 Data requirements

All information provided in this application, to the best of our abilities, has been obtained, described and referenced as indicated in Section E.1 Data Requirements of the FSANZ Application Handbook (FSANZ, 1-3-2016).

F Assessment procedure

DSM Nutrition Products Asia Pacific anticipates that this application should be considered according to subdivision F - *Modification of general procedure for developing new food regulatory measures and major variations*, as described in FSANZ Act 1991, Part 3, Division 1 (FSANZ, 1991).

G Confidential commercial information (CCI)

Not applicable

H Other confidential information

Not applicable

I Exclusive capturable commercial benefit (ECCB)

There is no exclusive capturable commercial benefit with respect to rapeseed protein. There are several industrial players active in the field of plant protein production for use in food, including rapeseed protein. This is substantiated by two US GRAS dossiers submitted by two distinctive companies (GRN000327 and GRN000386) and the EU novel food dossier that was submitted by another company than DSM.

It is expected that, in future, more companies active in plant protein will start producing rapeseed protein.

J International and other national standards

J.1 International Standards

In 1989 Codex Alimentarius adopted both a General Standard for Vegetable Protein Products (VPP) (ALIMENTARIUS, C., 1989) and General Guidelines for the Utilization of Vegetable Protein Products (VPP) in Foods (Codex Alimentarius Commission, 1989). The rapeseed protein as described in this application is within the scope of the VPP as described by Codex Alimentarius.

The rapeseed protein is isolated from press cake that remains after pressing oil from the conventional (non-GM) rapeseed cultivars *Brassica napus* or *Brassica rapa* for low erucic acid rapeseed oil as defined in Codex Standard 210-1999 on Vegetable Oils (Alimentarius, C., 1999) These *Brassica* varieties contain only low levels of erucic acid and glucosinolates and are also known as canola or Rapeseed-00 (OECD 2011).

J.2 Other national standards or regulations

DSM notified US FDA of the GRAS status of their rapeseed protein (GRN 000683) and received a No Questions letter from the FDA Center for Food Safety and Nutrition in the USA (US FDA, 2017). Rapeseed protein isolate produced by other manufacturers, was the subject of two prior GRAS Notices, GRN000327 in 2010 and GRN000386 in 2011. Both Notices received no questions letters (US FDA 2010, US FDA 2011).

The EU authorized the use of rapeseed protein isolate from a competitor as a novel food ingredient in 2014 (EU/424/2014) (EC, 2014). And since March 8, 2017 the use of DSM's CanolaPRO™ is also approved in the EU as being substantial equivalent to EU/424/2014 (EC, 2017).

K Statutory declaration

An Original signed statutory declaration that includes the following statements is provided with this Application herein.

1. The information provided in this application fully sets out the matters required.

2. The information provided in this application is true to the best of my knowledge and belief.

3. No information has been withheld that might prejudice this application, to the best of my knowledge and belief.

L Checklist

Checklists are enclosed with this Application that are of relevance based on Appendix 1

1. Checklist for General Requirements (3.1.1)
2. Checklist for Applications for New Foods, Novel Foods (3.5.2)

NOVEL FOODS (3.5.2)

A Exclusive use of novel foods

DSM does not seek exclusive use (see also above under I on ECCB).

B Technical information on the novel food

The data provided in this application are representative for the rapeseed protein intended to be marketed in Australia and New Zealand.

Throughout this dossier, the substance to be registered can be referred to as CanolaPRO™, rapeseed protein or rapeseed protein isolate. These terms can be used interchangeably. Instead of rapeseed, the term canola can be used to refer to the same substance.

B.1 Information on the type of novel food

The subject of this novel food notification is rapeseed protein isolate, known commercially as CanolaPRO™. It is a substance that has a protein content of ≥ 90% and is derived from rapeseed press cake, a byproduct of edible rapeseed oil production. The rapeseed used for the rapeseed protein production is from the *Brassica* varieties (*Brassica napus, Brassica rapa* and *Brassica juncea*) that are low in erucic acid and glucosinolates. It is also known as canola.

The rapeseed protein, CanolaPRO™, falls within the following categories of novel foods as identified by FSANZ:

- Plant or animals and their components
- Plant or animal extracts
- Dietary macro-components
- Food ingredients derived from new sources

B.2 Information on the purpose of adding a novel food ingredient to food

Rapeseed protein will be used as an alternative protein source as a food ingredient in different food applications. Furthermore, it can be used for its functional properties, a.o. thickener, water binder, emulsifier, gelling agent, foaming agent or texturizer. Primarily it is intended in food products for use in general population.

B.3 Information on the physical and chemical properties of the novel food or novel food ingredient

CanolaPRO™ is sold in powder form containing ≤ 7% w/w moisture. The powder dissolves very well in water and is stable if kept under dry storage conditions at moderate temperatures, <

25 °C.
CanolaPRO™ has a protein content of ≥ 90%. Carbohydrates, total lipids are below detection limit (see Table 7). The protein consists of two major protein fractions: cruciferins and napins. Cruciferins are globulins and are the major storage protein in the seed. They are composed of 6 subunits with a total molecular weight of approximately 300 kDa. Napins are albumins, low-molecular-weight storage proteins (14 kDa) composed of two disulfide-linked polypeptides (Tan, S.H. *et al.*, 2011). CanolaPRO™ rapeseed protein isolate contains approximately 40-65% cruciferins and 35-60% napins[9].

The results of amino acid analysis of multiple batches of CanolaPRO™ rapeseed protein isolate are summarized in Table 1.

[9] As analyzed by HP-SEC analysis: Samples were dissolved in a 500 mM NaCl solution and analyzed by HP-SEC using the same solution as mobile phase. Detection was done by measuring UV absorbance at 280 nm. The relative contribution of cruciferin and napin (%) was calculated as the ratio of the peak area of each protein with respect to the sum of both peak areas.

Table 1 Typical amino acid content in representative batches of CanolaPRO™

Amino acid	Unit	RPI-1536-01-G	RPI-1543-02-P**	RPI-1543-03-P**	RPI-1549-01-P**	RPI-1549-02-P**	Average	% of amino acid total (w/w)
Alanine	g/100g	4.09	4.13	4.19	4.22	4.21	4.17	4.4%
Arginine	g/100g	6.24	6.27	6.23	6.47	6.41	6.32	6.7%
Aspartate	g/100g	6.44	5.35	5.26	5.62	5.71	5.68	6.0%
Glutamate	g/100g	20.9	22.3	22.8	23.2	22.8	22.4	23.7%
Glycine	g/100g	4.81	4.76	4.79	4.90	4.88	4.83	5.1%
Histidine*	g/100g	2.84	3.08	3.06	3.17	3.12	3.05	3.2%
Hydroxy-proline	g/100g	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	n/a
Isoleucine*	g/100g	3.58	3.45	3.44	3.55	3.56	3.52	3.7%
Leucine*	g/100g	6.84	6.68	6.69	7.02	6.96	6.84	7.2%
Lysine*	g/100g	5.62	6.22	6.15	6.24	6.18	6.08	6.4%
Ornithine	g/100g	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	n/a
Phenyl-alanine*	g/100g	3.74	3.53	3.54	3.72	3.73	3.65	3.9%
Proline	g/100g	6.30	6.92	6.07	6.85	6.71	6.57	7.0%
Serine	g/100g	4.17	3.71	3.57	4.07	4.04	3.91	4.1%
Threonine*	g/100g	3.91	3.66	3.53	3.77	3.80	3.73	4.0%
Tyrosine	g/100g	2.03	1.95	1.83	1.98	2.01	1.96	2.1%
Valine*	g/100g	4.68	4.64	4.59	4.79	4.74	4.69	5.0%
Cysteine	g/100g	3.00	3.78	3.81	3.50	3.46	3.51	3.7%
Methionine*	g/100g	1.98	2.05	2.13	2.09	2.04	2.06	2.2%
Tryptophan*	g/100g	1.35	1.34	1.34	1.39	1.40	1.36	1.4%

*Essential amino acids

** Batches 1543-02 and 1543-03 were derived from the same batch of rapeseed press cake. Batches 1549-01 and 1549-02 were derived from the same batch of raw material but different from the two 1543 batches

Stability of CanolaPRO™ rapeseed protein isolate

Two representative batches of CanolaPRO™ rapeseed protein isolate were sampled during 16 weeks' storage at 20 and 40 °C[10]. Each batch was analyzed for protein content (Kjeldahl, N*6.25) and protein solubility, which is indicative of the functional performance of the protein in food applications. Solubility was determined at pH 6.8 and each 0 and 150 mM NaCl.

The results, as shown in Appendix 2 demonstrate that both protein content and protein solubility remain constant at the tested time and temperatures.

With no apparent degradation after 15 weeks at 40 °C, it is reasonable to presume that CanolaPRO™ will be stable at 25 °C for 15 months.

[10] In general, industry practice indicates that one week of shelf life at 40 °C represents 4 weeks of shelf life at 20 °C.

B.4 Information on the impurity profile for a typical preparation

DSM has investigated which impurities and contaminants could pose a safety concern.

Anti-nutritional factors

Rape plants are known to contain erucic acid in the oil and glucosinolates, phenolics and phytic acid and protease inhibitors in the seeds. CanolaPRO™ rapeseed protein isolate is derived from varieties of rapeseed low in erucic acid and glucosinolates. The concentration of anti-nutritional factors in CanolaPRO™ and their potential for adverse impacts from consuming is addressed in Section *C.1.4 Information regarding the potential adverse effects associated with the food or its ingredients.*

Heavy metals

The heavy metals content of the rapeseed press cake was analyzed using ICP-AES in three different batches (Table 2). Additionally, heavy metals have also been measured in the end product CanolaPRO™ using ICP-MS (Table 3). The heavy metals content in both the raw material (Rapeseed press cake) and the product RPI90 (Rapeseed Protein Isolate, traded as "CanolaPRO™") is within safe limits.

Table 2 Heavy metal content in three batches of the raw material rapeseed press cake.

Batch No.	Arsenic (ppm)	Cadmium (ppm)	Lead (ppm)	Mercury (ppm)
Limit of Detection (LOD)[11]	0.02	0.02	0.3 or 0.2	0.02
T - 94345	< 0.02	0.08	<0.3	< 0.02
L - 98285	< 0.02	0.11	<0.2	< 0.02
R - 95423	< 0.02	0.08	<0.2	< 0.02

Table 3 Heavy metal concentration in five batches of the product rapeseed protein isolate

Batch No.	Arsenic (ppm)	Cadmium (ppm)	Lead (ppm)	Mercury (ppm)
Limit of Detection (LOD)[12]	0.01	0.005	0.001	0.002
PB17151	< 0.01	0.022	0.013	< 0.002
PB17132	< 0.01	0.028	0.014	< 0.002
PB17092	0.01	0.014	0.020	< 0.002
PB17082	< 0.01	0.012	0.006	< 0.002
PB17072	< 0.01	0.011	0.024	< 0.002

[11] LOD based on analysis of a 0.5 g sample. For lead analysis, a sample solution was prepared by weighing an amount of sample, followed by digestion and dilution. The LOD of the sample solution prepared as such was 0.003 mg/L. The results were calculated back to a level in the dry matter, based on the weighted amount. Therefore, the LOD for lead in the sample was either 0.3 or 0.2 ppm, depending on the amount of sample weight (either 0.75 g or 0.5 g).

[12] LOD was calculated for a sample amount of 0.5 g.

Mycotoxins

DSM monitors the mycotoxins in the rapeseed press cake material and in the end-product CanolaPRO™. Table 4 summarizes the results of mycotoxins analysis of two representative batches of rapeseed press cake.

Table 5 provides the results of mycotoxin analysis of four lots of CanolaPRO™. The results show levels well below any level that would be of concern.

Table 4 Mycotoxins concentration in two different and representative batches of rapeseed press cake

Mycotoxins	Unit	LOQ	Rapeseed press cake batches	
			RPC-95423	RPC-95285
Aflatoxin B1[13]	ppb	< 0.1	< LOQ	< LOQ
Deoxynivalenol[14]	ppb	< 10	< LOQ	39 ppb
Fumonisins (sum)[15]	ppb	< 30	< LOQ	< LOQ
T-2 & HT-2 (sum)	ppb	< 20	< LOQ	< LOQ

Table 5 Mycotoxins concentration four different and representative batches of the product rapeseed protein isolate CanolaPRO™

	Lot Number	CanolaPRO-1536-01-G	CanolaPRO-1543-02-P	CanolaPRO-1549-01-P	CanolaPRO-1615-01-G
Test Method[16]	Toxin	Result µg/Kg	Result µg/Kg	Result µg/Kg	Result µg/Kg
BA-TM-03	Deoxynivalenol (DON)	<10	<10	<10	<10
BA-TM-03	Diacetoxyscirpenol (DAS)	<10	<10	<10	<10
BA-TM-03	3-Acetyldeoxynivalenol (3AcDON)	<10	<10	<10	<10
BA-TM-03	15-Acetyldeoxynivalenol (15AcDON)	<10	<10	<10	<10
BA-TM-03	Fusarenone X (Fus X)	<10	<10	<10	<10
BA-TM-03	Nivalenol (NIV)	<10	<10	<10	<10
BA-TM-03	Neosolaniol (NEO)	<10	<10	<10	<10
BA-TM-03	T2 Toxin (T2)	<10	<10	<10	<10
BA-TM-03	HT2 Toxin (HT2)	<10	<10	<10	<10
BA-TM-10	Aflatoxin B1	<0.1	<0.1	<0.1	<0.1
BA-TM-10	Aflatoxin B2	<0.1	<0.1	<0.1	<0.1
BA-TM-10	Aflatoxin G1	<0.1	<0.1	<0.1	<0.1
BA-TM-10	Aflatoxin G2	<0.1	<0.1	<0.1	<0.1
BA-TM-31	Fumonisin B1	<10	<10	<10	<10
BA-TM-31	Fumonisin B2	<10	<10	<10	<10
BA-TM-31	Fumonisin B3	<10	<10	<10	<10

[13] Analyzed by DIN EN ISO 17375:2006 method
[14] Analyzed by DIN EN ISO 15791:2009 method
[15] Analyzed by DIN EN ISO 15791:2009 method
[16] In-house method of the UK accredited lab PAS Premier Analytical Services, High Wycombe, UK.

Pesticides

Three lots of rapeseed protein isolate were sent to a third-party laboratory for analysis. The material was screened for residues of over 600 pesticides using GC-MS and LC-MS. No residues were found to be at a level of concern. The analytical reports can be found in Appendix 3.

Microbiological contaminants

The press cake used as raw material in the rapeseed protein production must be of sufficient microbial quality. The rapeseed oil crushing is done without water and the water content of the rapeseed is so low that no microbial growth will take place during or after processing.

The microbiological contamination level is monitored for all rapeseed press cake used to produce CanolaPRO™. Microbiological data of three independent and representative batches of rapeseed press cake are presented in Appendix 4.

The manufacturing process for rapeseed protein isolate contains several filtration steps to control the microbiological load. Additionally, the contamination levels are monitored during the manufacturing process and the end product, CanolaPRO™, is controlled for contamination (see results in table 7).

B.5 Manufacturing process for a novel food ingredient

CanolaPRO™ rapeseed protein isolate is produced from the press cake that remains after cleaned, flaked and conditioned seeds of any food-grade, canola-quality, non-GMO cultivar of rapeseed (as defined in Codex Standard 2010-1999) have been pressed to separate the oil.

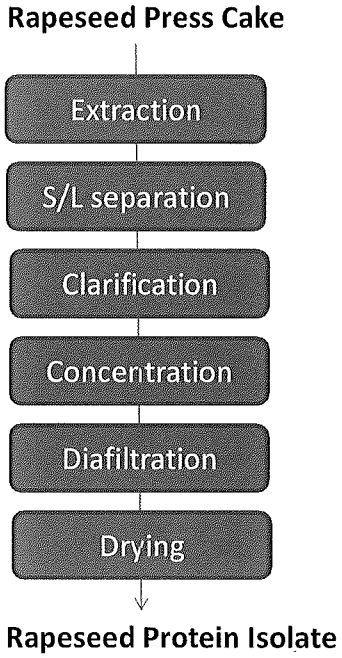
Figure 1 depicts the steps involved in the production process. All materials used in production of CanolaPRO™ are food grade, and each facility involved in the process.

The process starts with an extraction step, in which rapeseed cake/meal is mixed with an aqueous salt solution (cake/meal to water ratio: 1:5 to 1:20) (0-5% NaCl) at a temperature between 40 and 75 °C. After 5 minutes to 2 hours, the protein-rich solution is separated from the insoluble material. The protein-rich solution is hereafter referred to as the extract. The pH of the extract is adjusted, and the extract is further processed to clarify the material and remove non-protein substances. Citric acid and/or ascorbic acid may be used as buffers. The residual fat and formed precipitates are removed *via* a solid/liquid separation step (*e.g.*, membrane filter press or centrifugation). The extract is then concentrated and washed in an ultrafiltration/diafiltration (UF/DF) step. The UF/DF step concentrates the protein and removes anti-nutritional factors (*e.g.*, polyphenols, residual phytate, glucosinolates). Sodium bisulfite may be used to whiten the product, if necessary. If sulfite is used, the finished product will contain less than 10 ppm.

Finally, the washed concentrate can be dried in a suitable dryer, such as a spray drier (single or multistage) at an inlet temperature of 150-200 °C and an outlet temperature of 50-100 °C. The resulting powder is the ingredient that is the subject of this application.

Each processing aid used in the manufacturing of CanolaPRO™, including sodium chloride, pH adjustment titrants (*e.g.*, ascorbic acid, citric acid, hydrochloric acid, and sodium hydroxide), and divalent cations such as calcium chloride, is food grade. Maltodextrin or any other human food-grade carbohydrate might be used to formulate the end-product, depending on customer needs.

Figure 1 Overview of method of production of CanolaPRO™ from rapeseed press cake



S/L: Solid liquid separation (*e.g.*, filtration, centrifugation)

B.6 Specification for identity and purity for a novel food ingredient

Apart from the Standard on Vegetable Protein Products (VPP) in the Codex Alimentarius (Codex Stan 174-1989) (see also Section J International and other national standards), there is no standard or monograph specifically on rapeseed protein available in the primary or secondary sources as defined in S3-2 of the Code.

CanolaPRO™ complies with the following compositional parameters on VPP as given in Codex Standard 174-1989:

- Moisture : sufficiently low to ensure microbiological stability
- Crude protein (N*6.25) : shall not be less than 40% on dw basis
- Ash : shall not exceed 10% on dw basis
- Fat : residual fat content shall be compatible with GMP
- Crude fibre : crude fibre shall not exceed 10% on dw basis

In the EU, the European Commission authorised rapeseed protein as Novel Food and laid down specifications in the Commission Implementing Decision (EU/2014/424). DSM proved that its rapeseed protein meets these specifications and consequently obtained approval of marketing rapeseed protein in the EU (EC, 2017) (see also Section J.2 Other national standards or regulations on page 14).

The product specifications for CanolaPRO™ as given in Table 6 are in line with the Codex Standard on VPP, the EU requirements and the specifications as notified to the US FDA (see also Section J.2 Other national standards or regulations on page 14).

Table 6 Product specifications for CanolaPRO™ rapeseed protein isolate

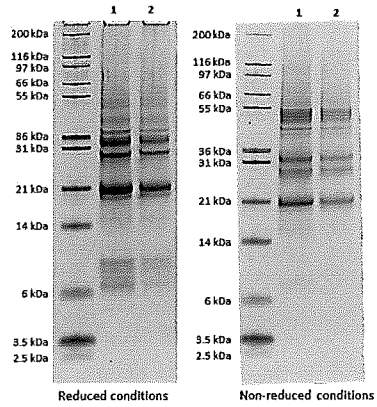
Parameter	Unit	Value	Method
- Appearance	-	Tan powder	Visual
- Composition			
Total Protein (% *via* N*6.25)	% w/w	≥ 90	AOCS Ba 4e-93
Carbohydrates	% w/w	≤ 7	By difference*
Fat (direct)	% w/w	≤ 2	FCC v10 appendix 2c; ISO 12966-2
Ash	% w/w	≤ 4	FCC v10 appendix 2c
Moisture	% w/w	≤ 7	FCC v7, 1133 [100%- dm]
- Purity			
Glucosinolates	µmol/g	≤ 1	EEC 1864/90
Phytates	% w/w	≤ 1.5	Ellis *et al.*, 1977
Lead	mg/kg	≤ 0.5	ICP-AES
- Microbiological criteria			
Total plate count	cfu/g	≤ 10^4	ISO 4833:part 2 2013
E. coli	cfu in 10 g	absent	ISO 21528
Salmonella spp.	cfu in 25 g	absent	ISO 6579:2002
Yeast and Molds	cfu/g	< 100	ISO 21527-2

*Carbohydrates are calculated by difference as follows: 100 % - [protein (as is) % + moisture % + fat % + ash % + fiber %]

B.7 Analytical method for detection of a novel food ingredient

The rapeseed protein can be detected using common protein analytical methods (AOCS Ba 4e-93), such as SDS page. This method is described in Appendix 8. An example of an SDS gel of a typical rapeseed protein batch is given in Figure 2.

Figure 2 Example of an SDS gel of a typical rapeseed protein batch



nr	Sample description (code)	Remarks
1	RZA 1692	Dried ccUF (RPI)
2	RZA 1693	Powder collected from filter of exhaust

C Information on the safety of the novel food

C.1 Plants or animals (or their components)

C.1.1 Information on the composition of the novel food

The results of analyses of five separate and representative batches of CanolaPRO™ rapeseed protein isolate, are summarized in Table 7, showing compliance with the specifications and consistency of the production process. Certificates of analysis are provided in Appendix 5.

Table 7 Analytical results of five representative batches of CanolaPRO™

Parameter	Unit	Spec.	RPI-1536-01-G	RPI-1543-02-P**	RPI-1543-03-P**	RPI-1549-01-P**	RPI-1549-02-P**
- Appearance	-	Tan powder	Tan powder	Tan powder	Tan powder	Tan powder	Tan powder
- Composition							
Total Protein (% via N*6.25)	% w/w	≥ 90	96.3	98.1	98.8	98.8	98.8
Carbohydrates	% w/w	≤ 7	0	0	0	0	0
Fat (direct)	% w/w	≤ 2	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Ash	% w/w	≤ 4	0.71	0.08	0.08	0.08	0.06
Moisture	% w/w	≤ 7	4.3	3.3	3.6	2.3	3
- Purity							
Glucosinolates	µmol/g	≤ 1	<0.1	<0.1	<0.1	<0.1	<0.1
Phytates	% w/w	≤ 1.5	< 0.14	< 0.14	<0.14	<0.14	<0.14
Lead	mg/kg	≤ 0.5	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
- Microbiological criteria							
Total plate count	cfu/g	≤ 10^4	110	270	110	70	20
E. coli	Absent in 10 g	absent	absent	absent	absent	absent	absent
Salmonella spp.	Absent in 25 g	absent	absent	absent	absent	absent	absent
Moulds	cfu/g	< 100	30	20	30	<10	<10
Yeasts	cfu/g	< 100	<10	<10	<10	<10	<10

**Batches 1543-02 and 1543-03 were derived from the same batch of rapeseed press cake. Batches 1549-01 and 1549-02 were derived from the same batch of raw material but different from the two 1543 batches.

More extensive information on anti-nutritional factors in CanolaPRO™ is given in Section C.1.4 Information regarding the potential adverse effects associated with the food or its ingredients. More data on heavy metals, mycotoxins, pesticide residues and microbiological contaminants in CanolaPRO™ are given in Section B.4 Information on the impurity profile for a typical preparation.

C.1.2 Information on the effects of food processing or preparation

CanolaPRO™ rapeseed protein isolate is derived from varieties of rapeseed low in erucic acid and glucosinolates. Furthermore, it has been demonstrated that technological processes used to manufacture rapeseed products significantly reduce the levels of anti-nutritional factors. For example, isolation of canola proteins has been shown to eliminate up to 95% of glucosinolates, 92% of phytic acid and 100% of tannic acid (Mansour, E. *et al.*, 1993).

The residual concentrations of anti-nutritional factors as analyzed in CanolaPRO™ and their potential adverse effects are discussed in Section C.1.4.

C.1.3 Information on the current use of this food or food component in population sub-groups or in other countries

Rapeseed protein isolate is a relatively new food ingredient that was the subject of several GRAS notices (GRN000327 in 2010, GRN000386 in 2011 and GRN000683 in 2017) that received no questions letters from the FDA Center for Food Safety and Nutrition in the USA (see also Section J.2). Additionally, it has been authorised for use a novel food ingredient in the EU since 2014 (see also Section J.2).

For a much longer period of time, the crop oilseed rape has been used as oil for cooking and salads and to produce canola meal as animal feed. In the late 1970s, the name canola (Canadian oil, low acid) was adopted in North America to distinguish the plant low in erucic acid from other types of rapeseed. Later on, rapeseed varieties low in glucosinolates were developed, notably for livestock consumption (Mansour et al., 1993).

Rapeseed oil, low in erucic acid, was recognized as GRAS in 1985 by the U.S. FDA (21 CFR §184.1555(c), which is the edible oil obtained from *Brassica napus* or *Brassica campestris*). Later, this GRAS status was extended to canola oil from *Brassica juncea*.

Canola varieties low in erucic acid were introduced in Australia in the 1980s and since then been further improved. FSANZ extensively examined the safety of the canola oil for consumption in 2003 and found no cause for concern (FSANZ, 2003)

C.1.4 Information regarding the potential adverse effects associated with the food or its ingredients

In this Section, potential adverse effects of anti-nutritional factors that could be present in the rapeseed protein are addressed. Potential allergenicity of the new protein is discussed in detail in Section C.6.1 of the NOVEL FOODS part of this dossier.

Anti-Nutritional Factors

As previously noted, rapeseed, along with other members of the *Brassicae* family, are known to naturally contain various anti-nutritional factors such as erucic acid, phytic acid, glucosinolates, and phenolic compounds.

CanolaPRO™ rapeseed protein isolate is derived from varieties of rapeseed low in erucic acid and glucosinolates. Furthermore, it has been demonstrated that technological processes used to manufacture rapeseed products significantly reduce the levels of anti-nutritional factors. For example, isolation of canola proteins has been shown to eliminate up to 95% of glucosinolates, 92% of phytic acid and 100% of tannic acid (Mansour et al., 1993).

Possible anti-nutritional factors originating from the canola are:

- Erucic acid
- Total phenolics
- Phytic acid
- Glucosinolates
- Protease (trypsin) inhibitors

The results of analyses of various batches of CanolaPRO™ for anti-nutritional factors are summarized in Table 8. Additional details are provided in the sections that follow.

Table 8 Concentration of anti-nutritional factors in five independent and representative batches of CanolaPRO™

Batch	Erucic acid	Total phenolics (expressed as sinapic acid)	Phytic acid	Glucosinolates	Trypsin inhibitor activity (TIA)
	%	ppm	%	µmol/g	mg/g
RPI-1536-01-G	< 0.005	605	< 0.14	< 0.1	18.5
RPI-1543-02-P	< 0.005	703	< 0.14	< 0.1	22.9
RPI-1543-03-P	< 0.005	881	< 0.14	< 0.1	25.5
RPI-1549-01-P	< 0.005	670	< 0.14	< 0.1	21.9
RPI-1549-02-P	< 0.005	600	< 0.14	< 0.1	24.4

Erucic acid

Erucic acid is a fatty acid present in the oil of cruciferous plants, including rapeseed and canola. While no negative health effects have ever been documented in humans, intake of rapeseed oil high in erucic acid has been associated with lipid and histological changes in the hearts of experimental animals (OECD, 2011). However, administration of vegetable fatty acids to rats has resulted in similar myocardial lipidosis (Neat, C.E. *et al.*, 1981), possibly due to rats having reduced ability to digest vegetable fats (containing erucic acid or not), compared to other animals (Chien, K.R. *et al.*, 1983).

The toxicity of erucic acid has been studied in short-term and sub-chronic feeding studies in animals. Most studies showed no adverse effects, despite the use of high concentrations and unnatural exposure scenarios. In one case, newborn piglets, which have limited ability to absorb these fats, received rapeseed oil in place of sow's milk as the sole source of nutrition (Food Standards Australia and New Zealand, 2003). Myocardial lipidosis occurred in the piglets very shortly after exposure began, increasing in severity in a dose-dependent manner. The severity of the lipidosis appeared to decline with time, whether or not the feeding of high erucic acid rapeseed oil continued, suggesting the liver responded by increasing enzyme levels to cope with the unusual diet. Myocardial lipidosis in animals might therefore be regarded as a short-term, reversible effect (Australia & New Zealand, 2003).

There are a number of epidemiological studies on the human consumption of oils containing high levels of erucic acid, but they do not indicate any association between erucic acid and the occurrence of heart disease (Australia & New Zealand, 2003). Nevertheless, Food Standards Australia New Zealand has defined a tolerable intake of 7.5 mg erucic acid/kg bw/day for humans (Australia& New Zealand, 2003). This tolerable intake was based on the level associated with increased myocardial lipidosis in nursling pigs.

In each Canada and the U.S., the term "canola oil" is used to describe an oil derived from the seed of the genus *Brassica*, containing less than 2% of all fatty acids as erucic acid. CanolaPRO™ is produced using a byproduct of edible rapeseed (*i.e.*, canola) oil meeting this criterion. Analyses of CanolaPRO™ showed erucic acid concentrations <0.005% (Table 8), at least 400 times below the 2% limit for canola oil. At this concentration, a person would have to consume 150 g CanolaPRO™/kg bw/day to reach the tolerable intake level of 7.5 mg erucic

acid/kg bw/day established by Food Standards Australia New Zealand. DSM's highly conservative estimates (see section D.3) indicate that CanolaPRO™ exposures would be well below this level and therefore do not represent any toxicological concern.

Total phenolics (expressed as sinapic acid)

Phenolic acids are common to all kinds of plants and are therefore present in a considerable part of the human diet. Rich sources of phenolic acids are blueberry (1881-2112 mg/kg), cherry (290-1280 mg/kg), pear (44-1270 mg/kg), apple (2-258 mg/kg), orange (21-182 mg/kg), potato (100-190 mg/kg) and coffee (56 g/kg dry weight) (CFSAN / Office of Food Additive Safety, 2010, GRN 000327). Phenolic substances are also present in soybeans (2.1-3.4 g/kg), and consequently in soy protein isolates (Tepavčević, V. *et al.*, 2010). In general, phenolic compounds are considered safe and have been investigated for their antioxidant properties. Concerns regarding their natural presence in rapeseed products is related to their potential negative impact on animal nutrition, notably for the pig and poultry industries. Phenolic acids are associated with poor palatability due to bitterness or astringency, thus affecting the feed intake of animals. In addition, they can interfere with nutrient uptake in the digestive tract.

Sinapine, the choline ester of sinapic acid, is the most abundant of all small phenolics in canola. Sinapine is converted into trimethylamine by the intestinal microflora and is then absorbed. Most animals have the ability to convert trimethylamine to trimethylamine oxide, a compound that is easily excreted. However, some animals cannot fully metabolize trimethylamine. This was notably the case for laying hens that produced eggs smelling 'fishy' or 'crabby' following consumption of canola meal. The problem was traced back to the sinapine content and to the leaching of trimethylamine into the eggs, giving them a fishy odor (Bonnardeaux, J., 2007, OECD, 2011).

Analysis of multiple batches of CanolaPRO™ (Table 8) showed total phenolics concentrations of 600-900 ppm, similar to levels found in commonly consumed foods and in other rapeseed protein isolates that were considered GRAS (notices GRN No. 327 and GRN No. 386) and are therefore not expected to be an issue.

Phytic acid

Phytic acid/phytate is the principal storage form of phosphorus in many seeds and it is ingested with many plant-derived foods. Soy protein isolate is reported to contain 1.6-2.0 % phytic acid (Honig, D. *et al.*, 1984). Lower values (0.49-0.84 %) were reported more recently (Hurrell, R.F. *et al.*, 1998). Tofu was found to contain 1.46-2.90 % phytic acid (on a dry matter basis). Phytic acid/phytate is a common component of cereals such as maize (0.72-2.22 %), wheat (0.39-1.35 %), rice (0.06-1.08%), barley (0.38-1.16%), sorghum (0.57-3.35 %), oat (0.42-1.16%), rye (0.54-1.46 %), millet (0.18-1.67 %), triticale (0.50-1.89 %) and wild rice (2.20%). Its presence has also been reported in several legumes such as kidney beans (0.61-2.3 %), broad beans (0.51-1.77 %), peas (0.22-1.22 %) dry cowpeas (0.37-2.90 %), chickpeas (0.28-1.60 %) and lentils (0.27-1.51 %). Several types of nuts contain phytic acid/phytate ranging from 0.17-9.42 % (on dry matter basis) (Schlemmer, U. *et al.*, 2009).

Phytic acid is a strong chelator of important minerals, such as calcium, zinc and iron, and could therefore contribute to mineral deficiencies by reducing their bioavailability. Phytate can also chelate the vitamin niacin (B3) which could contribute to vitamin B3 deficiency (Reddy, N.R. and Sathe, S.K., 2001). In regions of the world where unleavened bread makes up a large proportion of the diet, the phytase in wheat has been associated with zinc deficiency in humans (Jones, J., 1979).

Adverse effects have been reported in several studies with rats fed protein concentrates containing between 5 and 7.5% of the diet. Oral administration to pregnant rats was also associated with loss of appetite, wasting, apathy, reduced litter size and an increase in numbers of still-born pups (Eklund, A., 1973, Eklund, A., 1975, Jones, J., 1979). These adverse effects were attributed to chelation of zinc by phytate, resulting in zinc deficiency. Serum analyses obtained from treated rats revealed low zinc values, but normal levels of calcium, magnesium, iron and copper (Jones, 1979). Similarly, feeding rapeseed proteins containing high levels of phytate salts (1.61% of the total rat diet) to a group of female rats for two weeks before breeding was associated with significantly lower levels of zinc in maternal serum, liver, and femur, and in the pups, compared to other groups. In addition, body weights were reduced in these animals (Shah, B. *et al.*, 1979). Conversely, a group of female rats fed rapeseed proteins and supplemented with zinc did not show anorexia, and no significant differences were noted between the control and supplemented group in reproductive performance or zinc levels.

A similar experiment in male rats showed marked reductions of serum and femur zinc content in animals receiving rapeseed protein concentrates alone, compared to the control group; zinc levels were normal in animals receiving rapeseed protein concentrates along with zinc supplementation (Jones, 1979). No visible abnormalities were seen in the zinc-deficient animals, but these rats gained weight at a slower rate than those receiving zinc supplementation or than the control rats. These results suggest that male rats are less susceptible to the effects of zinc deficiency than pregnant rats.

Mejia *et al.* (2009b) did not observe any effects on plasma zinc concentrations in rats receiving a napin-rich protein isolate containing 3.34% phytate at up to 20% of the diet for 90 days.

As Table 8 shows, the level of phytate found in CanolaPRO™ was <0.14%, lower than the levels found in commonly consumed foods, and much lower than the phytate levels reported to cause adverse effects in male or female rats fed rapeseed proteins (Jones, 1979; Shah *et al.*, 1979). The available data therefore strongly suggest that the very low levels of phytate present in CanolaPRO™ are not of toxicological concern.

Glucosinolates

Glucosinolates are a class of water-soluble, sulfur- or nitrogen-containing glucosides that occur as secondary metabolites in virtually all species of *Brassica*. Glucosinolate concentrations have been reported for broccoli (47-121 mg GSL/100g), cauliflower (14-208 mg GSL/100g), cabbage (39-70 mg GSL/100g), turnip (99-230 mg GSL/100g) and radish (44-252 mg GSL/100g) (CFSAN / Office of Food Additive Safety, 2010, GRN 000327).

On their own, glucosinolates are innocuous, but when cells of the seed are ruptured, glucosinolates come in contact with the enzyme myrosinase naturally present in *Brassica* species, which hydrolyzes the glucosinolates by cleaving off the glucose group. The remaining unstable molecules are then quickly converted into a wide range of glucosinolate derivatives, including isothiocyanates, nitriles, thiocyanates and 5-vinyloxazolidine-2-thione (VOT), with the release of sulphur. Heating during the production process inactivates myrosinase, although this does not completely eliminate the effects of glucosinolates, because intestinal microflora also produces myrosinase (Tripathi, M. and Mishra, A., 2007).

The raw material used for production of CanolaPRO™ rapeseed protein isolate is canola or rapeseed bred for low glucosinolate content, *i.e.*, less than 30 µmol glucosinolates/g meal. In addition, the very low typical value of <0.1 µmol glucosinolates/g in CanolaPRO™ (see Table 8) suggests the process used for extraction of rapeseed protein reduces glucosinolate levels to an insignificant amount, consistent with the reports of Mansour *et al.* (1993).

In humans, isothiocyanates, thiocyanates and VOT are described as goitrogenic, reducing the ability of the thyroid to absorb iodine (Downey, K., 2005). Nitriles, on the other hand, can affect animal performance and can be toxic to the liver and kidneys (Tripathi & Mishra 2007). Nitriles lead to hypertrophy of the target organs, disruption of the normal lobular structure of the liver and irregular proliferation of the bile duct. They can also produce rapid kidney lesions, along with elevated plasma levels of nitrogen, urea and creatinine. Experiments performed in animals suggest that they interact with reduced glutathione, thus leading to substantial alterations in tissue glutathione levels in the liver, kidney, adrenals and lungs.

Due to their derivatives, glucosinolate in canola meal (18 to 30 µmol/g) have been shown to have antinutritional or toxic effects in animal studies. Conversely, exposure to lower glucosinolate levels has been reported to have a positive health effects (Tan *et al.* 2011) including cancer prevention (Lampe, J.W. and Peterson, S., 2002).

Feeding of rapeseed proteins containing high levels of glucosinolates to rats has been associated with anti-thyroidal effects and reduction of body weights (Tripathi & Mishra 2007). However, it has been shown that the purification of rapeseed proteins to remove glucosinolates eliminates the negative effects on the thyroid (Loew, F.M. *et al.*, 1976, Jones, J., 1979, Kroll, J. and Przybilski, H., 1991). While feeding rats 20 or 40% protein isolates containing 930 ppm glucosinolates led to slight anti-thyroid effects, purification of the proteins to 30 ppm glucosinolates abolished the adverse thyroid effects (Loew *et al.*, 1976). Studies in beagle dogs and rats fed a 20% protein diet containing 20 or 40% rapeseed protein concentrate (with 290 ppm goitrin and 900 ppm isothiocyanates) for 90 days showed no effects in dogs, but the higher concentration of rapeseed proteins led to anti-thyroid effects in rats (Jones, 1979). Repeating this study with rapeseed protein concentrates containing lower levels of residual glucosinolates (20 ppm goitrin and 30 ppm isothiocyanates) did not lead to any adverse effects in rats.

To further investigate the toxicological effects of rapeseed proteins and their components on the thyroid gland, rats were fed a diet containing 10% of one of three rapeseed protein products - industrial rapeseed meal, rapeseed protein isolate prepared from the meal by extraction, ultra- and diafiltration, or rapeseed extraction residue obtained by protein

extraction of the meal (Kroll & Przybilski, 1991). The effects of the three diets on the thyroid gland was tested with a thyroid stimulation test. While the industrial rapeseed meal containing high levels of glucosinolates led to a clear impairment of rat thyroid function, thyrotoxic effects were considerably reduced with each rapeseed protein isolate and rapeseed extraction residue. These results strongly suggest that the anti-thyroidal effects observed with rapeseed proteins can be attributed to the presence of a high level of glucosinolates.

The acceptable daily intake (ADI) of allyl isothiocyanates (AITC) derived by European Food Safety Authority (EFSA) is 20 µg/kg bw/day (EFSA panel on food additives and nutrient sources added to food (ANS), 2010). AITC levels in CanolaPRO™ were below the detection limit (< 3 ppm). Nevertheless, assuming CanolaPRO™ were to contain 3 ppm AITC (0.0003%), a person would have to consume approximately 6.67 g CanolaPRO™/kg bw/day to reach the ADI of 20 µg AITC/kg bw/day. DSM's highly conservative estimates (see section D.3) indicate that CanolaPRO™ exposures would be well below this level and therefore do not represent a toxicological concern.

Protease (trypsin) inhibitors

The *Brassica* genus of plants, like many other plants, contains protease inhibitors (Ceciliani, F. *et al.*, 1994). Consequently, CanolaPRO™ also contains a protease inhibitor. The trypsin inhibitor activity is in the range of 18-25 mg/g (Table 8). Napin is the primary source of protease inhibitors in canola (Puumalainen, T.J. *et al.*, 2006).

The presence of protease inhibitors in plants has been known for almost 100 years "Ever since Osborne and Mendel (1917) observed that soybeans would not support the growth of rats unless the beans were cooked for 3 h on a steam bath..." (Rackis, J.J. and Gumbmann, M.R., 1981). Soybean based products have been the primary concern as a source of protease inhibitors, but anti-nutritional compounds are also present in other legumes (Kadam, S.S. and Smithard, R.R., 1987, Carvalho, M.R.B. and Sgarbieri, V.C., 1997), wheat and potatoes (Habib, H. and Fazili, K.M., 2007). As was indicated by Osborne and Mendel, heat processing tends to inactivate inhibitors of protein digestion. In a market survey, Doell *et al.* (1982) reported that raw soy beans contained 49.6 mg trypsin inhibitor per gram of protein, but raw and cooked tofu contained 9.2 and 5.5 mg/g respectively. Based on analysis of Ultra High Temperature (UHT) processed soy milk using standard no commercial time/temperature programs, Yuan *et al.* (2008) reported that, in general, higher temperature and longer time resulted in lower residual trypsin inhibitor; concentrations ranged from a low of 10.9% for an experimental processing program to a high of 37.7% for one of the commercial processes. As an example, that even the most sensitive population is already exposed to these inhibitors, a survey of commercial soy-based infant formula in Canada revealed a trypsin inhibitor concentration ranging from 0.75 to 1.59 mg/g protein in ready-to-eat liquid formula and from 0.34 to 0.91 mg/g in powder formula (Xiao, C.W. *et al.*, 2012).

Many of the proposed food categories in which CanolaPRO™ is anticipated to be utilized are heat processed to varying degrees which will lead to some level of degradation of the protease inhibitor. But even if no degradation were to occur, the level of protease inhibitor would not be above 25.5 mg/g protein. This concentration is not unusual and is in line with

other food products such as cow's milk, cabbage and tofu (Doell et al., 1982) or soy milk, (Xiao et al., 2012). Since heat processing decreases the presence of protease inhibitors, the concentration in products containing CanolaPRO™ will be very low and not likely to be a concern.

C.2 Plant or animal extracts

C.2.1 Information on the method of extraction and the composition of the concentrated extract

The manufacturing process of the rapeseed protein, including the extraction process, is outlined in detail in Section B.5. The extraction process involves an aqueous extraction with a salt (NaCl) solution. This mild extraction process maintains the high quality functional properties of the canola protein. Apart from the potential contaminants discussed in Section B.4, no potential contaminants in the rapeseed protein originate specifically from the extraction process. The NaCl from the extraction liquid is washed out during diafiltration till typical levels around 0.15% w/w.

C.2.2 Information on the use of this plant or animal extract as a food in other countries

Please refer to Section C.1.3 for information on the current use of rapeseed protein as a food ingredient in other countries.

C.2.3 Information on the toxicity of the extract obtained from studies conducted in animals or humans

Rapeseed is a potential protein source for humans and has been the source of many safety studies. The first records of safety studies with rapeseed were from livestock animals exposed to rapeseed press cake. Appendix 6 provides an overview of studies performed in various animals using rapeseed meals and protein concentrates in an ascending chronological order. From this overview of studies, it appears that several toxicity endpoints have been identified. Some studies reported thyrotoxic effects in laying hens (Jackson, N., 1970) and rats (Kroll & Przybilski, 1991). Several studies have linked exposure to rapeseed protein concentrates with reduced weight gain in pregnant rats and reduced litter size (Eklund, 1973; Eklund, 1975; Shah *et al.*, 1979; Jones, 1979). Slightly lower body weights were reported for non-pregnant rats fed rapeseed proteins (Shah *et al.*, 1980, Mejia *et al.*, 2009b) and in rainbow trout (Teskeredžić, Z. *et al.*, 1995). Finally, changes in biochemical parameters and organ characteristics, notably in liver and kidneys, have been observed on several occasions in rats fed rapeseed protein meal (Thompson, L. *et al.*, 1982, Garg, S. *et al.*, 1982) and rapeseed proteins (Plass, R. *et al.*, 1992).

The analytical composition of the different rapeseed protein products and flours tested in these studies was not always available. Therefore, it was not possible to assess the purity of the protein preparations tested, or to determine if contaminants played a role in the adverse effects noted. However, the main adverse effects reported in these studies were most likely

related to the anti-nutritional factors discussed previously (glucosinolate derivatives and phytic acids) present in rapeseed meal and concentrates, rather than to the proteins themselves when isolated.

Administration of rapeseed protein meal/concentrate containing phytic acid to pregnant rats has been linked to loss of appetite, wasting, apathy, reduced litter size and an increase in numbers of still-born pups (Eklund, 1973; Eklund, 1975; Jones, 1979). These adverse effects were probably caused by zinc chelation by phytate, leading to zinc deficiency in these animals. Pregnant rats fed rapeseed proteins and supplemented with zinc did not show any anorexia or reproductive toxicity (Shah *et al.*, 1979). On the other hand, the slightly lower body weights and lower food consumption observed in non-pregnant rats fed napin-rich canola isolates containing 0.32% phytic acid, as described by Mejia *et al.* (2009b), was shown to be due to low palatability.

Glucosinolates and glucosinolate derivatives present in rapeseed protein preparations have been linked to anti-thyroidal effects and lower body weights in animals (Tripathi & Mishra, 2007), while the purification of rapeseed proteins to remove glucosinolates has been shown to abolish the negative effects on the thyroid (Loew *et al.*, 1976; Jones, 1979; Kroll & Przybilski, 1991).

Several glucosinolate derivatives have been linked to toxic effects on the liver and kidneys of treated animals. This is notably the case with nitriles. The mechanism that underlies their toxicity seems to be their ability to interact with reduced glutathione, thus leading to substantial alterations in tissue glutathione levels, as observed in the liver, kidneys, adrenals and lungs of rats after chronic ingestion or a single injection of acrylonitrile (Nugon-Baudon, L. and Rabot, S., 1994).

The toxic effect of nitriles manifests itself as hypertrophy of the target organs, disruption of the normal lobular structure of the liver and irregular proliferation of the bile duct. Nitriles have also been associated with enlarged nuclei of the epithelial cells lining the convoluted tubules of the kidneys, as well as rapid production of kidney lesions, along with elevated plasma levels of nitrogen, urea and creatinine, which could suggest functional alterations of the kidneys (Nugon-Baudon & Rabot, 1994). Another glucosinolate derivative, progoitrin, has also been shown to induce enlargement of the liver and kidneys in experimental animals, aside from effects on the thyroid (Nugon-Baudon & Rabot, 1994). Therefore, the presence of glucosinolates and glucosinolate derivatives most likely explains the adverse effects observed in animals fed rapeseed protein meal (Thompson *et al.*, 1982; Garg *et al.*, 1982). It is difficult to conclude on the slight absolute liver weight changes and reduced relative kidney weight observed in rats fed rapeseed protein isolates and rapeseed extraction residue (Plass *et al.*, 1992). In this case, the concentration of glucosinolate derivatives (progoitrin, VOT, butenyl, pentenyl and phenyl-ethyl isothiocyanate) in the rapeseed products were reportedly very low, but the nitrile content was not measured. The glucosinolate concentration of CanolaPRO™ is below that of previously notified rapeseed protein isolates (Table 8).

The safety of rapeseed protein isolates has been shown in 13-week toxicity studies in rats fed either a cruciferin-rich protein isolate (Puratein® from ADM/Burcon) or a napin-rich protein isolate (Supertein™ from ADM/Burcon) with low content of anti-nutritional factors (Mejia, L.A.

et al., 2009a and 2009b). These published and peer-reviewed safety studies can be used to bridge the toxicological assessment of DSM CanolaPRO™ rapeseed protein isolate and to support its safety. CanolaPRO™ contains the same major rapeseed storage proteins, cruciferin and napin, as the protein isolates used by Mejia *et al.* The composition of DSM and ADM/Burcon products is comparable, with protein contents of at least 90%, and levels of moisture, ash, carbohydrates, fats and fibre in the same range for these products. DSM and ADM/Burcon amino acid profiles are also comparable, as well as the levels of potentially toxic anti-nutritional factors (see data in Appendix 7), with phytase levels being even lower in CanolaPRO™. The 13-week toxicity studies reported by Mejia *et al.* (2009a and 2009b) are described further below.

EFSA accepted that CanolaPRO™ is substantial equivalent to products covered by these Mejia studies (see appendix 10).

Both ADM/Burcon studies were conducted according to FDA Redbook guidelines. In each 13-week study, Sprague-Dawley rats (20/sex/group) were fed *ad libitum* with an AIN-93G-based protein-free diet supplemented with 5%, 10% or 20% rapeseed protein isolate. It was reported that the control group received 20% vitamin-free casein as control (Mejia *et al.*, 2009a and 2009b).

Rats were observed for mortality, clinical signs, physical abnormalities, eye abnormalities, changes in body weights and food consumption. Functional observational battery and locomotor activity tests, and clinical pathology investigations (haematology, coagulation, clinical chemistry and urinalysis), were also performed in subsets of animals (10/sex/group). All rats were subjected to detailed necropsy at terminal sacrifice, and specified organs were weighed. Histopathological examination was carried out on the preserved organs and tissues of the 20% rapeseed protein isolate and control groups, and on gross lesions from all rats in the study.

No treatment-related changes were observed in either study in the functional observation tests, haematology, clinical chemistry or urinalysis, or gross and histopathological examination. No effects on body weight gains and food consumption were observed in rats receiving up to 20% cruciferin-rich protein isolates (Mejia *et al.*, 2009a). A slightly greater thyroid/parathyroid ratio was observed in the 20% cruciferin-rich protein isolate group. However, there were no correlated histopathologic changes, and the values of the ratios in all groups were within the laboratory's historical normal control range. In addition, statistical significance was not consistent between absolute and relative values in males *vs.* females, suggesting a random outcome. Therefore, this observation was considered of no toxicological relevance and was not considered an adverse effect (Mejia *et al.*, 2009a).

Taking into account these observations, the 10% level of dietary supplementation was considered a no-observable-effect level (NOEL), and the 20% level was considered a no-observable-adverse-effect level (NOAEL). The NOAEL level of cruciferin corresponded to 11.24 g/kg bw/day for males and 14.11 g/kg bw/day for females (Mejia *et al.*, 2009a).

Rats receiving 10% (males only) and 20% of the ADM napin-rich protein isolates in the diet had lower body weights and body weight gains. Lower food consumption was observed in males at

all levels of protein isolates, and in females at 10% and 20% (Mejia *et al.*, 2009b). The authors attributed the lower body weights, body weight gains, and food consumption to low palatability of the napin-rich protein isolates. Based on these observations, the NOEL for dietary administration of the napin-rich protein isolate was considered to be 5%, and the NOAEL 20%, the latter equivalent to 12.46 g/kg bw/day for males and 14.95 g/kg bw/day for females (Mejia *et al.*, 2009b).

C.2.4 Safety assessment reports prepared by international agencies or other national government agencies

The EU authorized the use of rapeseed protein isolate as a novel food ingredient in 2014 (European Commission, EU/424/2014). In March 2017, DSM's CanolaPRO™ rapeseed protein isolate was approved in the EU as being substantially equivalent to the rapeseed protein isolate in EU/424/2014.

The Scientific Opinion of the European Food Safety Authority on "rapeseed protein" as novel food ingredient and the Substantial Equivalence Opinion by the Food Safety Authority of Ireland (FSAI) can be found in Appendix 9 and Appendix 10.

DSM notified US FDA of the GRAS status of their rapeseed protein (GRN 000683) and received a No Questions letter from the FDA Center for Food Safety and Nutrition in the USA (US FDA, 2017). This letter can be found in Appendix 11.
Rapeseed protein isolate produced by other manufacturers, was the subject of two prior GRAS Notices, GRN000327 in 2010 and GRN000386 in 2011. Both Notices received no questions letters.

C.4 Single chemical entities and Dietary macro-components

C.4.1 Information on the toxicokinetics and metabolism of the single chemical entity and, where appropriate, its degradation products and major metabolites

Proteins are an essential part of the daily human diet as an integral part of many food products. As with other proteins, the rapeseed proteins cruciferin and napin will be digested through normal metabolic processes.

After ingestion, proteins are hydrolyzed in the gastrointestinal tract by proteolytic enzymes derived from the pancreas, resulting in the release of dipeptides, tripeptides, and free amino acids (Grimble, G.K., 1994). Carrier systems specific for the transport of either the amino acids or the di- and tripeptides are responsible for the efficient transport across the intestine wall. The amino acids of rapeseed protein isolate are relatively well absorbed (Galibois, I. *et al.*, 1989, Fleddermann, M. *et al.*, 2013) and utilized (Bos, C. *et al.*, 2007). The amino acids resulting from the digestion of foods are used as building blocks for formation and maintenance of body proteins.

DSM performed a rat study, which showed that the digestibility in the gastrointestinal tract was 94% for native CanolaPRO™ rapeseed (canola) protein isolate and 97.2% when heat-

treated. The study report is provided in Appendix 12. Due to the good amino acids profile, the Protein Digestibility Corrected Amino Acid (PDCAAS) in this study was 100%.

A human study investigating rapeseed protein found an ileal digestibility of 87% (Deglaire, A. *et al.*, 2009).

CanolaPRO™ contains relatively high levels of all indispensable amino acids (see Table 3). Based on a scoring pattern for a child between 0.5 and 3 years old, the Digestible Indispensable Amino Acid Score (DIAAS) of CanolaPRO™ was calculated to be 95%, which is comparable to the estimated DIAAS for soy protein isolate (FAO, 2013). A protein with a DIAAS of 75-99% is considered a good source of protein, according to FAO recommendations (FAO, 2013).

C.4.2 Information from studies in animals or humans that is relevant to the toxicity of the single chemical entity and, where appropriate, its degradation products and major metabolites

This information is given in Section C.2.3 of the NOVEL FOODS part of this dossier.

C.4.3 Safety assessment reports prepared by international agencies or other national government agencies

This information is given in Section C.2.4 of the NOVEL FOODS part of this dossier.

C.6 Food ingredients derived from a new source

C.6.1 Information on the safety of the source organism

C.6.1.A History of oilseed rape in the human diet

Oilseed rape species are derived from the *Brassica* genus of the *Brassicaceae* or *Cruciferae* family, also known as the mustard or cabbage family. *Brassica* species are one of the most widely cultivated species of plants used for human food. As sources of common vegetables in the diet, *Brassica* species, such as broccoli, cabbage, cauliflower, radish, and turnip, have been in use for centuries. Some of them are now recognized as having desirable health benefits.

Several species of the *Brassicaceae* or *Cruciferae* family have become important agricultural crops around the world. The seeds of these Crucifers are rich in oil and contain considerable amounts of protein that accounts for 20 to 35% of the seed dry weight. The predominant storage proteins of these Crucifers are cruciferin (11 or 12S) and napin. These proteins are expressed during seed development as precursors, and undergo co- and post-translational modifications, before being transported to membranous organelles (protein bodies), where they accumulate in large quantities and become a considerable fraction of seed biomass. The structural protein oleosin is associated with the oil fraction.

Among the *Brassicaceae*, rapeseed (*Brassica napus* and *Brassica rapa*, formerly *Brassica campestris*, also known as turnip rape or sarson), oriental and brown mustard (*Brassica juncea*, also known as Indian mustard), black mustard (*Brassica nigra*), and yellow mustard (*Sinapis alba* syn. *Brassica hirta*, also known as white mustard) are important in the global oilseed economy (see USDA, 2016 for taxonomic information).

Oilseed rape was first cultivated in India about 4,000 years ago. It was then introduced to China about 2,000 years later. The large-scale production of oilseed rape was first reported in Europe in the 13th century, but its consumption in the Western world is more recent and was first limited to the use of canola meal in the livestock industry and use of the oil for cooking and salads. Interest in rapeseed breeding intensified in Canada soon after the crop was introduced from Europe in the 1940s. The first efforts were concentrated on improving the agronomic characteristics and the oil content. Studies conducted in the late 1940s correlated a high consumption of rapeseed oil containing large amounts of erucic acid with heart lesions in experimental animals. These studies stimulated plant breeders to develop rapeseed varieties low in erucic acid. In the late 1970s, the name canola (**Can**adian **o**il, low **a**cid) was adopted in North America to distinguish the plant low in erucic acid from other types of rapeseed. Rapeseed varieties low in glucosinolates were developed later, notably for livestock consumption, when it appeared that glucosinolates contained in rapeseed had toxic effects on the animals and were responsible for the bitter taste of the rapeseed meal. Further breeding programs led to the development of varieties low in both erucic acid and glucosinolates. The term canola has since then been adopted to designate a cultivar of *Brassica napus*, *Brassica juncea* or *Brassica rapa*. Canola must contain less than 2% erucic acid in the oil and less than 30 µmol/g glucosinolates in the air-dried, oil-free meal. Current technological processes used to manufacture rapeseed products are known to further eliminate significant amounts of anti-nutritional factors.

Rapeseed oil (*i.e.*, the edible oil obtained from *Brassica napus* or *Brassica campestris*, low in erucic acid) was affirmed generally recognized as safe (GRAS) by U.S. FDA[17] in 1985. This GRAS status was then extended to canola oil from *Brassica juncea*.

C.6.1.B Allergenicity, hypersensitivity and immune response

At the request of DSM, the Food Allergy Research and Resource Program (FARRP) at the University of Nebraska reviewed data it had accumulated regarding allergenicity to rapeseed proteins. FARRP re-evaluated five rapeseed protein sequences DSM provided, using AllergenOnline.org version 16, released January 2016, with full-length FASTA and sliding 80mer FASTA (Goodman, R.E. *et al.*, 2016). FARRP also ran a comparison with the NCBI-Protein database using BLASTP with default values, and keyword "allergen," as well as no keyword, for confirmation.

[17] See 21 CFR 184.1555 at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1555.

Sequences were also run against the Celiac Database to evaluate potential matches of peptides and proteins using the Celiac Database (linked from AllergenOnline.org) with both exact peptide match and FASTA *vs.* the gluten/gliadin dataset. Below are the results of the evaluation. The complete report is in Appendix 13.

1. CRU1_BRANA.

MARLSSLLSFSLALLIFLHGSTAQQFPNECQLDQLNALEPSHVLKAEAGRIEVWDHHAP
QLRCSGVSFVRYIIESKGL YLPSFFSTAKLSFVAKGEGLMGRWPGCAETFQDSSVFQP
SGGSPSGEGQGQGQQGQGQGHQGQGQGQQGQQGQQGQQSQGQGFRDMHQKVE
HI RTGDTIATHPGVAQWFYNDGNQPLVIVSVLDLASHQNQLDRNPR PFYLAGNNPQGQ
VWIEGREQQPQKNILNGFTPEVLAKAFKI DVRTAQQLQNQQDN RGNIIRVQGPFSVI RPP
LRSQRPQETEVNGLEETICSARCTDNLDDPSNADVYKPQLGYISTLNSYDLPILRFLRLS
ALRGSIRQNAMVLPQWNANANAVL YVTDGEAHVQVVNDNGDRVFDGQVSQGQLLSIP
QGFSWKRATSEQFRWIEFKTNANAQINTLAGRTSVLRGLPLEVISNGYQISLEEARRVK
FNTIETTL THSSGPASYGGPRKADA

Allergen matches AllergenOnline.org FASTA: The sequence has relatively high (58%, E score 4×10^{-12} or 10^{-44}) identity match over essentially the full 500 AA length to two 11S globulin proteins in mustard, *Sinapis alba* white mustard (sometimes called yellow mustard), an annual mustard grown for seeds. In addition, the highest identity match in an 80 AA window was to the 11S globulins of this mustard. The 11S globulin is an allergen with ~41% to 45% sequence identity to some tree nut and seed 11S allergens (Cashew, walnut, hazelnut, pecan, almond, sesame, pistachio and Brazil nut). The only study clearly directed at potential cross-reactivity of 11S albumins of mustard, tree nuts and peanuts was by Sirvent *et al.* (2012), which showed some correlation by *in vitro* IgE testing to the Sin a 2, 11S globulin of mustard, but lower than IgE to 2S albumin (Sin a 1), and showed *in vivo* skin test co-reactivity to extracts of seeds of mustard and almond, hazelnut, pistachio, walnut and peanut, which was individually variable between subjects and nuts/seeds, but more pronounced in subjects with clinical reactivity to white mustard 11S and one or more nuts in a small number of subjects. There was not conclusive proof that the SPT reactivity was all, or even mostly, due to IgE binding to the 11S globulins.

Other studies have shown the 2S albumins and possibly Lipid Transfer proteins (LTP) are likely more important in clinical reactivity (Asero, R., 2014, Asero, R., 2011, Robotham, J.M. *et al.*, 2005, Pastorello, E.A. *et al.*, 2001). The amino acid sequence of the CRU1_BRANA was around 43-45% identical to 11S globulin sequences of the tree nut allergens that were matched by Sin a 2. The identity matches for 80 amino acid windows were 50-63% Identical. That is as expected, since some regions of large proteins are more likely conserved.

Due to the simple, repetitive sequences of some AA in the 11S sequence, there were low to modest identity matches to some gluten and gliadin proteins with 20-40% identity, especially over shorter segments. The highest 80mer sequences were higher than full length, which is a common finding. The E scores were markedly lower than for matches of the *Brassica* 11S.

Celiac Database evaluation: The moderate matches to wheat (*Triticum sp.*) raises the question of whether the similarities are sufficient to elicit symptoms of celiac disease (CD).

FARRP compared the five sequences to their Celiac Database of CD peptides using an exact match protocol that identifies query proteins that contain an exact match in any one CD peptide. The peptides were also tested using FASTA to the 68 representative gluten and gliadin proteins. The 11S globulin had low levels of identity and large E scores that are far from being at the levels of Identity that would be of concern (see database).

Confirmation of bioinformatics using NCBI with BLASTP. Searches of the NCBI Protein database with keyword limit of "allergen" gave similar results to the FASTA searches with AllergenOnline. The highest matches were to 11S globulin of *Sinapis alba*, and similar identity matches to tree nut, peanut and seed storage 11S globulins. When searched without any keyword the matches were very high in identity, with very small E scores to a wide variety of mustard and mustard relatives.

Searches of other 11S *Brassica* seed storage proteins.

2. CRU2_BRANA.

MARLSSLL YFSITVLIFLHGSTAQQFPNECQLDQLNALEPSHVLKAEAGRIEVWDHHAPQ
LRCSGVSFVRYIIESQGL YLPSFLNTANVSFVAKGQGLMGRWPGCAETFQDSSVFQPG
SGSPFGEGQGQGQQGQGQGQGQGQGQGKGQQGQGKGQQGQSQGQQGQGQGFRDM
HQKVEHI RSG DTIATHPGVAQWFYNNGNQPLVIVAVMDLASHQNQLDRNPSQFYLAGK
NPQGQSWLHGRGQQPQNNI LNGFSPEVLAQAFKI DVRTAQQLQNQQDNRGNIVRVQG
PFGVIRPPLKSQRPQETEANGLEETICSARCTDNLDDPSNADVYKPQLGYISILNSYOLPI
LRVLRLSALRGSIRQNAMVLPQWKSKSNAVL YVTDGEAQIQWNDNGDRVFDGQVSQG
QLLSIPQGFSWKRATSDQFRWI EFKTNANAQINTLAGRTSVMRGLPLEVIANGYQISLE
EARRVKFNTIETTL THSSGPASYGRPRKADA

The FASTA sequence comparison data results were very similar for CRU1 and CRU2 proteins, as were CD (no peptide matches and low identity matches) and to NCBI.

3. CRU3_BRANA

MVKVPHLLVATFGVLLVLNGCLARQSLGVPPQLGNACNLONLDVLQPTETIKSEAGRVE
YWDHNNPQIRCAGVSVSRVIIEQGGL YLPTFFSSPKISYVVQGMGISGRVVPGCAETFM
DSQPMQGQQQGQPWQGQQGQQGQQGQQGQQGQQGQQGQQGQQGQQGQQGQQ
QQGFRDMHQKVEHVRHGOIIAITAGSSHWIYNTGDQPLVIICLLDIANYQNQLDRNPRTF
RL.AGNNPQGGSQQQQQQQQNMLSGFOPQVLAQALKIDVRL.AQELQNQQDSRGNIVRV
KGPFQWRPPLRQPYESEQWRHPRGPPQSPQDNGLEETICSMRTHENIDDPARADVY
KPNLGRVTSVNSYTLPILQYIRLSATRGILQGNAMVLPKYNMNANEIL YCTQGQARIQW
NDNGQNVLDQQVQKGQLWIPQGFAYWQSHQNNFEWISFKTNANAMVSTLAGRTSAL
RALPLEVITNAFQISLEEARRIKFNTLETTL TRARGGQPQLIEEIVEA

The FASTA matches to *Sinapis alba* 11S albumins were much more significant (90% identity and E scores of 2 e-122) for CRU3. However, the sequence matches to tree nuts, peanuts and seed storage proteins were similarly low (~44% identity) as for CRU1. The CRU3 is evidently a closer homologue of *Sinapis alba* than CRU1 and CRU2. Again, there were no matches to CD peptides and very poor scoring Identities by FASTA.

4. CRU4_BRANA

MGPTSLLSFFFTFL TLFHGFTAQQWPNECQLDQLNALEPSQIIKSEGGRIEVWDHHAPQ
LRCSGFAFERFVJEPQGLYLPTFLNAGKLTFVVHGHALMGKVTPGCAETFNDSPVFGQG
QGQEQGQGQGQGQGQGFRDMHQKVEHlRSGDTfATPPGVAQWFYNNGNEPLILVAA
ADIANNLNQLDRNLRPFLLAGNNPQGQQWLQGRQQQKQNNIFNGFAPQILAQAFKISVE
TAQKLQNQQVNRGNIVKVQGQFGVIRPPLRQGQGGQQPQEEGNGLEETLCTMRCTEN
LDDPSSADVYKPSLGYISTLNSYNLPILRFLRLSALRGSIHNNAMVLPQWNVNANAAL YV
TKGKAHIQNVNDNGQRVFDQEISKGQLLWPQGFAWKRATSQQFQWIEFKSNDNAQI
NTLAGRTSVMRGLPLEVISNGYQISPQEARSVKFSTLETTL TQSSGPMGYGMPRVEA

The results with CRU4 were very similar to those with CRU1 and CRU2, no identity matches to CD peptide and poor alignments to glutens.

5. Gl:461840

MVKVPHLLVA TFGVLLVLNG CLARQSLGVP PQLGNACNLD NLDVLQPTET
IKSEAGRVEY WOHNNPQtRC AGVSVSRVII EQGGL YLPTF FSSPKISYVV
QGMGISGRW PGCAETFMOS QPMQGQQQGQ PWQGQQGQQG QQGQQGQQGQ
QGQQGQQGQQ GQQGQQGQQQ QGFRDMHQKV EHVRHGOIIA ITAGSSHWIY
NTGDQPLVII CLLDIANYQN QLDRNPRTFR LAGNNPQGGS QQQQQQQQNM
LSGFDPQVLA QALKIDVRLA QELQNQQDSR GNIVRVKGPF QWRPPLRQP
YESEQWRHPR GPPQSPQDNG LEETICSMRT HENIDDPARA DVYKPNLGRV
TSVNSYTLPI WIPQGFAYV VQSHQNNFEW ISFKTNANAM VSTLAGRTSA
LRALPLEVIT NAFQISLEEA RRIKFNTLET TLTRARGGQP QLIEEIVEAE QUENCE

The results with Gl:461840 were between identity matches for CRU3 and CRU1 for comparison to *Sinapis alba*. The identities to tree nuts and 11S proteins of seeds and peanut was 30-44%. There were no matches to CD peptides, and low identity scores to glutens.

Evaluation of allergenicity: The prospect of allergic cross-reactivity between 11S albumins of *Brassica sp.* and *Sinapis alba* is considerably higher than to tree nut and seed storage proteins. Species in these genera are within the mustard tribe *Brassiceae* of the family *Brassicaceae*.

Clearly, these species are genetically closely related, and the high sequence identities demonstrate conservation. As noted by many researchers, including Aalberse (2000), proteins sharing greater than 70% identity are highly likely to be cross-reactive, whereas those sharing less than 50% identity (overall) are not likely to share IgE cross-reactivity.

CanolaPRO™ rapeseed (canola) protein isolate had high sequence identity to known mustard proteins that are considered as allergens in parts of the world, such as EU, but not to any food allergens recognized in the Regulations in AUS/NZ and USA. Therefore, CanolaPRO™ is not expected to be allergenic, except possibly in individuals allergic to mustard protein. To address this issue, DSM will alert its customers (*i.e.*, facilities receiving CanolaPRO™ material for further industrial processing) through documentation (*e.g.*, Product Data Sheet) that

CanolaPRO™ may cause allergic reactions in individuals allergic to mustard. This will enable food manufacturers using CanolaPRO™ to adequately label the finished food product.

C.6.2 Information on the composition of the novel food ingredient derived from a new source

CanolaPRO™ rapeseed protein predominantly consists of protein. It contains minor levels of carbohydrates and fat. Typical levels are given in Table 7 in Section C.1.1 of the NOVEL FOODS part of this dossier.

C.6.3 Information on the toxicity of the novel food ingredient derived from the new source

This information is given in Section C.2.3 and C.4.2 of the NOVEL FOODS part of this dossier.

C.6.4 Safety assessment reports prepared by international agencies or other national government agencies

This information is given in Section C.2.4 of the NOVEL FOODS part of this dossier.

D Information on dietary exposure to the novel food

D.1 A list of the foods or food groups proposed to or which might contain the novel food ingredient or substance

DSM intends to market CanolaPRO™ rapeseed (canola) protein isolate to food product manufacturers as a direct protein replacement of animal- or vegetable-based protein such as soy, whey, and pea protein. CanolaPRO™ has broad functionality and can be used in a wide range of food applications, as a protein source, thickener, water binder, emulsifier, gelling agent, foaming agent, or texturizer.

CanolaPRO™ will be used in a variety of food products for the general population. Examples of the intended foods and the proposed maximum levels of use are provided in Table 9. Due to potential unpalatability (bitterness) and/or technological limitations associated with its water-binding capacity, use levels are not expected to exceed 30% in any one food.

Table 9 Proposed use of CanolaPRO™ rapeseed (canola) protein isolate in foods

Food Category	Examples	Proposed maximum amount in final food
Bakery products	Referencing to Standard 2.1.1.Cereal and Cereal Products Breads and rolls; doughnuts; cookies; crackers; cakes; pies; batters; muffins; cereal and granola bars; breakfast cereals	5%
Beverages	Referencing to Standard 2.6.1 Fruit juices and juice blends; Standard 2.6.2. soft drinks; Standard 2.9.4 energy drinks	5%
Dairy products	Referencing to Standard 2.5.1 - 2.5.6 Yogurt; cheese and cheese products; desserts and dessert toppings; ice pops and sorbets	5%
Other foods	Prepared food such as ready-to-eat meals, soups, and pasta; snacks	10%
	Meat analogues	30%
	Protein-enriched, bars, and pasta; protein-enriched powder and ready-to-drink beverages; energy bars	30%

D.2 The proposed level of the novel food ingredient or substance for each food or food group

Please refer to section D.1.

D.3 For foods or food groups not currently listed in the most recent Australian or New Zealand (NNSs), information on the likely level of consumption

To estimate CanolaPRO™ intakes, DSM used the dietary protein intake values reported by the Australian Bureau of Statistic in 2015 (Australian Bureau of Statistics, 2015) and the University of Otago and Ministry of Health in 2011 for New Zealand (University of Otago and Ministry of Health, 2011).

In its assessment of consumer exposure, DSM considered that CanolaPRO™ rapeseed (canola) protein isolate is intended for use as a direct protein replacement of animal- or vegetable-based protein currently in food; its use is therefore not expected to have a significant impact on the overall protein intake of the Australian and New Zealand population.

DSM also considered that intrinsic protein from poultry, beef, cheese, milk, *etc.* will continue to be the principal source of dietary protein for the foreseeable future.

The New Zealand Adult Nutrition Survey describes the food sources contributing to protein in the diet (University of Otago and Ministry of Health, 2011). The Bread group was the single largest contributor of protein to the diet (11.1%), followed by Poultry and Milk (each 8.8%), Beef and veal (7.8%), Grains and pasta (6.8%), Bread-based dishes (6.6%), Fish and seafood (6.0%), Pork (4.5%), Vegetables (4.3%), Sausages and processed meats (3.1%), Cheese (3.1%), Eggs and egg dishes (2.9%), Breakfast cereals (2.8%), Lamb and mutton (2.0%), dairy products (2.0%), Nuts and seeds (1.2%), and Other meat (0.5%). Assuming that rapeseed protein cannot replace proteins for meat, fish, milk, eggs, vegetables and nuts, there is a total of (poultry 8.8%, milk 8.8%, beef and veal 7.8%, fish and seafood 6.0%, pork 4.5%, vegetables 4.3%, sausages and processed meats 3.1%. cheese 3.1%, eggs and egg dishes 2.9%, lamb and mutton 2.0%, nuts and seeds 1.2%, and other meat 0.5% =) 53.0% of protein that cannot be replaced. The remaining 47% was from a variety of other foods, only a subset of which would be considered candidates for CanolaPRO™, specifically, bread (11.1%), grains and pasta (6.8%), and dairy products (2.0%). Although highly unlikely that all proteins in bread, grains, and dairy products will be replaced, these are applications where rapeseed protein could be added.

Based on the Australian Health Survey 2011-12, Foods and Nutrients[18] the major food sources of protein include: Meat, poultry and game products and dishes (34.4%), Cereal-based products and dishes (16.6%), Cereals and cereal products (14.1%), Milk products and dishes (11.8%), Fish and seafood products and dishes (5.4%), Vegetable products and dishes (4.5%), Non-alcoholic beverages (2.4%), Egg products and dishes (2.0%), Special dietary foods (1.5%), Soup (1.2%), Fruit products and dishes (1.1%), and Seed and nut products and dishes (1.1%).

Assuming that rapeseed protein cannot replace proteins for meat, fish, milk, eggs, vegetables, fruits, and nuts, there is a total of (Meat, poultry and game products and dishes 34.4%, Milk products and dishes 11.8%, Fish and seafood products and dishes 5.4%, Vegetable products and dishes 4.5%, Egg products and dishes 2.0%, Fruit products and dishes 1.1%, and Seed and nut products and dishes 1.1% =) 60.3% of protein that cannot be replaced. The remaining 39.7% was from a variety of other foods, only a subset of which would be considered potential candidates for CanolaPRO™, although it is highly unlikely that all protein in these applications will be replaced.

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[18] http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/by%20Subject/4364.0.55.007~2011-12~Main%20Features~Protein~706

CanolaPRO™ rapeseed (canola) protein isolate is not expected to have 100% of the market share for protein isolates or concentrates. However, to account for the limited information regarding current consumer exposure to other protein isolates and concentrates (which would provide insight into potential exposures to CanolaPRO™), DSM employed a worst-case approach that assumed CanolaPRO™ would be the only source of protein in the diet other than the 53% for New-Zealand and 60.3% for Australia intrinsic protein from meat, fish, milk, *etc.* A value of 47% for New-Zealand and 39.7% for Australia was therefore considered the highest possible contribution that CanolaPRO™ could make to the overall dietary protein intake. In reality, it is highly unlikely that CanolaPRO™ would represent 47% or 39.7% of all dietary protein consumed, especially since it will have to compete in the marketplace with other protein isolates and concentrates currently used in food.

To estimate CanolaPRO™ intakes, DSM used the dietary protein intake values reported by the Australian Bureau of Statistic in 2015 and the University of Otago and Ministry of Health from 2011. In New Zealand the highest protein intake is found in males between 19 and 30 years old and also for males 31-50 years old. The median usual daily protein intake in New Zealand was 111 g/day for males, with the 90th percentile 142 g/day. Assuming a default body weight of 70 kilogram, this results in a protein intake of 2.0 g/kg bw/day. Assuming that CanolaPRO™ represents a worst-case scenario of 47% of all dietary protein consumed, this would mean that maximally 0.95 g/kg bw/day is consumed.

The highest protein intake in Australia is also found in males between 19 and 30 years of age, who have a mean intake of 113 g protein/day and a 90th percentile intake of 143 g protein/day. Table 10 includes the average body weights and 90th percentile protein consumption in different age categories. It is assumed that CanolaPRO™ would represent maximally 39.7% of all dietary protein consumed.

Table 10. Total protein consumption and CanolaPRO™ consumption in males based on the **Australian Bureau of Statistic in 2015**

Age group (years)	Average body weight (kg)[19]	90th percentile protein consumption (g/day)	90th percentile protein consumption (g/kg bw/day)	90th percentile CanolaPRO™ consumption (g/kg bw/day)
2-3	15.8	76	4.8	1.9
4-8	24.3	82	3.4	1.3
9-13	44.0	114	2.6	1.0
14-18	65.1*	133	2.0	0.8
19-30	**79.9**	**143**	**1.8**	**0.7**

* Mean weight for boys 14-16 years of age.

[19] Mean body weight for male children (2-16) were obtained from the 2007 Australian National Children's Nutrition and Physical Activity survey. ISBN: 1-74186-756-8
Mean body weight for male adults (18-24 years) was obtained from the Australian Health Survey: First Results, 2011-12 (released in 2012). Adults > 24 years have a higher body weight.

The animal studies performed on rapeseed protein isolates can be used to assess the safety of CanolaPRO™. The NOAEL level of cruciferin corresponded to 11.24 g/kg bw/day for males and 14.11 g/kg bw/day for females (Mejia *et al.*, 2009a). The NOAEL level of napin corresponded to 12.46 g/kg bw/day for males and 14.95 g/kg bw/day for females (Mejia *et al.*, 2009b).

The worst-case exposure scenario for CanolaPRO™ is highly exaggerated as rapeseed protein will not have 100% market share as it will compete with other vegetable proteins, and it was assumed that 39.7 - 47% of total protein consumption is replaced which is highly unlikely. Even in this worst-case exposure scenario the anticipated dietary intake is well below the NOAEL as established in the animal studies. Therefore, with the intended use levels of CanolaPRO™, there is no safety concern.

D.4 The percentage of the food group in which the novel food ingredient is proposed to be used or the percentage of the market likely to use the novel food ingredient

CanolaPRO™ is expected to be used in food at levels up to 30%. Due to potential unpalatability (bitterness) and/or technological limitations associated with its water-binding capacity, use levels are not expected to exceed this 30% in any one food. CanolaPRO™ is expected to be marketed at a low market share in the beginning, and market share may increase in the following years. As the intended applications are broad, a large group of the population may consume CanolaPRO™.

The expected market share for CanolaPRO™ may be compared to other protein isolates, such as soy proteins. The soy industry in Australia is rather small, and soy is mainly used for milk and flour. Approximately 3 liters soy milk per annum is consumed by Australians. In general, soy flour makes up 1 -5% of bakery dough formulation[20].

The EFSA Comprehensive European Food Consumption Database contains information on the consumption of textured soy protein (EFSA, 2015). For consumers the mean consumption was up to 0.1 g/kg bw/day and the 90th percentile was up to 0.52 g/kg bw/day for consumers only (National Diet and Nutrition Survey from UK).

The National Health and Nutrition Examination Survey (NHANES) is a program that studies the nutritional status of adults and children in the United States. NHANES data from 2007-2010 indicate a similar maximum potential replacement (40.5%) of total protein by CanolaPRO™ as the Australian and New Zealand survey data. Of the total protein consumption in the US, only 3.2% can be attributed to plant-based protein foods (Phillips et al. 2015). Similar results may

[20] SOYBEANS in the AUSTRALIAN and GLOBAL MARKET 2011. An industry review and report update on Australian and global soybean production, current market situations and trends, and an analysis of the Australian industry, its potential and needs. Report compiled by Denis M'Gee, on behalf Soy Australia Limited, April 2011. http://www.australianoilseeds.com/__data/assets/pdf_file/0005/8177/Industry__and__Market_Review_2011.pdf

be expected as well in Australia and New Zealand. Rapeseed protein will become part of the plant-based protein foods, but is not expected to replace all plant-based or soy protein foods.

D.5 For foods where consumption has changed in recent years, information on likely current food consumption

Not applicable.

D.6 Data to show whether the food, or the food in which the novel food ingredient is used, is likely to replace another food from the diet, if applicable

CanolaPRO™ is not yet marketed, and therefore it is yet unknown how much of the food will be replaced with CanolaPRO™. CanolaPRO™ is not expected to result in a higher protein consumption, but could replace current protein isolates in the final food. The foods containing the protein isolates will remain similar, but may contain more vegetable protein and less animal-derived protein. In other words: no intention to increase protein intake, but merely diversification of protein intake

D.7 Information relating to the use of the novel food or novel food ingredient in other countries, if applicable

The use of rapeseed/canola protein in human foods has been previously described. For example, multiple generally recognized as safe (GRAS) notices have been submitted to and filed by U.S. FDA with no objections (see section C.2.4), including GRAS notice GRN No. 683, submitted by DSM for a canola protein isolate that is identical to the substance that is the subject of this novel food notification.

The EU authorized the use of rapeseed protein isolate as a novel food ingredient in 2014 (European Commission, EU/424/2014). In March 2017, DSM CanolaPRO™ rapeseed protein isolate was approved in the EU as being substantially equivalent to the rapeseed protein isolate in EU/424/2014.

However, neither in the USA nor in Europe there is broad marketing of rapeseed protein isolate at the moment of submission of this dossier.

E Information on the nutritional and health impact of the novel food

E.1 Information to demonstrate that the use of the novel food or novel food ingredient will not cause a nutritional imbalance in the diet

This information is given in Section C.4.1 of the NOVEL FOODS part of this dossier.

E.2 Information to demonstrate that the addition of the novel food ingredient will not create a significant negative public health impact

The novel food CanolaPRO™ rapeseed protein is not intended to effectuate a potential beneficial physiological or health-related effect. Therefore, this Section is not applicable.

F Information related to potential impact on consumer understanding and behavior

The novel food CanolaPRO™ rapeseed protein is not intended to effectuate a potential beneficial physiological or health-related effect. Therefore, the Sections F.1 and F.2 are not applicable.

F.3 Information to demonstrate that the food(s) containing the novel food ingredient will not adversely affect any population groups (e.g. particular age or cultural groups)

- Substitute of existing proteins - plant and animal-based proteins and not substituting or replacing one food group with another i.e. not replacing Carbohydrates or Fats. Macronutrient intake and contribution in a total diet is expected to be constant.
- It will provide an alternative source of protein to sub-population who do not need eat meat due to religious or personal choice (e.g. vegetarians and vegans).

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APPENDICES

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